THEILERIOSIS

Report of a workshop held in Nairobi, Kenya, 7-9 December 1976

Editors: J.B. Henson and Marilyn Campbell

Cosponsored by the International Laboratory for Research in Animal Diseases and the International Development Research Centre
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IDRC


IDRC pub CRDI. Report of a workshop on theileriosis, a tick-borne parasitic disease occurring in Africa and the Middle East - examines the effects of the disease on cattle production, various means of effective disease control, incidence in other species of bovidae; discusses research activities; and the need for scientific cooperation and information dissemination; includes recommendation/s.


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Theileriosis

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Foreword

Theileriosis is an important disease of cattle in Africa and the Middle East that results in the death of affected animals and increased production costs because of efforts that must be made to control the malady. The disease is found in up to 15 African countries and seven other countries in the Middle East and Indian subcontinent.

Theileriosis, which is transmitted by ticks, the most important vector in East Africa being *Rhipicephalus appendiculatus*, is caused by several members of the genus *Theileria*: *T. parva*, *T. lawrencei*, *T. annulata*, *T. mutans*, and possibly others. The principal species of domestic animals affected are cattle, although other species of ruminants are also affected, including wildlife. In fact, recent work has suggested that wildlife including eland and buffalo serve as reservoirs.

Effective control of the disease by vaccination seems feasible and protective immunity has been demonstrated with *T. parva* and *T. annulata*. Research on theileriosis has recently been intensified due to the creation of the International Laboratory for Research on Animal Diseases (ILRAD) in Nairobi, which is specifically mandated to conduct research on theileriosis and trypanosomiasis. IDRC is also supporting research on these two diseases in East Africa through a cooperative program with the East African Veterinary Research Organization (EAVRO), which is working in close collaboration with ILRAD.

To gain an appreciation of the global situation regarding theileriosis, ILRAD and IDRC cosponsored a 3-day workshop in Nairobi, Kenya. The main purposes of the workshop were to:

1. define the magnitude of theileriosis as a disease problem limiting livestock production in developing countries in Africa and the Middle East by providing a forum for discussion by representatives of countries where the disease occurs;
2. identify constraints to the effective control of the disease in these countries;
3. define current and projected research activities on the disease by national and international organizations, and recommend how ILRAD can interact with national organizations;
4. identify opportunities for interaction, information exchange, and cooperative research among individuals and institutions;
5. disseminate recent knowledge and information; and
6. publish the deliberations as a step in fostering continuing collaboration and as a means of focusing attention on the disease and ways in which it may be controlled.

Representatives from Burundi, Egypt, India, Iran, Kenya, Malawi, Pakistan, Rwanda, Tanzania, Uganda, Zambia, and Zanzibar presented state-of-the-art reviews of theileriosis in their countries, and papers were also received from Mozambique, Sudan, Somalia, and Turkey. Three specialists
also gave reports and four institutions detailed their present and future research aims.

After the presentation of reports, participants were divided into four committees, given guidelines on their particular topic, and each committee produced a set of recommendations, which appear at the end of this report.

It is hoped that the workshop will serve to focus attention on this important disease complex and will lead to a closer degree of international cooperation in research in theileriosis.

IDRC is indebted to the staff of ILRAD who were responsible for the scientific aspects of the workshop organization, and to Dr T. Chandler of IDRC's Regional Office in Nairobi who undertook responsibility for much of the planning and staff work that ensured a successful meeting.

Barry L. Nestel
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Agriculture, Food and Nutrition Sciences
International Development Research Centre
Welcoming Address

I.E. Muriithi

Director of Veterinary Services, Kenya

I feel very honoured to have been asked to come and give a word of welcome and open this meeting on theileriosis. I welcome you not only on behalf of myself, but also on behalf of my country and of the Board of Trustees of ILRAD.

Let me take a little of your time and remind you about a few things concerning East Coast Fever (ECF), one of the diseases caused by *Theileria* spp. ECF is a killer disease. If you introduce mature, fully susceptible bovines into an endemic ECF area, you will be extremely lucky if 80% or more of the animals do not die of the disease. In this part of the world, ECF is one of the three or four most important diseases limiting livestock production. I say this to impress upon you that it is an urgent problem requiring intensive and concentrated research. This is one of the functions that ILRAD has been assigned to perform.

Let me indicate what I might call a few significant thoughts for consideration during your deliberations. One of them is that research studies have been going on for 50 or more years on ECF, ever since the days of Koch and Theiler in this part of the world. Although more than 50 years have been spent on and off ECF research, we still do not have what I would call a practical chemotherapeutic agent to combat the disease. I am also not aware of any practical vaccine to control the disease — this despite 50 years. Also, many groups of varying size and composition have met in the past and discussed various aspects of the problem.

Regardless of the meetings and the research, we continue to rely on cumbersome dipping or spraying for controlling the disease. I have said this because the veterinary organizations working within ECF areas and the farming public being pestered by ECF expect a group like this to really look hard, think hard, and look back on those 50 years or so and define the gaps in the knowledge that have prevented us from developing practical solutions to reduce the problems of ECF. This is one thought to leave with you for deliberation.

Another one I ought to leave is this. If there are some of you who are convinced or even believe that a vaccine to control ECF is not the main, ultimate solution or one of the major solutions to the problem, I think you ought to say so clearly with reasons and give us the next alternative. If someone or an organization is working on vaccines, other people can be looking at this other alternative. If you can give us an alternative, it would be wise for you to indicate how best we could achieve that alternative.
A third thought is for those who may think that a vaccine is the best alternative. I think there are one or two guidelines that should be pointed out in regard to a vaccine. One is that a vaccine should preferably be completely avirulent but still immunogenic. The second point is that once an effective vaccine is available, it should not cost us any more than we are now paying for dipping or spraying. Finally, if a vaccine can be produced that is both immunogenic and avirulent, such a vaccine will definitely be useful because it reduces our burden of spraying and dipping very considerably.

Ladies and gentlemen, I am aware that these are hard landmarks to aim for, but landmarks are good. Even if such landmarks are far away, you can see them from a distance. It means that even if one meanders too much in a valley, it is still possible to know where the mountain is. I am also aware that these landmarks cannot be done at once or in one stage, but they may be good landmarks to aim at. One other point I ought to impress upon you is that any research on ECF or any other areas where theileriosis is as crucial as it is in our own part of the world, ought to be done in a great sense of urgency. I am not in any way saying that there has not been urgency, but there is no harm in repeating and emphasizing this urgency. Those with the problem have had it for all these many years; they have been expecting a solution and it is only fair that if we are able to produce a solution, we should produce it quickly and let us get on and use it. If we cannot produce a solution, there is no reason why we should not say so, because if it is a dead end and the problem is insoluble, at least that answer is known, however negative it looks. One can then turn to the next problem, for there are many requiring solution. I have every confidence in this group and I know that you will work toward and come out with some practical solutions.
Country Reports
India

Bovine Theileriosis in India

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The cattle population of India was about 176 million in 1966 and increased to approximately 179 million by 1972.

Though reliable statistics on the number of crossbred cattle at risk in India is lacking, it is well known that the number is large and is increasing fast. In Punjab State alone, there are about 1 million crossbred cattle at present, and about 50,000 crossbred calves are being added every year.

Theileriosis as a disease problem in India mainly concerns the exotic (*Bos taurus*) and crossbred cattle, as the Zebu (*Bos indicus*) is less susceptible to the indigenous *Theileria* parasites. Calves are generally more resistant than adult cattle. As both the vector ticks and the reservoir of *Theileria*-infected cattle are of ubiquitous distribution in India, calves get infected during the first few days of life, perhaps suffer a mild fever that goes unnoticed, recover, and become preimmune. The equilibrium between the host and the parasite is labile, which may be upset by stresses such as rinderpest, rinderpest immunization with “strong” vaccines such as goat-adapted virus, intercurrent infections, etc. The latent theilerial infection, as well as babesiosis and anaplasma infections, then flare up causing clinical disease and high mortality.

Whenever an attempt was made to upgrade the low-yielding local cattle by crossing them with high-yielding exotic breeds, theileriosis, babesiosis, and anaplasmosis emerged as clinical entities. Similarly, the present large-scale crossbreeding program under way all over India has precipitated clinical theileriosis. Although babesiosis and anaplasmosis, rinderpest, and foot-and-mouth disease have been controlled, theileriosis continues to pose the greatest threat to the successful crossbreeding program.

Indian cattle are infected with two species of *Theileria*, namely *T. annulata* and *T. mutans*. Both parasites occur throughout India.

Clinical *T. annulata* infection has been recognized at one time or the other on almost all farms stocking exotic or crossbred cattle. Information on the number of cases of the disease in the country and the losses due to it is lacking. The problem, however, is assuming tremendous proportions with the increasing number of animals at risk.

Theileriosis occurs usually as subacute or chronic infection in India. Mortality varies from farm to farm, depending on the virulence of the infecting parasite and the age and type of animals involved.

**Past Research**

*Theileria* organisms were regularly observed in the blood of cattle under rinderpest research at Mukteswar by Hugh Cooper and his colleagues during the first two decades of this century and were recorded as “small piroplasms.” The first definite recognition of these as *Theileria*..
organisms in the blood of Indian cattle was made at Mukteswar during the 2 yr ending March 1924 (Edwards 1927). All the theilerial forms were then put under T. mutans. On 12 June 1922, Hugh Cooper discovered lesions in a hill bull that bore “suspicious resemblance to East Coast Fever ...,” observed “extremely rich infection of red blood cells with piroplasms identical in appearance to T. parva” and “abundant structures quite indistinguishable from the so-called ‘Koch’s blue bodies’.” After 1 mo, he observed a similar picture in six other bulls. Undoubtedly, he was dealing with T. annulata infection, but deaths were erroneously ascribed to a virulent variant of T. mutans.

Cooper and his colleagues provided increasing evidence of theilerial infection in indigenous cattle, as during the year 1923-24, “of 5158 blood smears examined, T. mutans (and T. annulata?)* was definitely demonstrated in 1368.” Thus, they focused the attention of veterinarians in the country on the widespread occurrence of theilerial infection in Indian cattle in the “carrier” state, which could flare up during intercurrent infections and rinderpest immunization resulting in death of the beast. Cooper in 1928 (cited by Edwards 1930) and Edwards (1930) documented accounts of natural outbreaks of theileriosis in imported herds at Lahore, and at Bangalore, Allahabad, and Kirkee, respectively. The symptoms shown were fever, lachrymation, pica, loss of condition, enlargement of superficial lymph glands, anemia (red cell count reduced to one-fifth the number), diarrhea in later stages, and death. Variable numbers of Theileria in red blood corpuscles were seen in the blood. Up to 50% red cells were infected in the Kirkee outbreak. The disease followed a subacute or chronic course. Death rate in the outbreaks at Lahore and Kirkee was 25 and 30%, respectively.

Theileriosis remained under study at Mukteswar during the 1930s, and detailed accounts were published by Sen and Srinivasan (1937). The parasite responsible for theileriosis in India was for the first time identified as T. annulata. Successful transmission of a local isolate of T. annulata from a hill bull by blood inoculation to healthy bulls was reported; 47% of the bulls proved refractory (premune?). They described the symptoms; sequence of appearance of the parasites, piroplasms, and schizonts; changes in blood picture; and lesions of the experimentally produced disease in indigenous cattle. Symptoms of the disease were rise of temperature, which frequently became subnormal before death, progressive inappetence, pica, enlargement of superficial lymph glands, anemia, and the visible mucous membranes showed varying degrees of icterus and petechiae. Examination of blood at the first rise of temperature frequently revealed a few piroplasms and schizonts. But the piroplasms increased in number as the disease advanced and might have eventually invaded 50-100% of the red blood corpuscles, although the schizonts varied in number from “rare” to “numerous.” The constant postmortem findings were thin and watery blood; enlargement and edema of lymph glands and spleen; gelatinous condition of the body fat; petechiae on serous and mucous membranes, pericardium, endocardium, and respiratory tract; characteristic ulcers with central necrotic area surrounded by hemorrhagic zone in abomasum, which might be present throughout the length of whole intestine. They showed that infectivity of the blood at 21-22 °C remained for 96 h, and lost infectivity within 6 days when stored in a refrigerator. They also reported that 26 drugs, chosen because of their parasiticidal value, had no activity against T. annulata. Lastly, they demonstrated the value of premunition as a protective measure as the hill bulls recovered from experimental T. annulata infection withstood experimental challenge.

Accounts of typical symptoms of the natural infection in exotic and crossbred cattle have been provided by Edwards (1930) and Kathuria (1936), and in indigenous cattle by Sharma and Cautam (1971),

*Parentheses of present authors.
and by provincial Veterinary Disease Investigation Officers in their annual reports.

Ray (1950) at Mukteswar demonstrated that *Hyalomma savignyi* transmitted *T. annulata*. His conclusion that the infection was transmitted transovarially is not valid as experimental juvenile stages of the ticks used in the experiments were fed on calves that might have carried inapparent *T. annulata* infection.

Strains of *T. annulata* causing 25% (Edwards 1930) and more than 75% mortality (Sen and Srinivasan 1937) in adult cattle, and 66-75% in experimental infection (Gill et al. 1976a, b) and 13-23% in natural infection of calves (Raghavachari et al. 1945) have been recorded in India.

**Present Research**

The present mass-scale crossbreeding program has renewed interest in theileriosis in India. The Indian Council of Agricultural Research, New Delhi, has recently sanctioned ad hoc schemes aimed at evolving control measures of bovine theileriosis. These schemes are functioning at Veterinary Colleges at Khanapara (Assam), Madras, Ludhiana, and at the Indian Veterinary Research Institute, Izatnagar.

Recent research on theileriosis has included transmission of *T. annulata*, which has been experimentally transmitted by *Hyalomma anatolicum* anatolicum, *H. dromedarii*, and *H. marginatum issaci* (Bhattacharyulu et al. 1975b) and *H. detritum* (Gill et al. 1974) but not by *Rhipicephalus haemaphysalooides haemaphysalooides*, *Haemaphysalis bispinosa*, and *Boophilus microplus* (Bhattacharyulu et al. 1975b). Bhattacharyulu et al. (1975b) showed that infective nymphs of *H. a. anatolicum* did not lose infection when fed on a refractory host (rabbit) and that the infection was transmitted when the engorgement was disturbed after 24 h and the semigorged ticks transferred to the second calf. Furthermore, Bhattacharyulu et al. (1975a) demonstrated infective "particles" of *T. annulata* in the salivary glands of the infective adult ticks 24 h after engorgement and in the infective nymphs after 48 h of feeding.

Gill et al. (1976c) observed that engorgement of ticks (*H. dromedarii*) for 48 h was essential for maximum infectivity and that infectivity after 24 h engorgement was 80% (four-fifths of the calves became infected). Furthermore, tick-tissue suspension of infective unengorged ticks on inoculation to five calves transmitted the infection to two of them. Moreover, the infectivity was seen to vary from tick to tick of the same batch. Research on the following three methods of immunization is in progress at Punjab Agricultural University, Ludhiana, India.

1. **Infection with a "safe dose" of the infective material**

Gill et al. (1976c) observed that a quantitative relationship existed between the magnitude of the infective inoculum and the ensuing reaction. Infection with one or two ticks or one-tick equivalent tissue stabilitate produced mild reaction in fully susceptible calves. But infection with 10 and 100, and 20 and 200 ticks caused severe reaction. It was inferred that infection with one-tick tissue stabilitate might be a practical method of immunizing cattle.

2. **"Infection-treatment" method**

Gill et al. (1976a) experimentally immunized calves 6 and 9 mo of age by deliberate infection with 10-30 ticks per calf and treated them with chlorotetracycline at 16 mg/kg for 8 consecutive days commencing on the 1st day of tick infestation. The calves proved to be fully immune to 10-tick challenge after 50-73 days whereas the nonimmunized control calves suffered from typical acute theileriosis and 56-66% of them died of the infection.

3. **Attenuated tissue-culture vaccine**

Gill et al. (1976b) attenuated a virulent isolate of *T. annulata* by successive passage
of tissue culture. Injection of $1 \times 10^6$ schizonts produced only a mild fever in 10 of 15 calves. Piroplasms appeared in 8 of 15 calves. All the calves were found to be fully protected against 10-tick challenge given 30 days after the immunization.

**Future Research**

Work on the following lines is progressing at Ludhiana:

1. Immunization with "infection-treatment" method using chlorotetra-cycline and long-acting oxytetracycline. The infection is initiated with stabilates.
2. Activity of Menoctone (Wellcome) against *T. annulata* in in vitro experiments. (Dolan and Machardy (1976) have shown that Menoctone possesses considerable activity against *T. parva*.)
3. Study of antigenic relationship between isolates of *T. annulata* from different regions of India.
4. Testing of tissue-culture vaccination and "infection-treatment" method under natural conditions.

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Iran

Theileriosis in Ruminants of Iran

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Animal husbandry in Iran traditionally has been based on keeping and breeding mainly sheep. Sheep breeding was almost entirely in the hands of tribal people who looked upon their sheep as their wealth and the means of living. Dairy products consisted only of cheese, yogurt, and ghee, which were also produced from sheep and goats’ milk. Milk consumption in towns was restricted to sick people, and it was produced from the odd dairy cow. The breed of cattle kept was mainly for draught purposes, but at the same time a little milk was produced. A turning point in cattle husbandry came when attention was directed to milk production and with it the improvement of local breeds was found to be necessary. Total cattle population of Iran was 4.9 million in 1966 of which most were of the local breed. In 1966, about 1503 head of pure exotic breed of cattle were imported and at the same time about 20 000 head were exported. The imported animals were for dairy purposes and the exported cattle were mainly for beef consumption in the neighbouring countries.

In 1972, the exportation of beef decreased almost to nil due to a greater demand at home for beef. This was mainly due to the ever increasing price of mutton, which used to be the meat of choice for Iranians.

On the other hand the importation of pure exotic breed was escalated and, for example, in 1975-76 about 22 000 cattle were imported. According to the Ministry of Agriculture, the total importation of purebred cattle will reach 200 000 in the next few years.

At the present time the cattle population is estimated to be about 5.2 million of which most are still of the local breeds. The sheep and goat population has been estimated at 41.99 million head in 1966, but the population of these animals is decreasing due to many reasons, primarily because of the settling of the tribal people.

The species of Theileria reported in Iran are T. annulata, T. mutans, T. hirchi, and T. ovis.

Theileria annulata. This parasite, which is the cause of cattle theileriosis (“tropical theileriosis”), is an omnipresent parasite in Iran and has been reported from practically every part of the country. Cattle theileriosis as a disease was first reported in this country in 1935, when veterinary services had acquired a practical existence and the Razi Institute as a veterinary research laboratory had been established. This was at the same time that the authorities in the Animal Husbandry Bureau decided to import some pure exotic breeds of cattle for the improvement of local dairy animals as well as for milk production. Almost all of these animals died shortly after their arrival. The disease was diagnosed as theileriosis, and since that time the disease has been terrorizing cattle-breeding farmers.

T. annulata in Iran has been shown by Delpy to be transmitted by many species of the genus Hyalomma, namely H. ex-
cavatum, H. detritum, H. savigny, H. rufipesglabrum, and H. dromedarii. Theileriosis in adult local breeds is not a problem as these animals contract the disease at an early age and either die, or survive and become immune. This immunity is boosted every year by being exposed to the tick challenge.

It is not very clear what percentage of calves die from theileriosis, as calf mortality from other pathogens is very high, but it is believed that theileriosis does not impose a very high mortality. According to reports of the Veterinary Services, the number of cases recorded in 1968 was 391 and in 1975 this figure reached 9526. Unfortunately we do not have a clear view on the number of mortalities, as these cases have been reported from those that were brought to veterinary clinics all over the country. Most of them did not call back again to report the death or survival of their animals. Moreover, this figure is not representative of all the cases because some animals must have died before the owners had time to refer to a veterinary clinic.

Although in the past few years the number of cases due to theileriosis have decreased in large farms, they have increased considerably in the animals of small farmers who are buying young bulls to improve their local breeds. At the present time large farms keep their dairy cattle on the zero-grazing method: hay is brought to the animals and therefore the chances of animals becoming infected is greatly lowered. Furthermore, these farmers, in addition to the precautions they take to reduce the chances of introducing ticks into their farms, spray their stocks with acaricides almost regularly. The low incidence of theileriosis in these farms can also be attributed to new animal housing that ticks have not yet inhabited.

*Theileria mutans.* This is the non-pathogenic species of *Theileria* of cattle in Iran. This species, which differs from *T. annulata* morphologically as well as pathogenically, is only found in the Caspian Sea area. It is not known exactly whether this parasite is *T. mutans* or *T. sergenti* because the vector of *T. sergenti*, which is *Haemaphysalis innermis*, is found almost entirely in the area where the nonpathogenic *Theileria* occurs. Therefore, there are grounds to believe that this parasite is more likely *T. sergenti* than *T. mutans*. More studies are needed to elucidate the issue.

*Theileria hirci.* This is the pathogenic *Theileria* of sheep. The disease caused by this parasite used to be confused with other piroplasms of sheep. Only recently has it been identified as a significant disease that affects lambs in spring, summer, and early autumn depending on the geographical zone. This parasite is a pathogen in sheep less than 9 mo old and causes considerable mortalities. The lambing season in Iran is spread over several months, commencing in midautumn and extending well into early spring. Therefore, each year in spring, summer, and autumn those that survive become immune and resist further infection in the later years and are the source of infection for the vector ticks. This disease has been reported from southern, southeastern, southwestern, western, and central Iran, and the number of cases are increasing each year according to the reports. The number of cases reported in 1972 was 3078, but in 1975, 16 512 cases were reported. The significance of the disease is due to the considerable mortality it causes in the indigenous stocks. The vector of this parasite is probably the tick of *Hyalomma* species, as it had been shown that the parasite can be transmitted experimentally by *H. anatolicum anatolicum*.

*Theileria ovis.* The existence of this parasite is doubtful, as there has been no experimental work to show that this parasite is not the erythrocytic form of *T. hirci* found in most of the sheep in the endemic area.

**Control of Theileriosis in Iran**

So far, nothing has been done to control sheep theileriosis and the work has been entirely devoted to *T. annulata*. The
control methods consist mainly of tick control and vaccination of susceptible stocks against theileriosis. The Veterinary Services is actively engaged in regular spraying of animals and animal premises with acaricide. Generally speaking, the tick population has been considerably reduced in recent years. The vaccination is carried out in exotic breeds that are recently imported or bred in the country. The significance of this vaccination is yet to be evaluated, since there is not a clear-cut report to show the effectiveness of the vaccine in relation to the natural tick challenge.

The research on theileriosis in Iran is restricted to two institutions, namely the Razi Institute and the School of Veterinary Medicine, Pahlavi University, Shiraz. The research in the first institution is mainly concerned with improvement of the vaccine and in the second institution on the study of the mechanism of immunity in the bovine host and a study on the tissue culture.
Theileriosis in Turkey

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In Turkey, *Theileria annulata* and *T. mutans* are widespread; infection with *T. annulata* almost always results in death of the animal, but the mortality rate for *T. mutans*-infected animals is low. *T. parva* was once believed by researchers to exist in Turkey but studies have not proven this.

Mortalities from *T. annulata* mainly occur in imported dairy cattle from Switzerland, Germany, Holland, and the USA and represent an important loss to the economy of Turkey. *T. annulata* is found in 33 of 67 provinces, and *T. mutans* is found in all 67 provinces.

In 1930, Lestoquard researched protozoa-caused diseases at Bursa and his results are still valid today.

Through the support of the Turkish Scientific and Technical Research Council, nine bovine parasites were identified, including *T. annulata* and *T. mutans*. *T. annulata* is the most important of the nine, and although *T. mutans* was once thought to be a mild strain of *T. annulata*, morphological and biological studies recognized it as a distinct species.

*T. annulata* is carried by ticks of the *Hyalomma* genus: *H. detritum*, *H. excavatum*, *H. dromedarii*, and *H. savingny*. The tick *Boophilus annulatus*, which is common in Turkey, carries *T. mutans*, according to studies by Guler. Ticks belonging to the Ixodidae and Argasidae families thrive in the Turkish climate. The infected nymph stage of *H. detritum* can survive the winter in stables and infect cattle in the spring.

Our experimental research has mainly taken place in the Karacabey state farm, 75 km from Bursa. Although cattle had died from theileriosis in villages close to the state farm, no instances of theileriosis were witnessed on the state farm itself. However, thousands of cattle on the state farm were infected by *T. mutans*.

*T. mutans*-infected calves 14-16 mo old were brought from the state farm to tick-free stables and injected with blood from cattle in the neighbouring villages. All such treated cattle died of theileriosis. However, splenectomized cattle infected with *T. mutans* did not die.

In theileriosis caused by *T. annulata* many Koch bodies were found in the preparation made from lymph nodes. But even when *T. mutans* infection was very severe there were very few Koch bodies.

Imported cattle could be protected from infection if kept in tick-free stables and grazed in enclosed pastures. Native cattle are quite resistant to disease, and the 50% that have overcome the disease carry the sporocysts for the rest of their lives and enter a premunition period. These cattle are then carriers and continuously infect ticks that suck their blood. Therefore, if imported cattle graze the same pasture as village cattle, it would be impossible to prevent them from becoming infected.

If the disease is not in an advanced stage, an experienced clinician can save the life of
the animal. The organic disorders should be overcome and constipation must be avoided; stomachs and intestines must work regularly, and drugs against anemia and liver disorders must be given. Immunized blood, blood serum, and gamma globulin must be injected. Sick cattle must have the best food; and to prevent complications antibiotics (oxytetracycline) are advisable.

Because there is so far no efficient medicine for treating theileriosis, preventive precautions should be taken.

At present, there are studies being done in Turkey on a vaccine against theileriosis in tissue culture.
Egypt

Theileriosis in Egypt

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Theileriosis is a major threat to livestock in Egypt. The causative organism, *Theileria annulata*, affects most cattle at all ages. Buffalo may harbour the parasite but are not as seriously affected as cattle. The vectors of the parasite are ticks of the *Hyalomma* genus.

Cases of infection are encountered in the hot summer season when the weather is suitable for the propagation of ticks. Although more cases are found in the oases, some are encountered in the Delta region and in other parts of the Republic.

Past Research

There have been many papers published on theileriosis research in Egypt and a list of pertinent references is included at the end of this paper. However, no data are available at this moment on the monetary loss per year and over the past 10 years from theileriosis.

Present Research

At present, trials are being conducted on the diagnosis of latent infections of theileriosis with serological tests, using an antigen prepared from local strains. Such research requires 8 man-years for professional staff and 6 man-years for technical staff.

Future Research

Future research plans include:

1. preparation and production of specific antigens for detection of latent cases in both cattle and buffalo;
2. studies on the role of buffalo as carriers of the disease;
3. trials to immunize susceptible animals by using a vaccine produced by the tissue culture technique;
4. detailed studies on the effect of various insecticides on the animal host, on the insect vector, and their residual effect in the tissues and animal products used for human consumption;
5. control of vectors transmitting the disease as ticks:
   a. studies on different species of tick-intermediate hosts involved in localities where they are prevalent;
   b. studies on other vectors, such as blood-sucking insects (flies or mosquitoes), that may play the role of mechanical transmission of the parasite from one host to another;
   c. studies on new insecticides used for the control of ticks and methods of application: systemic, sprays, spray race, etc.

To continue theilerial disease control and research in Egypt, the establishment of a regional diagnostic unit for piroplasmosis in the Animal Health Research Institute in Egypt is needed to serve all Arab countries and the Middle East, including Egypt, Libya, Tunisia, Algeria, Morocco, Mauritania, Sudan, Ethiopia, Saudi Arabia, Syria, Lebanon, Jordan, and Iraq.

This unit would serve as a diagnostic
centre for piroplasmosis in the area, and would apply therapeutic agents to treat these diseases. The results would hopefully be the eventual destruction of blood-sucking insects and ticks; reduction of tick-borne diseases, mainly piroplasmosis, and other viral diseases; and an increase in the productivity of milk and meat-producing animals with the lowest costs.

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Pakistan

Theileriosis in Pakistan

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The livestock industry plays a vital role in the agricultural economy of Pakistan and provides 28% of the value of agricultural production. In 1972 there were approximately (in millions) 14.4 cattle, 9.7 buffalo, 12.8 sheep, and 14.9 goats in the country.

There are approximately 3.7 million milking cows and 5.6 million milking buffalo that produce about 2.2 and 6.2 million tons of milk respectively. The average milk production per animal (246 lb (112 kg) milk per capita per annum) is low although overall genetic quality of livestock, particularly buffalo, appears fairly good. Meat, which is the by-product of milk and draft animals, is unable to meet the national requirements. It is estimated that at an average off-take of 10% for cattle and 19% for buffalo, some 280 000 tons of meat were produced in 1972-73 and 294 000 tons during 1975-76. This produced 15.8 lb (7.2 kg) of meat per capita per annum, as compared with Iran 36.5 (16.6), UK 167.2 (76), West Germany 108 (49.1), USA 156 (70.9), and Australia 228 (103.6) per annum respectively.

The progress of this industry is very much handicapped by widespread contagious and parasitic diseases that occur sporadically. They have caused heavy losses in the past, estimated at about 50 000 to 100 000 head per annum in terms of mortality, and losses due to morbidity may be enormous as a considerable proportion of survivors suffer from loss of vitality and subsist with poor performance either for long periods or for the rest of their lives. Based on the realization of the need to improve the quality of our local cattle, an effective disease control program has been launched to combat several contagious diseases such as rinderpest, hemorrhagic septicemia, anthrax, etc., but parasitic disease continues to be a severe problem of productivity and vitality.

In Pakistan, two forms of theileriosis have been identified and these occur sporadically. Infection with *Theileria mutans* is commonly found in cattle but without producing symptoms. The organisms are rod-shaped in RBC and mostly dividing forms are seen. The other form is *Theileria annulata*, which is pathogenic. About 70% of the parasites are round or oval and the rest are rod-shaped. Schizogony is seen in the endothelial cells of capillaries. Two strains of the species have been identified. The first is the “J” form, which produces a mortality rate of 30-40% in calves of 2-12 weeks of age. The infected calves exhibit stomatitis and gingivitis. The second strain is identified as Mukteshwar strain, which affects both young and adult cattle with a high mortality rate up to 65%. This type produces enlargement of lymph glands, liver, and spleen, anemia, and petechiae in the alimentary canal.

An outbreak of theileriosis in crossbred cattle calves in a military farm located near Lahore has been detected and was suspected to be due to *Theileria parva*. Postmortem findings revealed various sizes
of hemorrhagic patches in the subcutaneous tissue, mesentery, intestinal mucosa, and the abomasum. The most characteristic lesions were found in the abomasum where numerous small, circumscribed, raised, red areas without a reaction zone in the mucosa were found. The liver was enlarged, friable in consistency, and had a few white spots. Petechial hemorrhages were also present in the gall bladder, urinary bladder, and on the pleura. The spleen was macroscopically normal.

Organisms microscopically indistinguishable from *T. parva* were seen in blood and lymph gland smears. The forms recorded in the blood were small comma-shaped or pear-shaped bodies. Koch’s blue bodies were also seen. In some cases rod-like masses of cytoplasm with a red chromatic dot at one end of the rod were seen. Schizonts in various stages of their development were also recorded in lymph gland punctured smears. *Hyalomma aegyptium* is suspected as the vector of the disease in this instance. The organism is recorded for the first time in Pakistan but a detailed study was not made.

**Present Research**

At the Veterinary Research Institute, Lahore, the Protozoan and Tick-borne Disease Division has initiated research on factors that determine susceptibility, resistance, and influence of ticks on the disease.

During a survey, 8547 tick specimens were collected from 770 host animals, i.e., buffalo, cattle, sheep, goats, and camels, in 11 administration divisions. Sixteen species of hard ticks representing five genera were found infesting domestic livestock.

This division also examines microscopically blood smears received from the field veterinarians for the diagnosis of protozoan diseases. Babesiosis and anaplasmosis have been recorded at various farms, most frequently in the crossbred exotic stock.

**Future Research**

Researchers should concentrate on serodiagnosis of protozoan diseases because the traditional method of diagnosis by blood smear examination does not yield the desired results. With the newer physical and biochemical methods, substantial progress has been achieved in the field of immunology. Antigen/antibody systems have been identified in several parasitic diseases such as malaria, amebiasis, and many helminth infections. The phenomena of antibodies and cross reaction of various species of parasite have been indicated through fluorescent antibody techniques (IFAT), gel-diffusion, IHA, and complement fixation tests (CFT). Some researchers have also studied IgG and IgM level, which is another area that requires further investigation.
Uganda

Theileriasis in Uganda

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The term “theileriasis” is used here as an alternative name for “theileriosis” to describe a disease syndrome caused by nonpigment-producing groups of piroplasms of the family Theileridae du Toit, 1918, in the genus Theileria Battencourt, Franca and Borges, 1907. It is an infectious tick-borne disease of domestic and (rarely) wild ruminants, affecting mainly blood and lymphoid tissues.

The disease occurs throughout Uganda (Oteng 1970, 1973a) and its distribution is closely associated with the distribution of the tick vectors, Rhipicephalus appendiculatus and rarely Amblyomma variegatum, which are found throughout the country (Wilson 1953; Matthysse and Colbo 1976). The incidence of the disease, however, varies with the amounts of rainfall received in each area of the country. The country may, therefore, be divided into three major areas on the basis of the incidence of the disease:

(1) areas of epidemic outbreaks, which receive an average annual rainfall below 800 mm, e.g., Karomoja;
(2) areas of sporadic outbreaks, which receive an average annual rainfall of 800-1000 mm, e.g., Ankole, L. Mobutu, and the Mobutu-Nile Valley areas;
(3) areas where the disease is endemic, which receive an average annual rainfall over 1000 mm, covering 80% of Uganda.

In addition to all clinical forms and reactions of the infected cattle to Theileria described by Henning (1956) and Neitz (1957), the following clinical forms and reactions are observed in Uganda:

(1) Turning Sickness: Described by Mettam (1933, 1934a, b, and 1936), Mettam and Carmichael (1936), and Woodson and Oteng (1968). Many more cases are still being recorded in the country.

(2) Unthriftiness in recovered calves and adults: Characterized mainly by weight losses, diarrhea, inappetence, and failure to gain even the original weight before infection (Wilson 1951a, b; Okao and Oteng 1973; Oteng 1973a) for at least 6 mo.

(3) Occurrence of up to three clinical reactions under field conditions before the recovered animals are actually immune to theileriasis (Wilson 1950a; Oteng 1973a).

(4) Durability of immunity on recovery from theileriasis appears to be dependent on the severity of the reaction on infection (Wilson 1950b). This is probably related to the number of infective ticks feeding on the susceptible cattle (Wilson 1950a) and the degree of infection of the ticks by the parasites (Wilson 1951a, b).

(5) A pathogenic T. mutans, which can be transmitted by inoculation of infected blood, is present in Uganda. The schizonts are readily demonstrable during the reaction period, and persist for at least 3 yr in the recovered animals (Oteng 1973a, b).

(6) Skin conditions, possibly an allergic dermatitis (Oteng 1973a) were observed
mainly in recovered adults on subsequent challenges.

There are about four pathogenic Theileria in Uganda and these include pathogenic *T. mutans* and three other pathogenic *Theileria* serologically (FAO Reports 1974, 1975 — Entebbe 1 & 2) but not immunologically (Guilbride and Opwata 1963; Oteng 1973a, b; Oteng et al. 1975; FAO Reports 1974, 1975) related to *T. parva* (Muguga).

There are four major results from theileriasis in Uganda:

1. **Calf mortality**: Theileriasis affects up to 50% of the annual calf crop in the theileriasis enzootic areas in Uganda. Intensification of disease control by adequate application of acaricides has reduced mortality to 5%, e.g., in the Bukedi District of Eastern Province during 1970-74.

2. **Unthriftiness in calves**: It is estimated that 5-10% of the calves that recover from clinical infections remain unthrifty and often stunted. Some of these animals may eventually die of other diseases. The steers often take up to 5 yr to reach economic slaughter weight and the heifers may take up to 2 yr before breeding. In any case, the mature adults always remain small, weighing between 200 and 300 kg.

3. **Mortality in adults**: This usually occurs in epidemic proportions, whereby 80-90% of the cattle die. Such incidences often occur in Karamoja and also in theileriasis-reclaimed areas on reintroduction of the disease, e.g., the Kyaggwe District in North Buganda Province, where the disease was almost eradicated by intensive use of acaricides.

4. **Unthriftiness in adults**: All the adults that recover are affected and it takes up to 18 mo before the animals start gaining condition even under good management. Female adults often cease breeding for at least 12 mo, and abortions occur in pregnant animals. Widespread dermatitis may occur in a few of the recovered animals.

All the visible (mortality) and perceptible (unthriftiness) losses due to theileriasis have a great tangible impact on the economy and health of the people of Uganda.

**Past Research**

Past research on theileriasis in Uganda has been reviewed by Oteng (1970a, 1973a). Bruce (1910) was the first worker to equate the local disease "Amakebe" with East Coast Fever (ECF) and this was confirmed by workers in South Africa (Mettam and Carmichael 1936). Further records on the disease were made by Richardson (1921-26), Mettam (1933, 1934a, b, 1936), Mettam and Carmichael (1936), Wilson (1950a, b, 1951a, b), Guilbride and Opwata (1963), and Stobbs (1966).

The research during this period aimed at finding ways and means of conferring immunity to susceptible cattle to reduce mortality caused by the disease, especially in calves and yearlings.

**Present Research**

The present work on Ugandan theileriasis started within the last 10 yr.

Current research on theileriasis has largely been on serological grouping of pathogenic *Theileria* under field conditions (FAO Reports 1976), using indirect fluorescent antibody (IFA) tests. An epidemiological survey has been based on records of field cattle mortality. Limited cross-immunity trials (Oteng 1973a; FAO Reports 1974, 1975; and Robson 1976) were also conducted. The present workers in Uganda have concluded, from the results obtained, that ECF is a disease syndrome caused by immunologically distinct types of *Theileria*.

Clinical and pathological examinations of ECF-infected cattle are still continuing in an attempt to recognize the various forms of the disease under field conditions.

Four professional staff have been involved in theileriasis research for a total of 14 man-years, assisted by two technical staff throughout this period.
Future Research

Future research will aim first at isolating and identifying the *Theileria* species commonly involved in theileriasis in Uganda, and secondly, together with other interested research institutions, at identifying the master or composite immunogenic strain(s) to be used subsequently as a component(s) of the vaccine against theileriasis.

Control

Uganda is adopting a two-pronged approach to control theileriasis, using both biological and chemical control methods. The main and immediate objective is to reduce gross tick infestation on the animals and in the environment (pasture), and ultimately to reduce and possibly eradicate the disease.

(1) Biological control: This may be visualized as consisting of: (a) Ecological control of ticks, i.e., creation of unfavourable environment for tick survival and possible discovery of predators for ticks; and (b) Vaccination against theileriasis (ultimately).

(2) Chemical control: Gross reduction of tick population by intensive use of acaricides is the main objective. It is hoped, depending on the carrier state of the disease, that theileriasis can also be effectively controlled, if not eradicated, from defined environments by this method. It is too early to consider treatment of theileriasis as another possible method of controlling clinical cases of the disease.

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Theileriosis in Malawi

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*Theileria parva* infection (East Coast Fever, ECF) is a major cause of cattle mortality in Malawi, a country in which the cattle population more than doubled between 1965 (318,000) and 1975 (700,000).

A Malawian-Danish cattle survey, using a sample of 2000 cattle over a 5-yr period, has shown that the mean calving percentage for breeding females of the Malawi Zebu breed is 63.5%. The number of calves dropped each year and the number of calves entered in the census records differ markedly and it is estimated that 66% of each year’s calf crop is lost before attaining the age of 2 yr.

Roughly 165,000 calves of 250,000 will be lost from the 1976 calf crop and the majority of these will die as a result of *Theileria parva* infection.

*T. parva* is prevalent in the Northern and Central regions of Malawi but is rarely seen clinically in the Southern Region. Although clinical ECF is rare in the south, serological evidence is available, using the indirect hemagglutination test (IHA) and indirect fluorescent antibody test (IFA), that indicates exposure of cattle to *T. parva*. The existence of an avirulent *T. parva* is a possibility.

*T. mutans* is widespread in all three regions but there is no indication as yet of the presence of a pathogenic strain.

*T. lawrencei* has not yet been identified in Malawian cattle or buffalo. Buffalo herds are confined to game parks and reserves and buffalo rarely graze the same areas as cattle. *T. lawrencei* is not thought to be a factor in the ECF picture in Malawi.

During 1975 there were 1466 cases of theileriosis confirmed at the Central Veterinary Laboratory and the regional laboratories.

Any estimate of the annual monetary loss due to theileriosis must perforce be approximate. Over the last 10 years it would be approximately 120,000 calves at 7 kwacha (K)* each and 10,000 adults at K60 each. The total loss of K1.44 million includes only losses through mortality and does not take into account production losses during nonfatal infections, which must be considerable.

Past Research

There has been little research into theileriosis in Malawi in the past, and little has been published in the scientific literature, with the exception of S. G. Wilson (1944. Veterinary Record (England), 56, 255-258). Information available has been obtained through routine examination of smears submitted from dipping stations and through a small amount of serological survey work carried out by Malawian-Danish and UNDP/FAO teams. From this information it has been possible to map roughly the incidence of theileriosis in the country.

*1 kwacha equals ca. U.S. $1.10.*
Present Research

(a) Nationwide Serological Survey: Roughly 600 bovine serum samples have been collected from all of the Government dipping stations in Malawi. These sera are being assayed, using IHA for titres to T. parva and T. mutans antigens. Bearing in mind the limitations of this test, it should be possible to map more accurately than before the incidence of theileriosis in Malawi and to delineate areas of ECF and of benign theileriosis.

(b) Three Tank Survey: Seventy-two village cattle divided equally between three dipping stations thought to be in high-, medium-, and low-incidence areas of ECF have been sampled monthly for the last 20 months. Blood for serology and blood and gland biopsy smears are collected. Serum is examined by IHA for T. parva and T. mutans and also by IFA for T. parva. Smears are stained by Giemsa and examined under oil immersion. A record is kept of past history and current clinical status of the cattle under survey. Useful information has been obtained from this prolonged survey through study of theilerial schizonts and of serological titres found month by month. This survey has also provided accurate base lines for interpretation of the nationwide serological survey already mentioned.

(c) Isolation of Malawi Strains of T. parva: The first Malawi strain of T. parva was isolated and characterized during 1976 using techniques developed at EAVRO, Muguga. Characterization studies continue and calves immunized with T. parva Malawi I are being challenged with infected field strain Rhipicephalus appendiculatus in the hope of isolating further distinct strains of T. parva.

(d) Stabilate Production: Initial steps have been taken in the development of a stabilate bank at Central Veterinary Laboratory. Using T. parva Malawi I, a small amount of ground-up adult tick stabilate (GUTS) was produced, which proved infective after 2 mo in storage at -70 °C.

(e) Tissue Culture: Techniques for growth of spleen monolayers and lymphoblastoid cells in suspension are being developed.

(f) ECF Postmortem Survey: Using a standardized routine, a postmortem survey of gross pathological findings in ECF cases is being conducted to define the Malawian ECF postmortem picture and to compare this with the ECF picture reported in the literature by other countries.

At present there are two professional and five technical staff involved in research on theileriosis.

Future Research

Plans for future research include: (a) continue to isolate strains of T. parva; (b) complete the nationwide serological survey; (c) continue the three tank survey; (d) complete ECF postmortem survey; (e) continue stabilate production and tissue culture; (f) immunize ECF-susceptible grade dairy cattle using the infection-and-treatment method prior to their issue from Government breeding stations into field conditions in ECF endemic areas, and compare the fate of these immunized cattle with nonvaccinated control cattle under the same conditions; (g) develop a reliable field test to measure an animal’s immune status relative to T. parva; (h) determine whether ECF immunity is in fact a sterile immunity or possibly a premunity that can be broken down with recrudescence of infection by stress factors; (i) challenge cattle from the Southern Region of Malawi, which have shown a strong IHA titre to T. parva antigen in the absence of any reported clinical signs, with T. parva Malawi I; (j) attempt isolation of T. parva from the Southern Region; (k) isolate strains of T. mutans and assess their pathogenicity.

Conclusion

Efficient tick control and animal movement control will always be of prime importance in theilerial disease control and steps are continually being taken to tighten up this control.
Artificial immunization of susceptible cattle that have not developed a natural immunity would assist greatly in this control by reducing the reservoir of susceptible cattle and so of infected hosts for the vector to feed on. Vaccination of young calves and of exotic and grade cattle would be of value in this respect.

The development of a reliable field test to measure the immune status of an animal is essential as is a vaccine as specific as possible to the challenge strains of *Theileria* to which cattle will be exposed. Research efforts in Malawi will be concentrated in these fields.
Kenya

Theileriosis in Kenya

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Kenya, with a land area of 569,000 km² and a human population of approximately 13 million, has an economy based primarily on agriculture. Approximately 15% of the land receives an annual rainfall in excess of 89 cm and is suitable for intensive agriculture. A further 10% of dry woodland with a rainfall between 64 and 89 cm forms a transition zone between high rainfall areas and areas suitable for ranching; the latter, the typical acacia grassland of East and Central Africa, forms 54% of Kenya’s land.

In 1966 the cattle population was estimated at 7.1 million; by the beginning of 1976 this figure had risen to 9.7 million. This figure can be further broken down to 650,000 dairy cattle; 900,000 beef cattle on large farms or in settlement areas; and 8.15 million cattle in pastoral/nomadic areas and in small holdings.

The current trend is toward the introduction of exotic (especially dairy) breeds, usually by repeated upgrading with artificial insemination, into areas with high agricultural potential, and, in drier areas, the development of large ranches often on a cooperative basis utilizing upgraded Zebu or Boran stock.

In 1973, the gross marketed production of cattle (including hides and skins) was 17.7 million Kenyan shillings (KSh)* and a further KSh 11.3 million for dairy products. This sum of KSh 29 million was out of a total agricultural production of KSh 123 million. Of the KSh 29 million, a sum of KSh 10.1 million was earned by exports.

The main species of theileriosis in Kenya are *Theileria parva*, *T. lawrencei*, and *T. mutans*. The latter is usually nonpathogenic but recent work at EAVRO has resulted in the isolation of pathogenic strains from various parts of Kenya.

It is estimated that 52.8% (i.e., 5.122 million) of the cattle are in areas where *Rhipicephalus appendiculatus* (the main vector tick of ECF) is present. Within this area there are four main situations where, in our experience, ECF is a problem:

1. Areas where the disease is enzootic but acaricide treatment, usually in the form of dipping or spraying, is readily available. Cattle in these areas are usually grade or improved indigenous stock. The reasons for ECF outbreaks are mostly due to faulty dip management. Deaths generally occur in cattle over 1 yr old.

2. Areas where the disease is enzootic but dipping is absent or irregular. Many such areas exist in parts of western Kenya where there is a dense cattle population. In such conditions, the main problem is ECF deaths in calves.

3. Marginal areas where *R. appendiculatus* does not normally occur. In times of heavy rain, the situation can change and large numbers of susceptible cattle can be exposed. Deaths occur in all age-groups.

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*KSh 8.10 equals ca. U.S. $1.00.*
Areas where susceptible stock are moved into enzootic areas without the proper acaracide control. Death occurs in all age-groups.

In areas where calf mortality occurs, figures are not readily available. However, it is estimated that in two provinces of Kenya where ECF is enzootic and dipping is not widespread, i.e., Nyanza and Western provinces, approximately 10 000-15 000 calves die every year of ECF. However, as the stocking rate is already very high in such areas, the potential financial loss is not as great as deaths in adult stock mentioned above. In fact, in our experience, calves surviving this initial exposure tend to show a lifelong immunity to ECF, as they are usually sedentary and exposed to a fairly constant T. parva challenge. Diseases such as helminthiasis are more important in these areas due to death and loss of production in adult stock.

To summarize, therefore, the greatest financial loss occurs in grade cattle or improved indigenous breeds in areas of relatively high agricultural potential, on farms often with access to dips or sprays.

The number of ECF cases per year in cattle over 1 yr old is probably between 50 000 and 70 000 but certainly not less than 40 000. As these deaths are in mostly mature stock, representing cash-in-hand to the farmer, the annual loss is between KSh2.5 and 3.5 million. Another example of the loss due to ECF is found in intensive follow-up studies of heifers produced by artificial insemination. In 1974-75 approximately 115 000 heifer calves were born as a result of A.I. It is known from previous results that 35% of these heifers will die before they reach calving age. Of these 35%, between 30 and 40% die of ECF. This means that at least 12 000 heifers are lost annually because of ECF, each with a potential value of KSh75, a total of KSh0.9 million. This loss of mainly dairy cows is a considerable drawback to the expansion of the Kenyan dairy industry, which, in 1973 for example, processed 275 million litres of milk.

Tick Control

As is clear from the above, the main reason for ECF deaths is faulty dip management. At the present time, there are in Kenya more than 4500 dips and spray races. It is estimated a further 1000-1500 are needed to provide a reasonable cover throughout the Republic.

The average cost of dipping once a week is approximately Kcents 18-21 per cow/year. However, the potential financial saving if ECF were no longer a problem would not be as great for two main reasons:

1) The loss due to other tick-borne diseases remains high. For example, in the country as a whole, reported cases of anaplasmosis are approximately double that of ECF cases. Although a proportion of anaplasmosis cases survive with or without treatment, the financial loss runs into millions of Kenyan shillings a year.

2) Although this is not generally recognized, dips in Kenya are becoming increasingly important as “meeting places.” For example, A.I. is often conducted at the dips, vaccinations are usually carried out, even dogs being vaccinated against rabies. Also our clinical services in providing routine treatment increasingly rely on cattle coming to the dips. Therefore, to the Veterinary Service in Kenya, the dips are invaluable.

To overcome our deficiency in dip management, the Veterinary Department is proposing to take over the entire supervision of cattle dips throughout the small-scale farming areas of the Republic. This project, partly financed from donor agencies, will come into operation in 1977 and by 1981-82 be operational in all districts. In this way properly controlled dipping should save thousands of cattle every year. The important factors in this project will be the introduction of free but compulsory dipping.

However, ECF will certainly remain an enormous problem even with well-managed dipping; and in the event of an ECF vaccine being successfully developed, the dipping interval could be increased.
Research

A summary of past research on theileriosis within Kenya is beyond the scope of this report, as innumerable articles based on such research have appeared in the scientific literature.

Currently, there are two professional staff and two technical staff engaged in theilerial research. The work is done in cooperation with the USDA immunology project at EAVRO. A general outline of the current research would include:

(1) Immunoglobulins: The immunoglobulins and immunoglobulin subclasses directed against *T. parva* are being studied. The composition of such immunoglobulins after both primary and secondary challenge (including field exposure) is being studied in relation to the potential immunity of the cattle. The specific immunoglobulins are also being examined for their mediation of several serological tests for *T. parva*. Lastly, another functional aspect of bovine immunoglobulin subclasses is being looked at, namely their importance in antibody-dependent cell-mediated cytotoxicity.

(2) Pathophysiology: This work includes detailed studies on the level of various blood components during the course of ECF infections. For example, C3, conglutinin, hemoglobin, and serum proteins are being looked at. A thorough hematological study, including the ratio of Ig-bearing cells amongst the lymphocyte population, is being carried out. The purified lymphocytes are also being studied for their ability to respond to mitogens such as phytohemagglutinin and pokeweed.

(3) Current research would also include studies on the apparent immunosuppression that occurs in ECF; this work is mainly on the primary and secondary antibody response to both live and dead antigens.

Future research will continue along the above lines, including the various aspects of immunization that are most likely to elicit a functional immunity.
Zanzibar and Pemba

Theileriosis in Zanzibar and Pemba

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Theileriosis, mainly East Coast Fever (ECF), is the biggest obstacle to productive cattle raising on the islands of Zanzibar and Pemba.

At present, ECF is endemic in both islands and is at a constant level throughout the year. Trials to introduce thoroughbred and crossbred animals to local farmers have failed because the animals have died within 3-4 mo from ECF.

All of the local-borne calves are infected before 6 mo old and the estimated calf mortality due to ECF is about 30%. The total calf mortality is more than 50%. The local people have accepted this situation and regard it as normal. On the other hand the adult cattle are all naturally immunized and rarely get sick.

The average number of adult *Rhipicephalus appendiculatus* found at tick investigations at different times of the year in various sites in Zanzibar and Pemba has been 10 per animal in Zanzibar and 100 per animal in Pemba. These seem to be fairly constant throughout the year.

At present no control measures are applied to privately owned cattle. Dip facilities exist but are closed due to low interest in dipping on the part of the farmers.

In the government-owned beef and milk ranches good tick control is in operation. Serological tests on the cattle for ECF antibodies confirm that the control is working well. Despite that, some cases of ECF still occur every year among calves and adults of these animals. This is probably due to insufficient fences around the ranches, which gives the tick-infested private herds access to the pastures.

The types of *Theileria* present are *T. parva* and *T. mutans*. *T. lawrencei* is not likely to be present because no wild hosts exist in the islands.

The number of cases of theileriosis per year is not properly recorded, but theileriosis can, as said before, be estimated as infecting all calves born each year, 5000 in Zanzibar and 8000 in Pemba. The annual loss due to theileriosis would then be 4000 calves a year, plus losses due to retarded growth and lowered production in surviving animals. The cost of being forced to keep low-producing animals instead of improved stock also has to be added. This figure has probably been quite constant during the last 10 years, since no changes in control measures have occurred during this period. The total monetary losses would therefore be around 2 million Zanzibar shillings per year.

Research

Some investigations on ticks were made during 1976. One investigation was done by Dr Newson from ICIPE in February, and another one by Dr Tatchel, consultant to the project FAO/URT/73/025. These reports deal with the distribution of the tick in different vegetation and under different climatic conditions.

The result, as reported earlier, was an average count of 10 *R. appendiculatus* per animal in Zanzibar and 100 per animal in
Pemba. The higher number of ticks in Pemba could have various explanations: microclima could be more favourable due to open pastures and less bushy vegetation, whereas the humidity in Zanzibar at ground level is probably too high to fit the life requirements of R. appendiculatus; the cattle density in Pemba is also higher, a fact that has to contribute to the higher counts. The reason for the large difference in tick numbers has not yet been investigated and could be an interesting field for further studies.

Present investigations are being done by the FAO (FAO project URT/73/025), which started its activity in Zanzibar in January 1976. These activities involve one FAO tick-control officer and two local veterinary assistants.

The work being done is a continual tick survey made every 2nd mo on the spots checked in the reports. The counts seem to be fairly stable, and the variations recorded at different months seem to be due more to variations in individual pickup rate than to different climatic conditions. At the same time a test on the antibody status of the cattle population is done by blood samples on filter paper, which are then dried and sent to ILRAD for analysis. These samples are tested for T. parva, T. mutans, and also for other tick-borne diseases and for trypanosomiasis. These tests are done to determine the distribution of disease in Zanzibar, since few sick cows are reported to the veterinary authorities.

Activities have also been started to establish a tick-control program with a complete eradication of ticks as the ultimate aim.

ECF vaccination with the infection-and-treatment method has been discussed as a supplementary method for ECF control in Zanzibar. A large-scale trial covering approximately 200 animals on various sites on the island is planned for spring 1977 and, if successful, is to be followed by a vaccination of all animals on the island.

Both these projects have as their aim the eradication of ECF and, if possible, ticks from Zanzibar. This should be possible because the areas are small and have no wild vectors for either disease or ticks. Also, as long as strict quarantine measures are applied to all imported cattle, re-infection of the area can be avoided.

Improvement of the present disease situation is necessary if the islands are to receive any reasonable profit from the labour and investment put into cattle production.

A dairy plant and an A.I. centre have been established but under present conditions use is limited since no improvement of the livestock is possible and the milk production of the present stock is low. Beef production is also far from sufficient for the needs of the island, and every year a considerable number of slaughter cattle has to be imported to meet requirements.

Theileria research and control are thus a necessity for cattle production development in Zanzibar.
Rwanda

Theileriosis in Rwanda

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Rwanda, the most densely populated country in Africa (150 persons/km²), is a pasture country where cattle breeding is traditional. However, the rapid population growth (2.9% per annum) has greatly reduced the pastures in quantity and quality, so that an increase in the number of cattle is hardly possible. The intensification of livestock production seems to be the only answer to meet present and future demands for milk and beef. Whatever steps are taken in this direction, it must include the control of animal diseases, the upgrading of indigenous stock, and also the introduction of exotic breeds. Some progress has already been made along these lines by various projects in the country. However, a major constraint in the development and profitable utilization of livestock is the presence of a number of diseases, mainly tick-borne, of which theileriosis is the most important.

In 1975 the Government of Rwanda together with the Food and Agriculture Organization (FAO) of the United Nations set up a project to control ticks and the diseases they transmit.

The breed of cattle in Rwanda are a type of half-Zebu, called “Ankole.” They have arisen from a crossing of long-horn cattle from North Africa and Asian-type Zebu with shorthorns and a cervical-thoracic hump. Their production under common rural conditions is fairly poor: 200 kg liveweight after 2½ yr, first calf at 5 yr, and a milk yield between 600 and 800 litres per lactation of 240 days.

In 1966 the official number of cattle, based on tax-return statistics, was 682,183 and in 1975, 686,813. However, their number is estimated to be at least 10% higher, between 750,000 and 800,000, due to tax evasion, border crossings, and illegal sales. Most cattle are concentrated in the so-called cattle belt, stretching from north to south with a density of up to 80 head per km². At this density the cattle population cannot be effectively increased and therefore priority should be given to upgrading their quality.

Tick-borne diseases, especially theileriosis, babesiosis, and anaplasmosis are prevalent, as well as heartwater, trypanosomiasis, anthrax, brucellosis, and foot-and-mouth disease, and a wide range of endoparasites. The country has been free of rinderpest and contagious pleuropneumonia for more than 20 yr.

The Veterinary Laboratories at Butare were established before independence and used to serve Rwanda, Burundi, and the western part of the Congo. After independence these spacious and expensive laboratories were found to be too large for the Rwandese veterinary service and the building was allocated to the Medical Faculty of the University. The veterinary service is now without diagnostic laboratories and even the FAO tick-control project is short of space at the National Scientific Research Institute (INRS) at Butare where it is based.

There are 165 dipping tanks and a number of hand-spraying platforms

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installed in Rwanda, but only 10% of the cattle population are regularly dipped (Sivren et al. 1974).

Unfortunately, little is known about theileriosis in Rwanda and no figures can yet be given on the exact distribution, number of cases, and monetary loss resulting from East Coast Fever (ECF). It is hoped to gain this information from an epizootiological survey being planned by the FAO tick-control project in the country. Despite the lack of authentic data, ECF must be considered as the most important disease in Rwanda and constitutes a constant threat to the livestock industries. It causes serious problems in trying to introduce exotic stock into Rwanda, and also at each attempt to distribute upgraded cattle from disease-free breeding stations to hillside farmers.

Apart from very small areas where intensive tick control is practiced, or with calves reared in tick-free surroundings, or where climatic conditions have drastically reduced the number of ticks, ECF is enzootic. Although Van Saceghem (1925) wrote that ECF did not exist on the high plateaus, e.g. Congo Nile, at an altitude of 2500 m and above, during the past 50 yr the situation may have changed and should be reexamined.

The prevalent Theileria species are *T. parva* and *T. mutans*. It is not known yet if *T. mutans* is a pathogenic or non-pathogenic form or if both types occur. Other *Theileria* species have not yet been diagnosed, but it is likely that *T. p. lawrencei* is present in areas where cattle have contact with buffalo.

Although the actual number of ECF cases per year is not known, it is estimated that calf mortality may be as high as 25% (Van Saceghem 1925). Figures of 30-40% mortality from ECF are often quoted, but certainly need to be verified.

Past Research

ECF was first reported in the area by De Greef in 1919 who observed it in the Kitega region among tick-infested animals concentrated along trade routes. Later Van Saceghem (1925) described the epizootiology of ECF in Rwanda-Burundi and studied its transmission. Other publications followed (Seraglia 1932; Schwetz 1933, 1934; Rodhain 1936) but were mainly observations on theileriosis from the Belgian Congo, as this area belonged to the same veterinary administration.

In 1959, Jezierski et al. (1959) described the immunization of cattle against ECF at the INEAC station (l'Institute national pour l'étude agronomique du Congo Belge) at Gabu-Nioka, using the infection and treatment method based on the work of Neitz (1953). The immunization was scientifically carried out and immunized cattle withstood field challenge. However, the lengthy drug administration (aureomycin) meant that the method was far from practical. Years later the same method was used at the Scientific Agriculture Institute of Rwanda (ISAR) to immunize selected breeding bulls. In 1970 the parasitological section of INRS in Butare delivered 35 000 infected *Rhipicephalus appendiculatus* ticks to ISAR (Songa) and four bulls were claimed to have been immunized successfully (INRS 1970). The annual report of ISAR for 1971 reported an immunization trial, based on the work of Jezierski et al. (1959), but did not mention the number of animals involved in the experiment. The INRS annual report for 1972 mentioned the delivery of 30 000 infected *R. appendiculatus* to ISAR to immunize 25 bulls. This intention failed, as no aureomycin for treatment was available after the cattle were infected.

Subsequent immunization attempts were made at the request of breeding stations and local farmers. This involved approximately 40 animals. A supernatant of ground ticks in physiological saline was inoculated into each animal, followed by four injections of whatever tetracycline was available at a dosage of 5 mg/kg bodyweight. None of the immunizations was monitored and results are very doubtful. Serum samples collected from the last group of 11 animals proved that eight had no antibody titres after
immunization and the other three were already positive to the IFA test before immunization.

With this background, FAO set up a tick-control project, appointing the author as epizootiologist to the project in late 1975.

**Present Research**

Because of the publicity surrounding the past INRS immunizations it became widely accepted that the method employed could control ECF. At the commencement of the project, therefore, the FAO epizootiologist was confronted with urgent requests to immunize cattle. Before any ECF immunizations could be carried out with justification, field trials had to be set up, employing the Muguga method of infection and treatment, which had proven to be successful in other parts of East Africa.

After an infected pasture for later field challenge was found, five experimental cattle and 20 breeding bulls were infected with the standard Muguga Project combination of three strains (*T. parva* Muguga, *T. parva* Kiambu 5, and *T. p. lawrencei* Serengeti transformed) and treated with Terra-ECF from Pfizer at a single dose of 20 mg/kg bodyweight in August 1976. Of the experimental animals, one died of ECF 34 days after immunization. Of the three stablate infectivity controls, only *T. parva* (Muguga) died. *T. parva* Kiambu 5 reacted but recovered, and *T. lawrencei* did not react to the stablate. Three months after immunization, the four immunized experimental cattle were exposed to field challenge together with four susceptible controls. As of 3 Dec 1976 (day 35), one of the immunized animals that failed to react to the immunization and one control have died of ECF. All others have contracted *T. mutans* infections, which complicates the disease picture. The trial is still in progress. The 20 immunized breeding bulls have not yet been exposed to field challenge.

On two occasions several thousand clean *R. appendiculatus* nymphs were infected on *T. parva*-diseased animals and delivered to Muguga in the hope of isolating the local strain to produce a stablate. Such stablate could be of use for future ECF immunizations, should the Muguga standard combination of three strains fail to protect against local theilerial strains.

Apart from the epizootiologist and a UN volunteer assistant, no other professional or technical staff are involved in theileriosis research in Rwanda. It is hoped, however, that at least one skilled laboratory technician will be assigned to the project for routine laboratory work and that competent staff will be engaged to help carry out an epizootiological survey in the country.

**Future Research**

This will involve the continuation of ECF immunization trials, using either the standard combination of three strains from Muguga or stablates produced from local strains in the hope that strains will be found to be successfully employed in ECF immunizations in Rwanda.

Investigations into the incidence and distribution of ECF and other tick-borne diseases in the country will be carried out, since very little is known about the epizootiology of ECF.

**Conclusion**

Theilerial disease control in Rwanda can only be achieved through improved tick-control measures and ECF immunization. A coordinated, national control scheme should be developed for better tick control. Present problems include bad dipping-tank management, continuation of toxaphene use in areas of high tick resistance, and low cattle-owner participation in dipping/spraying. These problems might gradually be overcome by training dipping-tank operators and informing cattle owners about the need to dip their cattle regularly. In areas where high toxaphene resistance is observed, a changeover to an organophosphorus compound should be recommended. The construction of more dipping tanks and handspraying crushes is advised in areas where little or no tick control is practiced. An investigation into the
distribution and seasonal abundance of ticks would also give valuable information as to whether strategic dipping might be employed in some areas.

ECF immunization, coupled with tick control, is necessary to protect improved breeding stock raised in ECF-free breeding stations and destined to be distributed to local farmers. A reliable method for ECF immunization would also aid farmers to upgrade their local stock and would enable valuable cattle to be maintained under once-weekly acaricide treatment relatively safely and with economic savings.

Theilerial research should include an epizootiological survey in the country, the installation of a serological test method for theilerial diseases, and investigations into the effective use of either the current standard combination of three strains (T. parva Muguga, T. parva Kiambu 5, and T. p. lawrencei Serengeti transformed) or of stabilates produced from local strains for immunization against ECF.

References


Theileriosis in Burundi

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Theileriosis is a serious hindrance to cattle raising in Burundi. Since 1952, a close network of dipping tanks has been set up for prevention purposes to replace the medical treatments we lack. Burundian veterinary medicine has concentrated on bacterial, helminthic, and sporozoan diseases. The current trend is to choose priorities on the basis of economic imperatives. Theileriosis warrants special attention. The practice of dipping to control ticks is familiar to breeders, but it does raise problems. We know little about the ecology of the vectors, the kinds of ticks, the species of *Theileria*, their biology, or the immunity aspects. The serious impact of this disease may stem from our raising methods, from the management of our pastureland and of our dips, as well as from the lack of research. The disease is widespread in young cattle between a few months and 4 yr old, which make up 50% of the livestock; the older cattle have premunition. The estimated death rate from ECF is between 10 and 30%. Plans for development or improvement of stock in Burundi must take the control of ticks into consideration, among other things. Methods are important; expenditures on acaricides must aim at economy, but must also fit in with an overall plan.

The most common species of *Theileria* in Burundi, from studies of morphology and pathogenicity, and from clinical observations, autopsy results, and microscopic examinations, is *T. parva*. In cattle over 4 yr old, premunition is observed. We know little about the results of recent studies on immunization tests and serological examinations using immunofluorescence. Our knowledge of other species, such as *T. annulata, T. mutans, and T. lawrencei*, is limited, and the economic impact of these species on our cattle raising is not known.

Because systematic research has not yet been done, an accurate assessment of distribution of cases throughout the country cannot be given.

Although there were more than 82,000 cases of theileriosis over the last 10 yr within Burundi, symptomatic treatment reduced these losses to one-third, to approximately 27,400 cases. At an average loss of 3000 Burundi francs (FBu)* per animal, this represents a total loss of 82.2 million FBu.

Our projects, based on the importation of exotic stock for crossbreeding with local stock to increase milk and meat production, are hampered by this disease.

In spite of the apparently close network of dipping tanks, some regions of the country do not have them; an attempt is being made to interest breeders in purchasing spray races. The serious impact of the disease is shown by the high death rate among young cattle, and our objectives are to encourage more regular use of the dips and to gain more objective knowledge of the reasons for changes in acaricides. A test must be made for each

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*1 FBu equals approximately U.S. $0.01.
acaricide, and this has not yet been coordinated; as well, resistance must be better assessed.

Research

The research service has yet to be organized. The field technicians deal with all the viral, bacterial, parasitic, and sporozoan diseases, and the others are responsible for food and the improved sanitation of the environment. If necessary, one technician takes charge of everything. There is no theileriosis research or specialized publication service. We are thinking of initially setting up a tick-control project, and then creating such a service.

The epizootiology of the disease and vector control are to be studied in a project that is awaiting financing.

There are no professional field teams or laboratory technical teams assigned exclusively to the study of this disease. This idea is among the future prospects of the project. Suitable equipment must be found.

Research being planned will deal first with proper management of pasture land, monitoring use of the dips, completion of the network of dips, chemical testing to monitor stability of the acaricides in the dips, accurate assessment of resistance, improvement of diagnostic methods by immunofluorescence, and immunization tests and training of sufficient managerial, field, and laboratory staff. We will attempt to minimize medical treatment in favour of preventive medicine (prophylaxis).
Tanzania

Theileriosis in Tanzania

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Tanzania, with an area of about 362,000 miles$^2$, has a cattle population of 15 million (A. Chagula, 1976, personal communication). This is an increase of 4.25 million cattle since 1966 when the cattle population was 10.75 million (Ministry of Agriculture, Tanzania, 1966) and represents an annual average increase in cattle over the last 10 years of approximately 400,000 animals.

Most cattle are located in northwestern Tanzania (Sukumaland and parts of Tabora), central Tanzania (Gogoland), northern Tanzania (Masailand), and the southern highlands (Iringa and Mbeya). Southern and western Tanzania have very few cattle because most of the areas here are infested with tsetse flies. The indigenous breed of cattle is the Tanzania Shorthorn Zebu (TSZ) and constitutes the greatest number of cattle. Some exotic breeds have now been introduced, particularly for the dairy industry, in the higher altitude areas of Tanzania (Arusha, Kilimanjaro, Iringa, Mbeya).

Theileriosis, and in particular East Coast Fever (ECF), is known to cause many cattle deaths in Tanzania. The causative agents of the disease syndrome in the country are *Theileria parva* (ECF) and *T. lawrencei* (cause of the so-called Corridor Disease). Pathogenic *T. mutans* was encountered during ECF vaccination trials near Dar es Salaam (Central Veterinary Laboratory 1976), but a countrywide confirmation of the existence of pathogenic *T. mutans* has not yet been done.

ECF is widespread in Tanzania and is considered a major cattle killer. Unfortunately precise figures on cattle deaths and hence economic losses caused by ECF are difficult to gather. In a 2-yr survey (1965-67) by McCullock (1968) in Sukumaland, calf mortality in enzootic and epizootic areas was 46% whereas mortality among adult cattle was 9% in enzootic areas and 15% in epizootic areas. In a survey by Mahlau (1968) done in Iringa District (southern highlands) for 1958-68, a 20% infection with *T. parva* was observed. In a survey of diseases at two holding grounds in Tabora region (an area south of Sukumaland), 47.4% of cattle deaths were due to ECF (Kohler 1974). At these two holdings grounds the stocking rate per month was 5000 cattle. At Pugu Holding Ground (PHG) near Dar es Salaam, which belongs to Tanganyika Packers (a slaughter and meat-packing company), of the cattle handled in 1974 (201,767), 5.8% died at the grounds; 47% of these deaths were due to ECF (Tanganyika Packers Ltd. 1974). In 1975, 5.9% cattle died at these grounds of 132,236 handled that year; ECF was responsible for 26% of these deaths (Tanganyika Packers Ltd. 1975).

Figures in the 1966-73 annual reports of the Veterinary Division of the Ministry of Agriculture on the number of deaths from ECF are compiled from regional or district reports. It is our opinion that these figures are low because some cases, even though observed in the field, are not
recorded by many field staff for monthly or annual reports.

ECF occurs in enzootic and epizootic forms. McCullock (1968) reported ECF in Sukumaland as occurring in enzootic form in the good rainfall, good grass-cover areas of Sukumaland (at the shores of Lake Victoria). He observed epizootic ECF as one went further inland and areas clean of ECF in southeastern Sukumaland. These clean areas are probably no longer so, owing to the free movement of cattle in most parts of Sukumaland as well as elsewhere in the country. In Sukumaland, with *Rhipicephalus appendiculatus* occurring throughout the year (Tatchell 1976), ECF is likely to be present most of the year. This is probably the case in other parts of the country with ECF (e.g., Arusha, West Kilimanjaro, and Tabora, Dodoma, and Singida regions). In Iringa, however, *R. appendiculatus* is found in high numbers during the rainy season (October to May) (Tatchell 1976); thus ECF is likely to be high during that time.

**Past Research**

Past research or investigations on theileriosis include those of Yeoman (1966) and McCullock et al. (1968), both dealing with ECF epizootiological surveys in Sukumaland, and the ECF survey by Mahlau (1968) in Iringa District, Southern Highlands. McCullock confirmed the work of Yeoman about ECF existing in enzootic form at the Lake Victoria shores and the epizootic form being further inland; McCullock, however, found the epizootic zone was far wider than Yeoman had observed for the period 1965 to 1966, this having extended into areas previously regarded as clean of ECF. Mortality in calves and adults due to ECF as observed by McCullock have already been discussed earlier. Mahlau (1968) observed 20% infection by *T. parva* and reported that a number of cattle recovering from ECF, and therefore thought to be immune, contracted ECF and some even died of the disease.

More recently (1973-76), research work done at the Central Veterinary Laboratory, Dar es Salaam, has been under FAO/UNDP Project URT/72/009: Improvement of Tick Control in Tanzania. Most of the basic research on immunology of ECF and biology of *T. parva* was left to the Regional Project at Muguga. The Tanzania project concerned itself with studies on:

(i) epizootiology of tick-borne diseases with emphasis on ECF and the evaluation of the effectiveness of the ECF vaccine cocktail developed at Muguga;

(ii) tick ecology studies including problems of tick resistance to acaricides;

(iii) acaricide chemistry studies including the development of better dipping methods and optimal dipping intervals as well as the development of programs of strength testing.

The Tanzania FAO/UNDP project (1976), which started in 1973 and ended in June 1976, had the following important findings:

(i) The vaccine cocktail developed at Muguga by the FAO Regional Project gave a good degree of protection against ECF. Trials were done in Iringa and at PHG near Dar es Salaam. At PHG cattle arrive from various parts of Tanzania and thus, though not proven by cross-immunity tests, a variety of strains of *T. parva* are likely to be encountered. Though the immunized cattle probably had a good degree of protection, some of the cattle exposed to challenge have shown poor health though they did not succumb to the disease (Semuguruka 1976). Whether this poor health is a result of subsequent challenge or other factors, e.g., poor nutrition or concurrent infections, has not been conclusively determined and should be determined in future ECF vaccination trials with the Muguga cocktail. Some animals in the third trial at PHG have, after 6 mo (April (end of exposure) to October 1976), continued to show poor weight gains despite supplementary feeding on concentrates.
(ii) (a) Pathogenic strains of *T. mutans* were encountered during one of the ECF vaccination trials at PHG. It is possible to protect cattle against pathogenic *T. mutans* by injecting blood from a carrier of a strain of low pathogenicity (FAO 1976).

(b) It was confirmed that *R. appendiculatus* was not a vector of *T. mutans* in East Africa; the proven vectors in East Africa were two species of *Amblyomma*.

(iii) *R. appendiculatus*, the vector of ECF, is seasonally present in Iringa (southern highlands) but is present throughout the year in other regions (Sukumaland, Arusha West Kilimanjaro, Tabora, Singida). This makes two different ECF epizootiological situations.

**Current and Planned Future Research**

Because of financial constraints, work on theileriosis and tick control that was to follow the studies started by the FAO/UNDP Project, URT/72/009, has had to stop for the time being. It is envisaged, however, within the current fiscal year, to carry out more ECF vaccination trials, during which time it shall be ascertained whether postvaccination challenge results in loss of body condition and slow weight gains as well as carrier status after postimmunization challenge. Also bulls and other cattle for distribution to ECF areas are to be immunized by the infection and treatment method and these shall be carefully monitored at their destinations.

Tick control, and tick resistance studies in particular, are being continued as a followup of the FAO/UNDP Project, URT/72/009, finished in June 1976.

In Tanzania we are aware of research work going on at EAVRO, Muguga, and ILRAD, Nairobi, and see no reason for duplication of this work at the Central Veterinary Laboratory. However, we believe that further research on theileriosis should include the following: elimination of the vector tick(s), prevention through effective immunization, and chemotherapy and/or chemoprophylaxis.

Currently in Tanzania, control of the vector tick is the only method in practice for the control of ECF. Poor dip management, development of resistance by ticks to acaricides, and deterioration of some acaricides are bottlenecks. Studies in elimination of these bottlenecks have been started but ought to be continued either at the national or international level.

It is our opinion that the infection and treatment method of immunization has potential if master strains for immunization can be easily identified and possible carrier status from immunization or postimmunization challenge are proven not to occur; further studies in this direction are necessary. Also a serological method of identifying strains of *T. parva* needs to be developed to ease these studies. At the same time tissue culture studies with *Theileria* parasites with a view to vaccine production need to be continued.

Immunization against ECF is not enough by itself. Chemotherapeutic and chemoprophylactic methods of control would be significant as complementary methods of ECF control. Studies leading to the development of these methods of control are imperative.

**References**


Future Research

Research in the field of theileriosis should include:

(a) At the causative agent level: adequate knowledge of its metabolism, both at the histiocytic and erythrocytic forms; the study of drug sensitivity at the various phases in the life cycle of the parasite in its vertebrate host.

(b) At the transmitting agent level: knowledge of the species responsible for their transmission, their biological cycles, and cartography; study of the cycle and metabolism of the parasites in the tick body.

(c) At the vertebrate host level: research of the game reservoirs of the parasites; study of the disease in experimentally infected animals; drug tests to find out the most effective and economic treatments.

To undertake the necessary research in theileriosis, one of the two parasitological laboratories (the Veterinary Faculty laboratory at Maputo, or the National Institute of Veterinary Research laboratory at Maputo) should be charged to receive the material considered indispensable for the research work outlined above. As well, stables should be constructed to carry experimental diseases, including an acclimatized chamber for tick cultivation.

References


The Sudan is a vast country with 2 million km² and a cattle population of 22 million. In 1966 the cattle population was estimated to be 17 million but the results of a recent nationwide livestock census showed that the country contains at least 22 million head of cattle. The cattle are mainly the short-horned Zebu type and a few crossbred European cattle are usually found in the organized dairy farms in Gezira Province and around the capital, Khartoum.

Animal resources in the Sudan rank second to agriculture in contribution to the national economy and welfare of the people. It was estimated that about 25% of the population depends entirely on animal breeding. Some of the most significant diseases of cattle in the Sudan are the tick-borne diseases amongst which theileriosis is considered the most menacing tick fever disease. However, our present knowledge about theileriosis is still incomplete. The disease is mainly caused by Theileria annulata and it is present throughout the country. Infection with T. parva was reported in 1955 in Equatoria Province bordering Kenya, where the tick vector Rhipicephalus appendiculatus is prevalent. This tick is not found in other parts of the Sudan, hence East Coast Fever (ECF) does not cause much concern in the country. Recently ECF was diagnosed in Equatoria Province among crossbred European cattle imported from Uganda and Kenya (Shommein 1976, unpublished report).

The main cause of cattle theileriosis in the Sudan is T. annulata. The most common ixodid ticks involved in transmitting the disease are Hyalomma detritum, H. excavatum, H. dromedarii, and H. marginatum. The first two species of Hyalomma are the most important and are found all over the country (Hoogstraal 1956). Other species of Hyalomma, namely H. truncatum and H. turanicum, are found in the country but their role in transmitting the disease is insignificant because they rarely live on cattle (Neitz 1959).

Artificial transmission of the disease has been established by intradermal and intravenous inoculation of infected blood into susceptible cattle (Shommein and Obeid 1974). However, in natural transmission the course of the disease was observed to be much more severe than in the artificially acquired infection. Schizonts were seen in both types of infection, but there were very few endoglobular forms of the parasite in the artificial infection when compared with those found in the natural infection. Sometimes after several passages of some strains of T. annulata in cattle, only Koch's blue bodies appear but no erythrocytic forms.

Similar to other diseases that require an arthropod vector infection, T. annulata is more or less a disease of place. The density of the ticks transmitting the disease, the availability of the carrier cattle, and the presence of susceptible animals determine the outbreaks and spread of the disease. All
those factors are available in the Sudan throughout the whole country. The highest incidence of theileriosis is from June to October, but experience has shown that some variations in the seasonal incidence of theileriosis do occur and few outbreaks were reported in winter.

The mortality rate in cattle seems to be linked with the virulence of the *T. annulata* strain. It has been estimated that the mortality rate varies from 20 to 25% in adult cattle and 15% in young calves. The indigenous Zebu cattle are more resistant than the European breeds and the crossbred animals (Shommein 1976, unpublished report). The acute form of the disease in adult susceptible cattle shows intermittent rise in temperature with inappetency, swelling of the superficial lymph nodes, and sometimes respiratory distress. If the cow is milking, a marked drop in milk yield is observed and animals in late pregnancy may abort although no lesions have been observed on the fetuses.

After a few days, marked anemia develops and the mucous and serous membranes may later become icteric. The disease runs a course of 10-15 days with rapid loss of condition. On post-mortem, the liver, spleen, and lymph nodes are enlarged. The mucous membranes are pale and may show some petechial hemorrhages. Ulceration of the abomasum is the most common finding. The epicardium and the endocardium in most cases are ecchymotic.

Some animals, however, do not show any of the particular symptoms and lesions of the disease apart from rise in temperature and swelling of the superficial lymph nodes. Some cattle contract the disease following, for example, inoculation of vaccines or subjection to stress factors.

Diagnosis is dependant primarily on the recognition of the characteristic symptoms such as fever, swollen superficial lymph nodes, and sometimes respiratory distress. The diagnosis is confirmed by examination of blood and lymph node smears.

Attempts have been made to treat *T. annulata* infection with tetracycline and other chemotherapeutics but without any appreciable success (Shommein and Obeid 1974). Successful treatment up to now seems to be improbable under field conditions and hence treatment is not advised. Proper evaluation of treatment is always obscured by the fact that many recoveries take place spontaneously.

The present prophylactic measures practiced in the country are quarantine and elimination of vector ticks. However, these methods are practiced in a very limited scale only in government and some private dairy farms.

**Past Research**

There has been very little research on theileriosis of cattle in the Sudan. In the past, efforts were mainly directed toward surveys of the incidence of theileriosis in organized dairy farms and government research units. No attempts were made to survey the incidence of theileriosis in nomadic and wild animals. In 1967 treatment trials of *T. annulata* infection were attempted using tetracycline, quinine, and pirevan alone and in combination with bacteriostatics and antipyretics but the trial was unsuccessful.

**Present Research**

At present the Central Veterinary Research Laboratory is launching research programs into identification of different strains of *T. annulata* with the objectives of finding a suitable immunizing strain to be used as a vaccine strain. Similarly treatment trials are continuing using different chemotherapeutics. Tick control is one of the major projects of the Ministry of Agriculture in the coming 6-yr plan.

Three veterinary entomologists and five assistant research officers are engaged in the tick control program, and two specialists, two assistant research workers, and five technicians are involved in research on theileriosis in cattle.

**Future Research**

Future research on theileriosis will be centred on two items, viz. tick control and
research on theileriosis. The tick control program was preceded by a survey of the distribution of ticks of cattle all over the country. The survey was completed and will be published soon by the Ministry of Agriculture Department of Epizootic Diseases. This will provide the basic data and information for a comprehensive ecological study of the ticks infecting cattle and other animals. It is hoped that this will indicate guidelines for future investigations with possible methods of tick control.

Future research on theileriosis will aim at the following objectives:

(a) to isolate representative strains of *T. annulata* from various field outbreaks in the Sudan and make these strains available for study by specialists in the country and for interested research workers abroad;

(b) to determine the significance of the carrier animals and their role in transmitting the disease to susceptible cattle;

(c) to attempt immunizing animals with a suitable immunizing strain of *T. annulata*.

**References**


Somalia

Theileriosis in Somalia

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There is little information available regarding ticks and tick-borne diseases in Somalia. To date continuous and organized research has not been undertaken in this field. Whatever little work has been done has been limited to the occasional naming of tick species.

There have been only two statistical surveys of the animal population of this country, the first one in 1952, and estimates are still awaited.

As no investigation has been carried out, the impact of theileriosis cannot be estimated. However, there have been reports of *Theileria parva* in the northwest part of the country.

**Future Research**

The proposed research activities will include all tick-borne diseases as well as identification of tick species. This will start in a small way and will consist of a laboratory and a mobile unit that will collect specimens to be examined at the laboratory. There are a number of veterinary graduates who could be given a short training locally while working under the guidance of an experienced scientist.

The tsetse and trypanosomiasis control schemes have now two laboratories, one of which will be involved in trypanosomiasis research, and it is hoped that this new tick unit will be closely associated with the trypanosome so that they can share the facilities.
Zambia

Theileriosis in Zambia

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Zambia, a land-locked country covering 752,600 km² and forming a part of the great African plateau whose average height above sea level ranges from 900 to 1200 metres, lies between the latitude 8° to 18° south and stretches from longitude 22° to about 34° east. Climatically, it is a tropical country with two distinct seasons, rainy and dry, whose average rainfall ranges from 80 to 120 cm. The rains occur from mid-October to March.

The total cattle population of Zambia stood at 1.749 million at the end of 1974, of which 1.541 million are kept by the traditional sector and the rest by commercial enterprises. This was an increase from 1.112 million head in 1965. Indeed, the potential of the livestock industry in Zambia is vast, wide, and boundless.

Theileriosis, mainly East Coast Fever (ECF) caused by *T. parva*, is the most severe form of disease and is responsible for limiting the expansion of the cattle industry in the Eastern and Northern provinces of the country. The disease falls under the scheduled diseases of Zambia and is one of the most important cattle diseases, taking into account the high mortality, the control measures related to the vector, and the recent trend in its spread. It is of great concern to the Zambian Government in general and to the Department of Veterinary and Tsetse Control Services in particular. Conservatively estimated, 150,000 Zambian kwacha (K) were spent in 1974-75 in controlling and confining the disease within its enzootic areas. The disease appears to be confined to those areas where the three-host tick *Rhipicephalus appendiculatus* is common. Outside of the Northern and Eastern provinces, the disease has not gained a foothold in other provinces, even in the presence of other ticks capable of transmitting it.

The records of the Department show that the disease was first recorded in Zambia in 1922 at Fife in the Northern Province. It then spread south and west and now covers the district of Mbala, parts of Kasama Chinsali, and Isoka in the Northern Province. In the Eastern Province, the disease is present in Lundazi, Chipata, Chadiza, and parts of Katete district. Up to now the disease has been confined to these provinces only where defined enzootic areas have existed. The outbreaks have remained low, ranging from 5 to 27 from 1965 to 1973. During the past 2 yr the disease has begun to increase. A total number of 42 outbreaks were recorded in 1974 and the number up to the end of July 1975 had already reached 56 outbreaks. Each outbreak is usually associated with high mortality. For example, 900 animals died in 27 outbreaks during 1965. Similarly 700 cattle died in 23 outbreaks during 1966, 384 animals in 11 outbreaks during 1967, and so on. During early 1975 about 610

*1 K equals approximately U.S. $1.26.
animals died in a single outbreak in the Northern Province. With the exotic or grade animals the effect of ECF is even more disastrous and has effectively retarded the expansion of the most needed projects of milk and beef schemes in these two provinces.

Despite the efforts to keep the disease under control, ECF is increasing. The inability to control it, by conventional methods, is probably related to the insufficient epizootiological knowledge of the disease under local conditions. The Department is immensely concerned about the possible development of resistance to the ixodicides and the spreading of the vector in the areas that have been hitherto uninfected.

It is because of this recent trend in its spread to nonenzootic and disease-free areas and the increasing economic importance that a detailed investigation was considered imperative for the development of more effective management measures.

**Plan of Work**

The project, in collaboration with the pest research of the National Council for Scientific Research, was designed to study various aspects of ECF with a view to understanding the epizootiology of the disease under local conditions in the Eastern and Northern provinces. The planned immunological investigation will not only provide the badly needed and efficient diagnostic methods, but is also likely to at least contribute toward the development of effective prophylactic and curative measures, thereby helping greatly to control ECF and check its spread to adjoining disease-free zones.

The plan of work was divided into two phases. Phase I was a disease survey (description follows), and phase II is to include: (a) intensive studies on the vector, to establish tick infestation patterns in cattle and incidental hosts in the enzootic, recently infected, and disease-free zones through monthly collection of ticks from at least 15 animals in each herd. These data are to be related to physical factors (rainfall, temperatures, humidity), vegetation types, and altitude within the study area. This would also include investigations on the effects of ixodicides. Initial studies on the efficacy of the existing control measures (dipping and spraying) by pre- and posttreatment tick counts will be done and ticks will be screened for resistance to ixodicides. An experimental area, sufficiently large, with a number of cattle within the ECF enzootic area, will be selected to portray the importance of tick control measures; (b) standardization of various serological techniques available for the studies; (c) characterization of *T. parva* strains; (d) chemotherapy and possibly chemoprophylaxis; (e) tick biology.

**Disease Survey**

Phase I of the project was started during August 1974 and completed in late 1976. Although the disease exists more or less throughout the year, it usually flares up with the onset of rains, probably linked with the activity of the adult *Rhipicephalus appendiculatus*. The disease flares up in November/December, is usually at its peak in January and February, starts declining in March, with only a little trouble in the form of sporadic outbreaks in April, May, and during the dry season. It is because of this pattern of the appearance of the disease that we decided to carry out two surveys (one in the dry season and the other in the wet season) to delineate the existing geographical limits of the enzootic zones of infection in the Eastern Province.

**Dry Season Survey**

This survey was carried out from August to October 1974. Approximately 14,000 blood and gland smears were collected, usually in duplicate couples, from each animal at various crushpens in Chipata, Chadiza, Katete, and Petauke districts. One gland puncture smear and one blood smear were normally examined from each animal. In cases of doubt, duplicates were also examined and additional smears were examined where necessary.
Interpretation of results — It was important to adopt some criterion for the interpretation of the results because of the morphological similarities amongst various species of the genus *Theileria*. The following criteria were adopted:

(a) Blood smear = positive for intraerythrocytic forms, and corresponding gland puncture smear negative = *T. mutans*;
(b) Blood smear positive, corresponding gland puncture smear not available = +ve for intraerythrocytic or theilerial blood forms;
(c) Koch’s blue bodies in the gland puncture smears, corresponding blood smear negative or positive for intraerythrocytic forms = ECF;
(d) In ECF positive cases, the intraerythrocytic forms were designated as *T. parva*.

Only 8 of the 188 crushpens sampled showed evidence of the disease. In five of these cases the blood smears were positive for the blood forms and the corresponding gland puncture smears for Koch’s blue bodies. In three of the positive cases blood smears were negative.

Absence of ECF revealed by this survey, especially in Chipata, Chadiza, and parts of Katete districts may not be true. It may be due to the fact that smears were collected during dry season when, as pointed out earlier, the disease is not active. The second important feature of the survey was the comparative high incidence of the so-called *T. mutans*. Seven of the 26 crushpens in Chadiza, 2 of the 19 in Chipata, 19 of the 61 in Katete, and 7 of the 84 in Petauke were positive for *T. mutans*. This high incidence of *T. mutans*, especially in Katete District, could be significant. It has been shown that in certain conditions blood forms of *T. parva* can multiply without any indication of the presence of persistent schizogony and such animals may act as reservoirs of infection and also suggest the possibility of premunity in ECF. In other words, the *T. mutans* recorded here may be blood forms of *T. parva*. The third important result was that the survey showed no extension of ECF areas.

Wet Season Survey

The second part of the disease survey took into consideration the difficulties encountered during the dry season survey, especially regarding cataloging and documenting the smears and samples collected. It was planned that samples would be taken from about 5000 head of cattle at 200 crushpens in Petauke, Katete, Chadiza, and Chipata districts. Two blood smears, two gland puncture smears, and one filter paper sample of blood from each animal was to be collected. The survey started in the middle of January 1976 and the field aspect was completed in May. Samples from over 7000 cattle at 269 crushpens were collected. The initial laboratory studies were started side by side, but to complete examinations of at least 14,000 smears will take some time. Laboratory studies of the survey are still in progress.

Interpretation of results — The criteria used here are the same as used in the dry survey studies. Very little ECF was revealed during the dry season survey and this absence was thought to be related to the activity of the adult *R. appendiculatus*. The disease covers a wide area and a total of 28 outbreaks were confirmed as compared to eight in the dry season. Even this survey has revealed a high incidence of *T. mutans*, especially in Katete and Chadiza districts, which are ECF areas, and a rather low incidence in Petauke, which is disease-free at present. It is possible that some of these *T. mutans* could well be *T. parva* and this vast presence of the organism in an ECF area is probably indicative of premunity in the disease, particularly where the mortality in many of these areas has been low. Further laboratory studies on the samples collected might augment these results.
Mozambique

Theileriosis in Mozambique

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Mozambique, in spite of its vastness (784,961 km²), its physiography (plains and highlands, covered with many plants of great feeding value), its climate (annual average temperature between 22 and 23 °C in the south and 25 and 26 °C in the north, and a rainfall from 310 to 1500 mm), has a very low cattle density (2 head/km²), especially when compared with some of its neighbours, e.g., Swaziland (47 head/km²), Tanzania (15/km²), and Malawi (7/km²).

One of the major reasons for such low density is the presence of the tsetse fly, predominantly Glossina morsitans, G. longipalpis, and G. pallidipes, over extensive areas of the country. This can be easily seen by comparing the Glossina distribution and that of cattle.

Although trypanosomiasis is the principal cause of this low production, babesiosis, anaplasmosis, and theileriosis, the topic of this paper, also significantly affect production.

The first recorded outbreak of theileriosis occurred in southern Mozambique in 1901 after the introduction of infected cattle from Tanganyika through Beira and Lourenco Marques ports. The disease spread rapidly and soon infected the herds of neighbouring areas.

The causative agent was Theileria parva confirmed at the Veterinary Laboratory of Onderstepoort, which was then under the direction of Arnold Theiler. The disease was widespread and the mortality high. Unfortunately, due to scarcity of veterinary staff at that time, it was not possible to study the disease in detail. However, a number of strict measures were taken:

(a) obligatory dipping with sodium arsenate every 72 h in infected areas;
(b) interdiction of cattle movements from or to the infected areas;
(c) fencing of the affected pasture areas;
(d) slaughter of the infected animals as well as those that were in contact with sick ones, with complete destruction of the skins;
(e) slaughter of all calves born from infected cows extending up to 18 mo after the last registered case;
(f) controlled fires of the pastures in the affected areas.

Such drastic methods, disliked by a great number of farmers, were sufficient to eliminate for 40 years additional cases of theileriosis south to the Zambezi River.

Later, in spite of the intense sanitary measures, the species T. parva was discovered in Angonia district (Tete Province) affecting a number of cattle.

In 1960 an outbreak of theileriosis by T. lawrencei was registered at Mapai Alto Limpopo District, Gaza Province, due to the existence of buffalo in that same area.

1In 1971, we believed that the designation of Theileria kochi should be used instead of T. parva. To avoid confusion we have adopted the traditional designation, T. parva.
The disease caused a great number of deaths, but the adoption of known measures of control, as well as the establishment of two hunting reserves in the proximity, prevented new outbreaks in that region.

Shortly afterward, in a region ecologically similar to that of the Limpopo at Mossurize, Manica Province, north to Save River, there was an outbreak, and again in 1970 and 1971 in Chimoio Manica Province new outbreaks were registered and attributed to *T. lawrencei*.

It seems, therefore, that two species of *Theileria* are responsible for occurrences in Mozambique: *Theileria parva* at Angonia and *Theileria lawrencei* at Gaza and Manica provinces.

The infections caused by these parasites may be assigned to two well-defined separate biological cycles:

(a) *cattle cycle* — the parasite is transmitted only by cattle and to cattle with evidence not only in the blood but also in the histiocytic system;

(b) *buffalo cycle* — the parasites are carried by the buffalo and are transmitted to cattle by infected ticks, the parasites being detected only in the peripheral blood of buffalo.

The distribution of theileriosis in Mozambique has two distinct aspects: permanent endemic foci, in Tete Province due to *T. parva*; sporadic epizootic foci, in Mapai, Mossurize, Chimoio, and Manica due to *T. lawrencei*.

*T. mutans*, in spite of being found in practically every livestock area, has not been associated with theileriosis outbreaks. We often recognize its association with *Babesia bigemina*, but as the sick animals react satisfactorily to the drugs Babesan and Berenil, we may attribute the illness to *B. bigemina*.

It is not easy to estimate the economic losses caused by theileriosis. If we consider the sporadic outbreaks in Mapai, Mossurize, Chimoio, and Manica, where there were some 200 head of cattle involved, in Angonia we may assume that the annual losses from East Coast Fever are probably less than 50 head. This low mortality in Angonia reflects the prophylactic measures instituted, such as weekly dipping. As we can see, theileriosis, in comparison to other protozoal diseases, such as trypanosomiasis, babesiosis, and anaplasmosis, do not constitute a major problem in Mozambique.

Past Research

Because theileriosis in Mozambique was relatively unimportant, it has never received special attention as a research subject, as can be seen by the scarcity of scientific literature. The existing reports of the outbreaks merely describe the symptomatology and methods used in the control of the disease.

In 1972 a review was published by Dias on piroplasmosis, which detailed the ecology, etiopathogeny, symptomatology, therapeutics, and prophylactic measures about this disease (Dias 1970, 1971, 1973).

Present Research

Recognizing that the problem of theileriosis, especially the enzootic incidence in Angonia, requires a solution, the University of Mozambique sent one of its assistants to Scotland where he is preparing a Ph.D. degree on the subject of histochemical reactions of the salivary glands of *Rhipicephalus appendiculatus* caused by the different phases of development of *T. parva*. The biochemical research being carried out deals with occurrence and models of distributions of nucleic acids, proteins, lipids, acid, and alkaline phosphatases, nonspecific esterases, beta glucuronidases, aril sulfatases, and cytochrome oxidases.

At present there is no one working on theileriosis in Mozambique and further research awaits the return of our assistant from Scotland.
Specialist Reports
Basic Principles of *Theileria annulata* Control

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**Epizootiology and Transmission**

Reports on epizootiology of tropical theileriosis (*Theileria annulata* infection) during the last two to three decades are very scanty. Epizootiological studies have never been carried out in large areas where the disease occurs. It appears, however, that wild animals do not play any role in the epizootiology of this disease. Cattle should be considered the main reservoir of infection, although in some areas the possible involvement of the water buffalo has to be clarified.

All breeds of cattle are susceptible to tropical theileriosis, but a degree of innate resistance has been reported for some breeds (Rafyi et al. 1965). In practice the most susceptible cattle appear to be dairy cows from *Theileria*-free areas. When dairy and beef cattle imported from *Theileria*-free countries are immunized in the same manner, less protection is achieved in the dairy cows.

Ticks of the genus *Hyalomma* transmitted tropical theileriosis under experimental conditions or were associated with outbreaks of the disease in the field (Sergent et al. 1931; Daubney and Sami Said 1951; Neitz 1956; Gill et al. 1974; Bhattacharyulu et al. 1975a; Samish and Pipano 1976).

In most countries the disease has a clearly seasonal character with most of the clinical cases appearing from July to September (Sergent et al. 1945; Delpy 1949; Hadani 1955).

It is quite clear that two ecological situations exist for the disease: the barn and the field. In some areas, all development stages of the two-host tick *H. detritum* are found in various kinds of buildings harbouring cattle. Consequently the disease occurs in animals kept in these buildings (Sergent et al. 1931). Not all the barns of a settlement situated in an enzootic area are infested with *H. detritum*, which means that a susceptible cattle population may occur in such an area. There is no evidence that three-host ticks of the genus *Hyalomma* transmit theileriosis in barns.

In the alternative ecological situation in which all stages of the vector develop in the field, two- and three-host *Hyalomma* ticks are involved. In this case theileriosis affects mostly grazing and range-raised cattle. Contrary to barn theileriosis, all cattle grazing in an infected field during the summer time are exposed to the infection.

The transmission of theileriosis in nature depends on the host preference of the preimaginal stages. For instance, in Israel *H. excavatum* transmits *Theileria* readily under experimental conditions (Samish and Pipano 1976), but in the field the larvae or nymphs infest small rodents but not cattle.

According to Markov et al. (1948) the one-host tick *H. scupense* transmits *T. annulata* when adults that have not yet engorged move from an infected animal to a susceptible one. This situation is similar to transmission by a two-host tick except that *H. scupense* molt on cattle and not on the ground.

Taxonomy of the *Hyalomma* group is rather confused and the various species transmitting *T. annulata* will not be
discussed here further. A revision of the taxonomy of ticks transmitting *T. annulata* and a study of their ecology in various geographical areas are needed. This would contribute considerably to the planning of control measures for the vectors of tropical theileriosis.

*T. annulata* infection is transmitted transtadially. Reports on transovarial transmission have not been confirmed. *H. detritum* becomes infected when larvae and nymphs engorge on cattle carrying erythrocytic forms. Adults developing from such preimaginal stages then transmit the infection to susceptible cattle. When unfed nymphs issued from infected larvae are artificially transferred to a susceptible animal, they transmit the infection (Sergent et al. 1931; Gill et al. 1974), but there is no evidence that this method of transmission occurs under natural conditions. However, in the case of three-host *Hyalomma* species, both nymphs and adults may transmit the infection if the previous stage had engorged on a infected animal (Delpy 1949; Daubney and Sami Said 1951; Bhattacharyulu et al. 1975a; Samish and Pipano 1976).

Adults of *H. excavatum* may be infective if the larvae were fed on infected cattle, even though the intermediate nymphs were fed on a nonsusceptible host (Bhattacharyulu et al. 1975a; Samish and Pipano 1976), but not when the nymphs are fed on cattle (Bhattacharyulu et al. 1975a).

Salivary glands of nymphs and adults of *H. anatolicum* issued from infected preimaginal stages showed masses of undifferentiated chromatin dots surrounded by cytoplasm. Following a feeding period of 24-48 h, distinct chromatin particles, which appear to be the infective stages, were seen (Hadani et al. 1969; Bhattacharyulu et al. 1975b).

Transmission experiments confirmed that ticks become infective a short period after attachment. In one instance, homogenate from unfed adult *H. dromedari* transmitted infection to cattle (Mazlum 1969). On the other hand, homogenate of unfed adult *H. detritum* and *H. excavatum* derived from infected nymphs did not infect susceptible calves (Samish and Pipano 1976).

Adult *H. anatolicum* transmitted theileriosis during the first 24 h of feeding (Bhattacharyulu et al. 1975a), and adult *H. detritum* and *H. excavatum* during the first 2-3 days of attachment (Sergent et al. 1945; Samish and Pipano 1976).

Homogenate of salivary glands from infected adult *H. detritum* fed until repletion on a nonsusceptible host was still infective for cattle (Samish and Pipano 1976). Repleted infected males of *H. detritum* transferred from calf to calf induced theileriosis (Sergent et al. 1945).

It is thus clear that *T. annulata* infection starts being transmitted a very short period after attachment of the ticks, and that infective forms are released throughout the period of feeding. These facts may explain the heavy infection induced by a single tick. The rate of infection in ticks derived from infected preimaginal stages has not yet been evaluated. However, it appears from transmission experiments with ticks collected in the field that a very high proportion of them carry the infection.

**Cultivation in vitro**

A new field in the study of *T. annulata* was opened by Tsur-Tchernomoretz (1945) when he cultivated the lymphoid stage of this parasite in tissue culture. In the initial trials small pieces (explants) of liver or spleen obtained from *Theileria*-infected animals were attached on the bottom of a culture vessel using bovine plasma and chicken embryo extract. The growth medium consisted of Tyrode solution complemented by glutamine, pyridoxine, riboflavin, and calf serum.

Subsequent experiments in growing schizonts in vitro were done (Tsurt-Chernomoretz 1947) but cultures were viable for 1-3 wk only and after this period necrosis of the centre of the explant occurred, which led to destruction of the culture. Attempts to obtain serial sub-cultivation failed during the first several passages.
Encouraging results were obtained by Brocklesby and Hawking (1958) when implant cultures were maintained during a period of 2 mo. A more sophisticated medium (Morton, Morgan, Parker 1950) was used and supplemented with 40% calf serum.

When analyzing these results on the basis of our present experience in cultivation of *T. annulata* schizonts, it is surprising to find that the schizont-infected cells did not proliferate and spread around the explant and later over all the surface of the flask. One possible explanation might be that cells were blocked by the coagulated plasma and that in most instances a very poor medium was used.

Monolayer cultures containing schizont-infected cells were obtained for the first time in 1960 at the Veterinary Institute in Bet Dagan by A. Kimron (unpublished) when kidney from a calf dying with theileriosis was trypsinized and the cells cultivated in 0.1% lactalbumin hydrolysate and yeast extract plus 30% calf serum.

Tsur and Adler (1962) cultivated *Theileria*-infected cells from liver, spleen, and lymph node of infected calves and later from the buffy coat of blood drawn during the acute stage of theileriosis (Tsur and Adler 1965). Infected cultures developed even when no schizonts could be detected in blood smears from these animals, indicating that a very small number of infected lymphocytes are able to give rise to *Theileria*-infected culture.

*T. annulata* schizonts were cultivated later by several investigators (Hulliger et al. 1964; Hooshmand-Rad and Hashemi-Fesharki 1968; Zablotsky 1967; Ende and Edlinger 1971b; Mutuzkina 1975). Some authors claim that the cells they cultivated had a tendency to grow in suspension. This tendency is strongly manifested in cultures of *T. parva* (Malmquist et al. 1970; Moulton et al. 1971). It may be useful to compare the growth characteristics of *T. annulata* from various geographical areas.

Hooshmand-Rad (1975) obtained an enhancement of the growth of *T. annulata*-infected cells and made establishment of cultures with small numbers of infected cells possible by adding lactalbumin hydrolysate and yeast extract to Eagle’s medium. However, in this context there is little information on the growth requirement of *Theileria*-infected cells and the optimal conditions for their multiplication.

A prolonged in vitro cultivation of schizonts leads to attenuation of their virulence. This phenomenon was reported by Tsur et al. (1964) who observed that cattle inoculated with schizonts grown in cell culture for several months exhibited a mild clinical reaction with only rare schizonts in the lymph nodes and liver. Later it was found that complete attenuation could be achieved, so that susceptible cattle inoculated with completely avirulent schizonts showed neither clinical symptoms nor schizonts in lymph node and liver (Pipano and Tsur 1966).

These initial experiments were done with the virulent “Tova” strain that had been isolated from a field case of theileriosis and was being maintained by needle passage in calves. When the culture experiments were initiated this strain had already been passaged more than 200 times in calves, and for years no erythrocytic forms were detected in the inoculated calves. Therefore no conclusion could be drawn at this stage during the first days of cultivation, even if these noninfected cells did not multiply in vitro.
of the experiments concerning the ability of cultivated schizonts to produce erythrocytic forms. Later trials with wild field strains of *T. annulata* showed that complete attenuation was accompanied by loss of the capacity to produce erythrocytic forms (Pipano and Israel 1971).

Three changes in the attenuation of cultured schizonts can be assessed by inoculating susceptible cattle: (1) inoculated cattle show clinical theileriosis but with a lower mortality rate down to zero than caused by the homologous schizonts from infected blood; at this stage schizonts are always detected in the lymph nodes or liver, and erythrocytic parasites always appear in peripheral blood; (2) inoculated animals show no fever, or at most a slight rise of temperature, during 1-2 days and schizonts are barely found by microscopic examination of smears from lymph nodes or liver biopsy; (3) complete attenuation is reached when inoculated calves show no clinical symptoms, and neither schizonts nor erythrocytic forms are detectable.

Different field isolates of *T. annulata* require different periods of growth in culture before reaching complete attenuation. It appears that the number of passages is not as important as the length of time that the parasites are grown in vitro. A period of 130 days to 2 yr was required for attenuation of four different field isolates (strains). In two instances a quite different period was needed when a new culture from the same strain was initiated and schizonts were grown under apparently the same conditions. There is no evidence that the time needed for attenuation is proportional to the initial virulence of the schizonts. In all experiments performed up to this date no reversal back to virulence has been found.

We know too little concerning *T. annulata* in cell culture to explain the phenomenon of attenuation. However, the studies of the kinetics of replication in *T. parva* by Jarret et al. (1969) may provide some clues for the process of attenuation of *T. annulata*. According to these authors, when the number of schizonts in a calf reaches $7 \times 10^9$, a rise in body temperature is noted; when the number reaches $2.4 \times 10^{10}$, the schizonts are detectable by microscopic examination. The number of schizonts needed to induce immunity is $10^9$, i.e., less than the number provoking clinical symptoms or allowing detection of schizonts by microscopic examination.

If these data are also valid for *T. annulata* it may be that the attenuated schizonts grown in culture undergo only a limited number of replication cycles when inoculated into cattle. Thus, they do not reach either the number required to provoke a rise in temperature or the number allowing detection by microscopic examination. Furthermore, they do not reach the stage yielding erythrocytic forms. However, if a sufficient number is inoculated initially, the schizonts can attain the quantity needed to induce immunity.

It follows from this that if the number of attenuated schizonts inoculated is great enough, they should be able to provoke fever or be detectable microscopically, even after a limited amount of multiplications. In point of fact the intravenous infusion of about $5 \times 10^9$ schizonts caused a transient fever accompanied by rare schizonts, but as expected no erythrocytic forms were ever developed.

### Immunology and Immunization

#### Antibody

Infection with *T. annulata* elicits production of a specific antibody that reacts in vitro with theilerial antigen. Circulating antibody has been detected by complement fixation (Schindler and Wokatch 1965; Markov et al. 1966; Tutushin 1966; Konyukhov and Poluboyarova 1967; Stepanova 1968), hemagglutination (Tutushin 1969), and fluorescent antibody techniques (Schindler and Wokatch 1965; Pipano and Cahana 1968, 1969; Askarov 1975). Erythrocytic parasites as well as schizonts have been used as antigen.
In most instances the antibody appears during the acute stage of the disease and peak titres are reached shortly after clinical recovery. If reinfection does not occur, antibody levels decline, but positive reactions are still detectable several months after recovery (Konyukhov and Poluboyarov a 1967; Stepanova 1968; Pipano and Cahana 1968).

Although cattle inoculated with attenuated schizonts from culture show neither clinical reaction nor parasites, a considerable antibody reaction is obtained. Such cattle exhibit a similar antibody production to that seen in cattle infected with virulent schizonts from blood passages (Pipano and Cahana 1968). On the other hand similar numbers of schizonts killed by freezing do not induce antibody when inoculated without adjuvant. This difference in the effect of inoculating living attenuated schizonts and killed schizonts may be considered additional evidence that attenuated schizonts multiply in cattle.

It appears that circulating antibody plays little if any role in protecting against the disease. Although there are reports on successful treatment of Theileria-infected field cattle using blood from animals recovered from the disease (Gilbert 1925; Agoev 1958) this has not been confirmed by laboratory trials. Furthermore some cattle that exhibited high antibody titres (1:16 000) died when challenged with living virulent parasites, whereas others that showed only a rather low titre (1:16 to 1:64) were resistant to the infection (Pipano et al. 1976).

Recently a thermolabile factor causing clumping of Theileria-infected cells from culture was found in the plasma of recovered cattle. However, the homologous serum had no effect on the cells (Hooshmand-Rad 1976).

Antibody against T. annulata was demonstrated in the colostrum of cows carrying this parasite by the complement fixation test (Tutushin 1967) and by the fluorescent antibody technique (Cohen and Pipano, unpublished data). This antibody may be responsible for the innate immunity observed in calves in some enzootic areas. A question of major importance is whether this antibody is able to block the multiplication of living attenuated schizonts used for vaccination, and thus to interfere with the development of immunity against the disease.

Although antibody detected in the serum of immunized cattle seems to have no protecting capacity, serological tests remain useful tools for assessing the response to immunization against theileriosi s (Pipano and Cahana 1969). A high antibody level following inoculation of live vaccine testifies to multiplication of the schizonts, and this process always stimulates protective immunity.

Immunity Engendered by the Different Developmental Stages of T. annulata

There are three main stages in the life cycle of T. annulata: one in the tick and two — the lymphoid and erythrocytic stages — in cattle. Parasites of all three stages can produce infection when inoculated into susceptible cattle.

Most of the tick stages have been elucidated recently (Schein 1975; Schein et al. 1975; Bhattacharyulu et al. 1975b). It is believed that the stage derived from the tick, when inoculated into cattle, penetrates the lymphocytes and yields the schizont. At least two types of schizonts (agamont and gamont) are observed in tick-transmitted infections.

Transfer of schizonts to susceptible cattle invariably produces infection. Agamonts are not always seen when schizont-infected blood is inoculated by needle.

Contrary to the situation with T. parva, no gamonts (microschizonts) are observed in cell culture of T. annulata. However, if cattle are inoculated with virulent T. annulata schizonts from culture, erythrocytic forms appear, indicating that the gamont stage had developed in these animals. On the other hand, since no erythrocytic parasites are ever detected in cattle inoculated with attenuated schizonts, it may be concluded that the gamont stage does not occur in them.
It may be assumed that in tick-transmitted infection immunity is induced by all the developmental stages of the schizonts, whereas in infection with blood or cell culture, immunity is induced by only some of these stages.

Erythrocytic forms are able to multiply when inoculated into cattle and splenectomy enhances their multiplication. In contrast to schizonts, erythrocytic forms are detectable for years after recovery from acute theileriosis. The mechanism of survival of this form in the immunized cattle is not known.

Cattle recovered from tick infection are immune against subsequent infection by ticks, needle-transmitted schizonts, and erythrocytic forms.

Cattle recovered from infection with living schizonts derived from blood or cell culture are protected against infection with schizonts from the homologous strain. If the schizonts used for primary infection yield erythrocytic forms, the animals are also protected against infection with these forms, but if erythrocytic forms are not produced by the primary infection with schizonts, then the animals remain susceptible to infection by subsequently inoculated erythrocytic forms (Pipano and Hadani 1974).

Infection with needle-transmitted schizonts does not engender full protection against tick-induced infection, but it prevents severe clinical symptoms and death.

Cattle infected with erythrocytic forms are not protected against infection with schizonts or the forms derived from ticks.

Trials with dead Theileria parasites show that full protection against the homologous stage can be achieved. Cattle immunized with dead schizonts plus adjuvants were resistant to challenge with virulent living schizonts, but they remained susceptible to Theileria particles from ticks (Pipano et al. 1976).

It appears, therefore, that each developmental stage of T. annulata elicits a homologous immune response that may provide only partial or no protection against infection with the other stages.

**Antigenic Variations**

Different field isolates of T. annulata have been found to be immunologically heterogenous. Summarizing the results from immunization trials, Sergent et al. (1945) pointed out that premunition to a homologous strain is stronger than premunition to a heterologous strain. Some investigators obtained relatively mild reactions when they challenged immunized cattle with a heterologous strain (Rafyi et al. 1965; Pipano et al. 1974). A total lack of protection was reported by Sergent et al. (1945) and Adler and Ellenbogen (1935) between Algerian and Israeli strains. However, Rasulov (1963) reported that experimental infection of cattle with Azerbaijani or Uzbekistani strains of T. annulata conferred reciprocal immunity.

Immunization with attenuated schizonts and challenge with virulent schizonts seems to be a highly sensitive method for detecting antigenic variations in T. annulata. Pipano (unpublished) immunized splenectomized calves with cultured attenuated schizonts from three field strains, and then challenged the animals with homologous and heterologous virulent schizonts. No reaction was detected upon challenge with the homologous schizonts, but animals challenged with heterologous schizonts showed the following range of response: (a) rise in antibody titre only; (b) rise in antibody titre plus erythrocytic forms in peripheral blood; (c) rise in antibody titre, plus schizonts and erythrocytic forms accompanied by fever. Death occurred only in nonimmunized cattle infected with the challenge material.

Wide variation in virulence is observed among T. annulata isolates from the field. Some investigators have ascribed the absence of cross-immunity to difference in virulence of the strains tested (Sergent et al. 1945; Rafyi et al. 1965). However, since completely avirulent schizonts stimulate a total protection against homologous virulent schizonts, it may be that virulence
is related to a biological feature like speed of replication rather than to antigenic structure.

It is obvious from the above that vaccine against *T. annulata* should be tested against local strains before being used in an enzootic area. Furthermore, if breakdown of immunity occurs, the protective capacity of the vaccine against the strain that provoked this breakdown must be verified.

**Immunization Procedures**

In the past, cattle were immunized against tropical theileriosis by inoculating them with blood from an animal in the acute stage of an infection with a relatively mildly virulent strain of *T. annulata* (Sergent et al. 1932; Adler and Ellenbogen 1934; Delpy 1937). Despite the generally low virulence of the inoculum, some of the recipient animals sometimes died. Since the strain used for immunization was not antigenically identical to the local strains (Adler and Ellenbogen 1935; Tsur 1949) the animals were reinoculated with a local strain to reinforce the immunity. Later experiments with blood or homogenate of organs from infected calves were not followed by field application of these methods (Rafyi et al. 1965, 1967; Mirzabekov et al. 1969; Askarov 1975).

Gill et al. (1976) infected calves using ticks (*H. anatolicum* and *H. dromedari*) and administered chlorotetracline at a dosage of 16 mg/kg. Eight days of medication from the beginning of the infection prevented severe reaction to the immunizing infection, but allowed the development of solid resistance to homologous challenge.

Some of the problems associated with the use of infected blood for immunization are avoided with vaccines produced in cell culture. So far it appears that any virulent field strain may be attenuated by a sufficient number of passages in vitro. This opens the way for each country to produce vaccines from local strains. The cultured material is safer than infected blood. No clinical reactions or other harmful effects have followed the inoculation of attenuated schizonts into all kinds of cattle, including pregnant dairy cows (Pipano et al. 1973).

Hashemi-Fesharki and Shad-Del (1973a, b) reported *Theileria* organisms and febrile responses in animals inoculated with schizonts from cell culture, although this occurred with less frequency than in animals infected with virulent schizonts. It would appear that the schizonts used by these workers still possessed some degree of virulence, and complete attenuation could probably be achieved by further cultivation in vitro.

Immunization of cattle from *Theileria*-free countries using attenuated schizonts conferred sufficient protection to allow these animals to survive in enzootic areas in Israel. Rare cases of apparent breakdown of immunity have been observed, but it was not certain that these animals were immunized properly.

Dairy calves immunized with attenuated schizonts are protected against lethal theileriosis and withstand the infection without economic losses. On the other hand, immunized cows exposed to tick infection suffer significant clinical reactions, leading in some of them to loss of milk production and abortion.

Duration of immunity in absence of reinfection has not yet been evaluated. It appears that resistance to reinfection varies with time, but in practice a protection of 2-3 years after the first exposure may be expected (Sergent et al. 1945). Cattle immunized with attenuated schizonts from cell culture did not show signs of infection when challenged with homologous virulent schizonts 18 mo later.

**Storage of Living *T. annulata* Parasites**

Sergent et al. (1945) reported that blood kept at temperatures of 0-25 °C remained infective for 9 days. Schizont-infected cells in minimum essential Eagle's medium showed 50% survival after 3 days storage at 22 °C and 70% at 4 °C. A suspension of 5 X 10^6 infected cells in the same medium kept 6 days at 4 °C induced full protection in cattle.
T. annulata schizonts have been successfully preserved at -70 °C for various periods of time (Tsur and Pipano 1962, 1963; Rafyi et al. 1967; Hashemi-Fesharki and Shad-Del 1973a, b).

A method for storage and transport of T. annulata vaccine has been elaborated in the Veterinary Institute Bet Dagan (Pipano et al. in preparation). The vaccine is frozen in the form of small pellets and stored in liquid nitrogen. Each pellet contains 5-10 doses of parasites in a volume of about 0.5 ml. Vaccine is transported in field liquid nitrogen containers. For use, the pellets are thawed and diluted in PBS and inoculated within 30 min of thawing.

Treatment

Antimalarial drugs (Neitz 1951; Cordassis 1956) and Berenil (Mahmoud et al. 1956; Pipano 1964) showed a selective action on the erythrocytic forms of T. annulata. No drugs active against the schizonts have yet been marketed.

Vector Control

Since no trials to prevent tropical theileriosis in susceptible cattle introduced into an infected area have been carried out by control of ticks, discussion on prophylaxis of this disease by vector control has only a hypothetical character.

As already mentioned, in the laboratory adult infective ticks transmit the infection within 48 h after attachment or, in some experiments, even before that. Consequently, trying to prevent the disease in the field by periodically killing the adult Hyalomma ticks that infest the cattle has little chance of succeeding.

A regular treatment of cattle with acaricides during the period when preimaginal stages occur seems more promising, especially with the two-host tick H. detritum. Larvae and nymphs of this species remain on cattle about 16 days. Consequently, an effective treatment with acaricides every 10-12 days will prevent the development of engorged nymphs. Such treatment must kill nearly all the preimaginal stages, because even if only a small number survive, the adults issuing from them will be able to provoke clinical theileriosis in susceptible cattle.

Larvae and nymphs of the three-host Hyalomma species require 3-5 and 6-8 days, respectively, for engorgement. Thus treatment with acaricides should be done, similar to the regimen used in control of East Coast Fever.

In barn-transmitted theileriosis, control of ticks in the barns should be attempted. Dusting the floor and walls with acaridical powder may kill most of the ticks. A highly volatile acaricide should be used to attack ticks hidden in the cracks and crevices of the buildings. However, there is no evidence that a concentration high enough to kill the ticks can be reached by this means.

Most Hyalomma species also infest rodent and wild mammals, a fact that makes the eradication of the tick by pasture regulation impossible.

Prospects for Control of T. annulata Infection

Tick control as a preventive measure has a smaller chance of succeeding with tropical theileriosis than with other piroplasmosis, e.g. babesiosis. This is especially true where three-host ticks are involved.

When the disease is barn-transmitted, control can be attempted by clearing the barns and surrounding yards of ticks, provided that the disease is not also transmitted on the pasture.

As a practical matter, therefore, it appears that immunization will be the method of choice for controlling tropical theileriosis at least for the near future. Since the cell culture vaccine has proved to be safe for any kind of cattle, efforts should be made to improve the protective potency of the immunization procedure.

Some breeds of cattle from Theileria-free countries can be protected from death in enzootic areas by immunization with
attenuated schizonts. It can be expected that such vaccination will probably also protect susceptible indigenous cattle. The Friesian dairy cow continues to present a problem in immunization programs because these animals, even when immunized, do not withstand heavy tick challenge during the period of lactation and pregnancy. On the other hand, vaccinated Friesian calves (young animals before pregnancy) show at most a mild reaction to tick challenge.

In areas heavily infested with *Haemaphysalis*, young immunized calves will be infected naturally shortly after being introduced and will build up a strong immunity. In areas in which there is only a low probability of infection during the first year, a reinforcement of immunity induced by the attenuated schizonts should be considered.

It is evident from laboratory and field experience that *Theileria* infections derived from ticks engender better protection than infections derived from virulent schizonts. Therefore it is suggested that tick stages of this parasite should be used in a two-stage immunization method. In stage one, young calves would be immunized with attenuated living schizonts, and in stage two about 2 mo later, a stabalate of *Theileria* from ticks would be inoculated.

Considerable experience has been accumulated in preparation of stabalate from *T. parva*-infected ticks. Application of these methods to *T. annulata* should be the main objective of research related to control of tropical theileriosis.

In summarizing the situation with regard to control of *T. annulata* infection, it must be emphasized that the current method using a cultured vaccine provides significant protection and that this method is applicable now for use wherever tropical theileriosis is a problem. Thus, application of the technique in countries where *T. annulata* continues to take its toll of the cattle population represents the single most effective step that can be taken to control this disease.

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Immunization of Cattle against *Theileria parva*

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Throughout its distribution in East and Central Africa, East Coast Fever (ECF) is considered to be the most important of the tick-borne diseases that inhibit the development of the livestock industry. At present, the only method of controlling the disease is by close-interval application of acaricides to cattle, in dips or sprays, to kill the tick vector. Susceptible cattle not protected in this way have a morbidity and mortality approaching 100%.

The disease is caused by a protozoan parasite, *Theileria parva*, which is transmitted by ticks, the most important of which is *Rhipicephalus appendiculatus*. Although only cattle and closely related species are susceptible to infection with *T. parva*, other species of *Theileria* are commonly observed in wild African bovidae. *T. lawrencei*, a common parasite of African buffalo (*Syncerus caffer*) is also pathogenic for cattle, causing a syndrome known as Corridor disease. Because of the many similarities observed between these two parasites, some investigators consider that *T. parva* and *T. lawrencei* are different biological strains of the same parasite (Barnett and Brocklesby 1966; Uilenberg 1976; Young and Purnell 1973).

*T. parva* in the salivary gland of the unfed tick is not infective for cattle, but when the tick attaches and feeds on an animal, the parasite undergoes a cycle of maturation and maximum numbers of the mature infective stage are excreted by the tick between 3 and 5 days (Purnell and Joyner 1968) after attachment. After a latent period of at least 4 days, the macroschizont stage of the parasite is found intracellularly in lymphoblastoid cells obtained from a lymph node adjacent to the site of attachment of the tick. The macroschizont appears to stimulate the host cell to divide, producing two schizont-infected daughter cells. This process continues until more than 50% of lymphoid cells contain parasites. In the early stages, the division of the infected cell and the growth of the macroschizont appear to be synchronous. Approximately 12 days after infection, a proportion of the infected cells is no longer capable of responding to stimulation, and the macroschizont increases in size. At this stage the macroschizont switches to microschizont, the host cell is disrupted, and micromerozoites are released to invade the red blood cells as piroplasms. It is this stage of the parasite, the piroplasm, that is considered to be infective for the tick vector.

It is well established that in ECF enzootic areas where no tick control methods are practiced, a proportion of cattle naturally acquire an immunity against the disease, therefore vaccination should be possible. Based on this information many attempts have been made to produce a method for vaccinating cattle.

Three main approaches have been:

1. **Inoculation of susceptible cattle with macroschizont-infected lymphoid cells harvested from animals dying of ECF**
This method was used on a large scale in South Africa at the beginning of this century (Theiler 1912; Spreull 1914). Between 200 000 and 300 000 cattle were inoculated with suspensions prepared from spleens and lymph nodes taken from infected cattle. Approximately 25% of the inoculated cattle died, and approximately 75% of those surviving the inoculation withstood natural challenge. Comparable results were obtained by later workers using smaller numbers of cattle, although some workers, for example Walker and Whittleworth (1930), reported 100% success with suspensions obtained from particular cattle. Jarrett et al. (1969) at the Veterinary Faculty, Nairobi, estimated that 1010 schizont-infected lymphoid cells were required to immunize cattle and confirmed their estimates in a small number of cattle.

(2) Concurrent infection of cattle and treatment with tetracycline drugs — Neitz (1953) found that when infected ticks were applied to cattle that were simultaneously treated with Aureomycin, the cattle underwent mild reactions and were immune on challenge. The drug was administered intravenously at a dosage rate of 10 mg/kg, starting 24 h after tick infestation and continuing on approximately alternate days for 2-3 weeks. Neitz also reported the efficacy of Terramycin used in the same manner. This was confirmed by Jezierski et al. (1959) in Zaire. Brocklesby and Bailey (1965) found that daily oral administration of Aurofac (and other related drugs) to infected tick-infested cattle for 28 days produced immunity comparable to that produced in cattle naturally recovered from ECF.

Jarrett et al. (1969), using Aureomycin orally at a dosage rate of 16 mg/kg, found that 10 daily treatments to infected tick-infested cattle markedly suppressed the reaction, whereas 14 daily treatments gave complete suppression and subsequent immunity.

Other workers (Robson et al. 1961; Roe 1962a, b; Stobbs 1964) treated cattle orally with Aurofac while exposed to natural infection. To produce immunity, since the attachment of infected ticks was random and unpredictable, continuous daily treatment for as long as 3 mo was required.

(3) "Quantum of infection" hypothesis — The quantum of infection hypothesis was put forward by Wilde et al. (1968). It is based on the observations of Lowe (1933) in enzootic areas in Tanzania that under climatic conditions unfavourable to tick survival, there was a reduced calf mortality to ECF. Lowe thought that the reduced mortality could be attributed to the small numbers of ticks feeding on the calves. Lewis (1950) reported that infection rates in ticks became reduced with age, and that old ticks were more likely to produce mild reactions in cattle. Wilson (1950a, b, 1951) found that when the number of infected ticks applied to cattle was limited, increased numbers of mild reactions, with recovery and subsequent immunity, were observed. Barnett (1957) suggested a direct relationship between numbers of parasites inoculated and the severity of the ensuing reaction but decided that because one infected tick could kill a susceptible animal, this approach had no practical application. Wilde et al. (1968), however, found that the infective stage of the parasite could be obtained in suspension from the salivary glands of partially fed ticks, that cattle could be infected by inoculation, and that the parasite survived freezing to approximately -70 °C and retained its infectivity for cattle.

Based on the above observations, we considered that to investigate any or all of the three approaches it was first necessary to achieve two objectives: (1) obtain suspensions of protozoites from prefed ticks that would regularly infect cattle by inoculation, that could be preserved alive at low temperature, and whose infectivity could be established by titration in cattle; (2) grow schizont-infected lymphoid cells in vitro.

If this were possible, we would then be in a position to: (1) investigate the quantum of infection hypothesis; (2) improve on the infection and treatment approach; (3) investigate the effects of irradiation of
protozoites; (4) investigate the possibility of vaccinating cattle by inoculation of schizonts grown in tissue culture.

**Results**

The objectives outlined above have been achieved.

**Stabilates**

Suspensions of the infective stage of the parasite can be harvested from ticks, which will regularly and reproducibly infect cattle by inoculation. These suspensions can be viably preserved at low temperatures as stabilates without loss of infectivity for at least 600 days (Cunningham et al. 1973b).

**Tissue Culture**

Using the method developed by Malmquist et al. (1970), macroschizont-infected cell lines can be regularly isolated from infected cattle and established in vitro. The cell lines can be grown in both static and suspended culture yielding 1-3 X 10^6 cells/ml, approximately 95% of which contain macroschizonts of the *Theileria* species and strain isolated. With a Log10 growth rate of 3 days, cultures up to 4 litres in volume can be harvested twice weekly, making mass suspension cultures a conceivable prospect. More than 100 cell lines have been isolated from cattle, buffalo, and eland infected with different theilerial parasites originating from all over East Africa.

Recently Brown et al. (1973) established protozoites from infected ticks in primary cell lines of thymus, bone marrow, spleen, and lymph nodes from embryo cattle and of spleen and lymph nodes from young and adult cattle. After a dormant stage of 5-8 weeks, lymphoblastoid cells begin to proliferate, the majority of which contain macroschizonts.

**Attempts to Vaccinate Cattle**

*(1) Investigation of “quantum of infection” hypothesis —* By serial dilution of stabilates and inoculation into groups of cattle, we have shown a direct relationship between the numbers of parasites inoculated and the severity of the ensuing infections. Large numbers of parasites cause acute, severe reactions whereas small numbers cause mild, transient reactions, thus supporting Barnett’s prediction and Wilde’s hypothesis (Cunningham et al. 1974b).

*(2) Irradiation of protozoites harvested from ticks —* In a series of experiments, we found that increasing doses of gamma irradiation caused increasing mortality of the parasites, so that irradiation merely produced a dilution effect as in *(1)* above (Cunningham et al. 1973a).

*(3) Infection and treatment —* Four different drugs in the tetracycline series have been shown to be active in inducing immunity when used at a dosage rate of 5 mg/kg administered as a daily dose on the day of inoculation of the stabilate and on the following 3 days. The drugs used are: N-Pyrrolidinomethyl tetracycline (Reverin); Chlortetracycline (Aureomycin); Oxytetracycline (two formulations: PVP Terramycin and PG Terramycin); and Tetracycline. More than 200 cattle have been immunized by this method, all of them solidly immune to lethal homologous challenge (Brown et al., in preparation).

Depending on the drug used, the duration of its administration, and the magnitude of the infecting dose, some cattle developed patent transient infections, with a few macroschizonts in the local drainage lymph node and a few piroplasms in the red cells.

Further investigations indicated that cattle can be immunized by the infection and treatment method, using Terramycin at a dosage rate of 10 mg/kg administered on days 0 and 4 following a sublethal stabilate challenge (Radley et al., in preparation).

Recently a long-acting formulation of oxytetracycline (Terra ECF) has been produced by Pfizer Corporation that will immunize cattle when inoculated at a dosage rate of 20 mg/kg simultaneously with stabilate (Radly et al. 1975c). More
recently, an improved formulation of long-acting terramycin has been produced that is easier to administer and is more efficacious when used in the immunizing procedure.

(4) Tissue culture — In attempts to vaccinate cattle with tissue culture material, the C2 cell line has been the most commonly used. This is the original cell line isolated by Malmquist et al. (1970) from an animal infected with *T. parva* Muguga. It was quickly established that only viable cells could be used to immunize cattle, and although high antibody titres were produced when inactivated material was inoculated, cattle were completely susceptible when challenged.

When optimal doses of low passage C2 cells were inoculated into cattle, almost 90% were immunized and of the remaining 10%, a proportion died and some were susceptible when challenged. Many different methods were used in trying to attenuate the C2 cell line, but the only method that gave some promise of success was extended passage. When passage levels in excess of 50 were inoculated into cattle, no mortality occurred even when $10^9$ C2 cells were used. When $10^8$ cells were used, at passage levels 49-205, all 37 cattle were successfully immunized against lethal challenge. At passage levels 205-287 using $10^8$ cells, increasing numbers of cattle were not successfully immunized. For example, $10^8$ cells from passage levels 282-286 failed to immunize three of six cattle. However, when $10^9$ cells from passage 287 were used, all of five cattle were successfully immunized. These results are summarized in Table 1.

So far, extended passage has been applied to only one other cell line, C379, which was isolated from an animal infected with a strain of *T. parva* obtained at Aitong in Kenya Masailand. C379 has now been passaged more than 300 times, which represents about 4 yr work, and results comparable to those recorded for the C2 line in Table 1 were obtained.

Thus it appears that a successful method of immunizing cattle against ECF has been developed. However, before embarking on

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*Table 1. Summary of immunogenetic results using living cells of a single *Theileria parva* Muguga cell line (C2) and homologous stabilate challenge.*

aSummary of results of 19 experiments using 260 cattle.

bIn challenges of 19 experiments, 65 susceptible control cattle were challenged using 7 *T. parva* Muguga stabilates. Mortality in controls was 98% (i.e., 64 of 65).

cNumbers in parentheses indicate total numbers of cattle in the relevant group.
the time-consuming procedure of extended passage, it is felt that the strains required to give protection against field challenge must be identified.

Challenge of Animals Immunized by the Preceding Procedures

During the investigation and development of the above methods of vaccination, surviving cattle, along with susceptible controls, were challenged with *T. parva* Muguga stabilate (Cunningham et al. 1973b). More than 95% of control cattle died of classical ECF. In addition, animals immunized by methods 1, 3, and 4 were exposed to natural challenge as described below.

**Method 1**

The immune animals had all recovered fortuitously from reduced stabilate challenge with *T. parva* Muguga, during investigation of the Quantum of Infection hypothesis. Prior to exposure, 14 of the 20 immunized cattle were exposed to a lethal homologous stabilate challenge. Along with susceptible control cattle they were exposed to natural challenge at Aitong in Kenya Masailand, as described by Snodgrass et al. (1972). All of the cattle, immunes and controls, died of an ECF-like syndrome, the main difference from classical ECF being that the majority of cattle died with severe anemia associated with a high piroplasm parasitemia. Other parasites identified from blood smears taken from the cattle during the experiments were babesia, anaplasma, trypanosoma, and borrelia. This complex situation resembled that described by Thomas and Neitz (1958), who considered that the syndrome observed by them was produced not only by the pathogens present, but was contributed to by the large numbers of rhipicephalid ticks feeding on the cattle. Subsequent investigations at EAVRO (Irvin et al. 1972, and Burridge and Kimber 1972) confirmed that the cattle at Aitong had been exposed to a pathogenic *T. mutans* challenge (later shown to be transmissible by *Amblyomma variegatum*, Young et al. (1976 / 77)), and also to a strain of *T. parva* that produced reactions in *T. parva* Muguga cattle, but did not kill them. Since large herds of wild buffalo were present in the exposure area, it is probable that the cattle were also exposed to *T. lawrencei*.

To obviate the complications experienced at Aitong, a paddock was established at EAVRO (Purnell et al. 1975) and infested with *Rhipicephalus appendiculatus*, which were infected with *T. parva* Muguga. No other pathogens were known to be transmitted by these ticks. This paddock was used to challenge cattle immunized by methods 3 and 4.

**Method 3**

Four cattle immunized against *T. parva* Muguga by simultaneous inoculation of stabilate and a single dose of long-acting oxytetracycline were exposed in the *T. parva* Muguga paddock for 60 days (Radley et al. 1975d). All of the immune cattle withstood this challenge and improved in condition during the period of exposure. Three groups of four susceptible cattle were introduced into the paddock with the immune cattle on days 0, 20, and 40. All of the susceptible cattle died of ECF between 14 and 21 days after entering the paddock, indicating that the immune cattle had withstood a continuous high challenge with *T. parva* Muguga.

Some time later, another experiment was carried out in the paddock to investigate whether cattle immunized against *T. parva* Muguga by infection and treatment would accelerate the disappearance of the parasite from the tick population in the paddock (Radley and Newson 1977). The paddock was divided into two parts, designated Paddock A and Paddock B.

**Paddock A**

Control cattle were introduced into Paddock A at regular intervals over 316 days and allowed to die there, thus allowing continued transmission of the parasite
through the larvae and nymphs that engorged on the cattle.

**Paddock B**

Six cattle were immunized against *T. parva* Muguga by infection and treatment using a single dose of Terra ECF. Two months later, without stabilate challenge, the immune cattle were introduced to Paddock B, and remained there for 316 days. Six pairs of susceptible cattle were also exposed in Paddock B at various intervals. All six pairs of cattle were removed from the paddock after 6 days to preclude the possibility of transmission. The first three pairs were sprayed with acaricide immediately after removal from the paddock, and because the challenge appeared to be greatly reduced, the last three pairs were not sprayed to allow the attached ticks to complete their engorgement to enhance the challenge. Details of the experiment and the results are shown in Table 2.

It is considered likely that exposure of the immune cattle accelerated the disappearance of *T. parva* from the ticks in the paddock. It should be noted that the last groups of control cattle had a very heavy infestation of ticks. Both animals in Paddock A had a very severe anemia at the time of death, which is very unusual in cattle dying of *T. parva* Muguga infection, and it is thought that the anemia was caused by the heavy tick infestation. The control cattle from Paddock B recovered from a transient ECF reaction, but were also anemic. One of them died 11 days after disappearance of *T. parva* schizonts and is considered likely to have died of “tick toxicosis.”

**Method 4**

Four cattle were immunized against *T. parva* Muguga by inoculation of $10^8$ C2 cells of the 25th passage. They were exposed in the *T. parva* Muguga paddock for 75 days. During the first 30 days of exposure all of them underwent a significant ECF reaction, and all of them recovered. Four control cattle were introduced to the paddock at the same time as the immunes, and another four cattle 30

<table>
<thead>
<tr>
<th>Month of experiment</th>
<th>Control cattle</th>
<th>Immune cattle</th>
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<tbody>
<tr>
<td></td>
<td>Paddock A</td>
<td>Paddock B</td>
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<tr>
<td></td>
<td>Death due to ECF</td>
<td>Tick load Adults / ear Day 6</td>
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<tr>
<td>1975</td>
<td></td>
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<tr>
<td>Nov</td>
<td>2 / 2</td>
<td>446</td>
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<td>Dec</td>
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<td>1976</td>
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<tr>
<td>Jan</td>
<td>1 / 2a</td>
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<td>Mar</td>
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<td>Apr</td>
<td>1 / 2b</td>
<td>487</td>
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<td>June</td>
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<tr>
<td>Sep</td>
<td>2 / 2</td>
<td>1454</td>
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</tbody>
</table>

aThe surviving animal had a positive titre to *T. parva* antigen and was used as a control by mistake.

bDied of acute anaplasmosis 11 days after introduction to the paddock.

71
days later. All eight control cattle died of ECF within 20 days of introduction to the paddock, demonstrating the presence of a severe and continuous challenge.

These results indicate that without doubt two successful methods of vaccination against *T. parva* Muguga have been developed that will successfully protect cattle against severe and continuous natural challenge.

The experiments carried out at Aitong gave us the first indications that different strains and species of *Theileria* are involved in the ECF syndrome, and brought home to us the complications that are likely to arise when cattle are exposed to continuous and unlimited tick worry and to a mixture of tick-borne pathogens.

**Cross-Immunity Trials with *T. parva* and *T. lawrencei* Isolated in East Africa**

Although the IFA test can be used to distinguish *T. mutans* from either *T. parva* or *T. lawrencei*, there are no serological tests available that can be used to identify the immunogenic specificity of different strains of the last two parasites. It was necessary, therefore, to embark on a costly and time-consuming series of cross-immunity tests in cattle. Approximately 25 isolates of *T. parva* and *T. lawrencei* were prepared, from all over East Africa, with the cooperation of the National Veterinary Services of each of the three countries. In all of the cross-immunity trials, animals were immunized by infection and treatment; we could not afford to use tissue culture material since the early passages of many of the strains caused a high mortality in cattle. Most of the results of these cross-immunity trials have been reported by Radley et al. (1975a, b, c). As a result of this work, three strains have been identified that can be used as a combination to immunize cattle. Aliquots of stabilates of the three strains are pooled and inoculated into cattle simultaneously with Terra ECF. Cattle so immunized have withstood stabilate challenge with 15 strains of *T. parva* that were obtained from all over East Africa.

When “cocktail” immunized animals were challenged with *T. lawrencei* stabilates, some strains produced up to 50% mortality. The cocktail therefore appears to give very good protection against *T. parva* but not against *T. lawrencei*. We anticipate no difficulty, however, in adding the breakthrough *T. lawrencei* strains to the cocktail to achieve complete protection.

**Exposure of Cocktail-Immunized Cattle to Natural Challenge**

A paddock infested with *R. appendiculatus* has been established at EAVRO in which two adult buffalo were maintained. These buffalo were carriers of *T. lawrencei*, and the parasite became established in the ticks that fed on them. When cocktail-immunized animals were exposed in this paddock, 50% of them died, and all controls died of classical *T. lawrencei* infection.

When five cocktail-immunized animals that had withstood stabilate challenge with a *T. lawrencei* strain isolated from the paddock were exposed in the paddock, all of them were found to be completely immune.

In cooperation with the Kenya Veterinary Department, eight cocktail-immunized cattle and eight controls were exposed to natural challenge at Ngong near Nairobi. The control cattle died of typical ECF. Only two of the eight immunized cattle survived. No theilerial schizonts were demonstrable in several of the immune cattle at post-mortem, and it is considered probable that tick worry and other tick-borne pathogens contributed. In addition, buffalo were seen in the paddock some months before the cattle were exposed, and it is possible that a *T. lawrencei* challenge was present.

In cooperation with the Tanzania Veterinary Department and an FAO Project, a large trial was carried out at Pugu near Dar es Salaam, at the holding ground of the Tanzania Meat Packers Company (Uilenberg, personal commun-
ication). Here there is a continuous introduction of tick-infested cattle that leave ticks with *Theileria* strains from many regions of Tanzania. There is no tick control at the holding ground but the number of *R. appendiculatus* do not become overwhelming since the climate is only marginally suitable for this species.

In the last of three experiments carried out at Pugu, 50 cattle immunized with the cocktail by infection and treatment and 19 control cattle were exposed for 2 mo. All of the control cattle died of ECF. None of the immunized cattle died of ECF, but 10 died of other causes. Two of the 10 died of accidental crushing, three of heartwater, one died a week after treatment for acute *Trypanosoma vivax* infection, one of suspected snake bite, and three of unidentified causes, but not ECF.

**Conclusion**

Assuming that there is a limit to the number of immunogenic types of theilerial parasites involved in the ECF syndrome, and assuming that they can be identified and isolated, it is likely that chemoprophylactic vaccination can be used in the control of the disease.

When the strains required to immunize cattle in a particular area are identified, tissue culture vaccination can be used, possibly enhanced by the use of adjuvants.

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The Control of Theileriosis

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There have been, in my own professional lifetime, considerable changes in our knowledge of the theilerioses, and relevant knowledge has emerged during the last two decades that has produced a variety of possibilities that simply did not appear credible 20 years ago. The changes can best be followed in respect to *Theileria parva*, the cause of East Coast Fever (ECF), because the history of this parasite, since its recognition and description at the end of the 19th century, is well documented and has attracted the interest and efforts of some of the great names associated with Africa and its disease problems. Also, *T. parva* is the species with which I have been most closely associated.

History of the Control of ECF

First Period

The history of the control of ECF falls into three main periods. The first period is that from the turn of the century to the middle 1950s. During this period, the causal agent was recognized, described, and named (Stephens and Christophers 1903; Theiler 1904; Koch 1905, 1906; Bettencourt et al. 1907; and Gonder 1910, 1911). There was considerable activity in the field of theileriosis for several decades and many of the characteristics of ECF and those of the causal parasite were recognized and established. Indeed, in the 1940s, those of us working in East Africa took for granted certain fundamental beliefs about *T. parva* that have had to be modified recently.

Efforts were concentrated, particularly in South Africa, by Theiler, du Toit, Koch, Spreull, Reichenow, and many others, on the control and treatment of the disease. It was realized that attack on the vector tick would provide a method that promised most expeditious and effective control, and the dip tank with sodium arsenite as acaricide came into widespread use. The limitations of the method were recognized from the early days of its implementation and much attention was paid to improvements involving variations of dipping interval and dip strength and in the use of hand dressing with a variety of preparations (du Toit and Viljoen 1929). It was recognized that dipping alone would not control ECF, and strict quarantine measures were brought into effect. The incidence of the disease was considerably reduced in both number and size of outbreaks. Eventually a slaughter policy was introduced in the remaining pockets of infection and ECF was eradicated from South Africa. During this period efforts were made — some of them heroic — to bring about artificial immunization and therapy. Immunization with suspensions of infected tissues was attempted and prominent in this work were the names of Meyer, Theiler, Spreull, Sergent and his colleagues, Walker and Whitworth, and du Toit. Although this work met with limited success in that some animals could be infected by the parenteral administration of infected tissues and that some of these recovered and attained a state of immunity, the method was far too unpredictable and
likely to cause too high a level of mortality, both from the original injection and the subsequent challenge, to be of any practical value.

Sporadic attempts were made to cure ECF by specific chemotherapy and other remedies but in spite of many claims to success, no authenticated cure of the disease was found. In 1953, however, Neitz reported a specific effect against the schizonts of *T. parva* by administering a prolonged course of tetracycline therapy, commencing at the time when infective ticks attached to the bovine host. This work marks the end of what I designate as the first period, and the emphasis, with the eradication of ECF from South Africa, moved to East Africa. Appropriately at this time, Neitz collected together the main features of the diseases caused by all the then known *Theileria* spp. in an exhaustive work which was published in 1957.

**Second Period**

The second period in which a new attack was made on ECF might well have commenced as a result of Neitz’s success with the tetracyclines. This success was confirmed by several workers in East Africa (Barnett, Brocklesby, Wilde and his colleagues) but although it gave hope of possible successful chemotherapy, it was obviously not a practical method. In East Africa, dipping had been introduced and in certain areas where this could be strictly enforced and where cattle movement could be strictly controlled, the disease was kept down to insignificant proportions. In other areas, where such strict and expensive control could not be maintained, the disease was endemic with the generation of immunity in large populations of cattle.

In 1957, the Wellcome Foundation Ltd. established a laboratory in Kenya specifically for the purpose of working on ECF and primarily to be involved in chemotherapeutic screening against the disease. This was the Wellcome Research Laboratory (East Africa) (WRL(EA)). There began what was possibly the most concentrated investigation of the chemotherapeutic of ECF ever attempted, backed by a large organization capable of producing a great variety of substances of possible value as remedies against *T. parva*. The work was carried on for more than 10 yr in spite of the expense incurred and the succession of failures in the search to find an effective therapeutic agent. However, at the end of this period it was stated that “the fact that some of the substances used exercised an adverse effect on the parasite gives hope that *T. parva* is not entirely invulnerable and that further search may reveal a drug or drugs that will be reliably curative” (Wilde 1967).

During this second period, the accumulation of knowledge in the WRL(EA) and also in the Kenya Veterinary Department at the East African Veterinary Research Organization (EAVRO) by Barnett, Brocklesby, Bailey, Ross and Löhrl, the Wellcome team, and others led to work on the immunology of the disease with the particular object of finding a means of artificial immunization against ECF. Early and exhaustive efforts using the schizogonous phase of the parasite and the infective particles from the tick in the living and the inactivated form led the workers at WRL(EA) to believe that the key to the immune process lay in that part of the parasitic cycle extending from the “switching on” of infective particle development in the tick salivary glands to the point at which the first schizonts appeared in the local lymph node of the susceptible bovine animal (Wilde 1967). It appeared to me that the parasite would be most vulnerable to chemotherapy in this phase. This view was reinforced by the demonstration in tissue culture at WRL(EA) that the multiplication of theilerial macroschizonts took place synchronously with the division of the host lymphocytes, thereby making it possible for the parasite, once the schizogonous phase had become established, to be protected by investment within the cells of the host. Evidence that this also occurred in vivo was found (Wilde 1967). Work was therefore begun on the
separation of the tick infective particles with the object of investigating their effect on the susceptible bovine host (Wilde et al. 1968). This led to recent work at EAVRO, which emphasized the importance of the “preschizogonous phase” of the parasite *T. parva* in the process of immunogenesis.

In 1967, a report was issued by FAO on the findings of the East African Livestock Survey (UNDP/SF/FAO 121 REG) and this stressed the importance of ECF as the most serious threat to livestock development in East Africa. As a result the UNDP/FAO project at EAVRO was established and the team appointed took over the research program on ECF; this marks the beginning of my third period.

### Third Period

There is no doubt that the concentrated investigation into many aspects of *T. parva* and ECF mounted by FAO and carried out at EAVRO has advanced our knowledge more significantly than could possibly have been envisaged 10 years ago. Details of this work and the amount of in-depth research are too voluminous to be listed here. A résumé has been ably presented by the Project Manager Dr Matt Cunningham (Cunningham 1977). I would, however, like to pay tribute to the workers and their valuable results and express regret that their work, as a team, is so shortly to come to an end. However, as a result of the efforts of the team, we now have several promising possibilities before us. Incidentally, although these have been worked out specifically for *T. parva*, there is no doubt in my mind that much of the findings will be applicable to *T. annulata* and any other *Theileria* species as and when circumstances indicate that the time is ripe to deal with them by the means suggested.

The possibilities of the *T. parva*/*T. lawrencei*/*T. mutans* complex are:

1. **Immunization** — (a) the use of a known quantum of infective particles to bring about a reaction that while being safe will be capable of stimulating immunogenesis; (b) the infection and treatment method; (c) the use as immunogens of lymphoblast cells, transformed by macroschizonts of *T. parva*, which have been attenuated in tissue culture passages; (d) a more remote possibility at present is the exploitation of the work of Irvin et al. (1975) on the adaptation of tissue culture macroschizonts to irradiated nude athymic mice.

2. **Chemotherapy** — The application of the tissue culture of lymphoblasts infected with macroschizonts as a primary drug-screening technique has already shown that successful chemotherapy of the disease ECF is now possible.

The above methods will have to be considered in the light of any particular situation but it is not suggested that their use will obviate the necessity for some form of acaricidal practice. This will still be required to reduce the losses due to the ticks themselves and to the other diseases transmitted by them.

The directors of veterinary services in the countries where ECF exists will soon have before them the choice of several lines of action, some of which have hitherto been unavailable. The decisions as to the policies to be adopted will undoubtedly depend on the conditions that obtain in their countries. The factors that must influence them will depend upon the policies dictated by the state and development of their livestock industries in various areas. For example, does the area call for intensive dairy production for the benefit of the people of the area or for the country as a whole, or is beef, with possible export potential, the objective? Whatever the situation the authorities are likely in the not too distant future to have much more freedom of choice, thanks to the research of the last decade. The choice, of course, will be subject to variations even within a country and no doubt directors of veterinary services will define zones in which the policies will be laid down.

In considering the future policies that might be adopted in the control of the ECF complex, the possibilities might well, therefore, include the following:
(1) In endemic areas where nomadism is practiced and where cattle movement control is limited it could be decided to "leave well alone" and permit the development and maintenance of immune populations by natural means. This will mean greater or smaller losses in the calf crop but in certain areas the local people are prepared to sustain this in return for the freedom of action that they enjoy. It is, therefore, a case of living with a reasonably stable presence of the disease.

(2) Artificial immunization — (a) There is now a method, developed by Radley and his colleagues, that utilizes the principle of infection and treatment in order to bring about immunity (Radley et al. 1975a). This has been made possible by the exploitation of the stablate method of preserving infective particles from infected ticks and the establishment of infection by their inoculation on a unique occasion, and the development of a long-acting tetracycline that can attack the parasite in this vulnerable stage and prevent its development into a schizont. This method currently offers the most promising practical means of artificial immunization. It is, however, beset by two major objections:

(i) The method is cumbersome and could present difficulties in the logistics of its administration on a wide scale. It does not show, at present, characteristics that are optimally desirable in a good field vaccine. I fear, also, that it would be expensive to apply on a large scale and could suffer from the disadvantage of some degree of lack of standardization.

(ii) During the last few years of work on *T. parva* and *T. lawrencei* it has become apparent that different antigenicmakeups exist, thereby leading to the recognition of different "strains" of the parasite. This has come as a surprise to some of us who worked with *T. parva* many years ago, although the concept of strains should have been less unexpected than is the belief that there should be one immutable strain of an arthropod-borne protozoan. This is especially so since the different strains of *T. annulata* have long been accepted. Unfortunately, the recognition of strains of different antigenicity has added to the problems of artificial immunization and although it is highly probable that the spectrum of antigenic characteristics in this group of parasites is limited, probably quite sharply, and although the overlap of different strains is sometimes quite large, it has been shown to be necessary for practical purposes to use a mixture of strains in any field-immunizing process. This mixture, for better or worse, has been referred to as a "cocktail." In recent work, two "strains" of *T. parva*, *T. parva* (Muguga) and *T. parva* (Kiambu), have been used, together with a transformed strain of *T. lawrencei* (*T. lawrencei* (Serengeti) transformed). This "cocktail" has immunized in the field against the parasite in various areas (Radley et al. 1975b).

It appears, therefore, that the method of infection and chemotherapy, if properly refined and used with discretion, does present the possibility of practical artificial immunization. There must, however, be some apprehension associated with its use in that it would be reasonably easy to fail to immunize due to inadequate viability of the mixed stablate and also that strains of parasite not currently present in an area could be introduced. I think that these objections could be overcome by careful attention to the planning, logistics, and follow-up observations in any immunizing campaigns, but they should not be ignored.

(b) Brown and his co-workers have developed lines of *Theileria* spp. in tissue culture following the initial success in the isolation of *T. parva* (Muguga) by Malmquist et al. (1970). It has been shown that these cultures of lymphoblasts transformed by the macroschizonts of *T. parva* can be established in the susceptible bovine host and produce an episode of disease
identical with ECF. Passage of these cells in vitro has brought about attenuation but there has been, in the cultures tested, a corresponding diminution of immunogenicity. Thus a certain level of attenuation could be attained in which a given dose of infected lymphoblasts would cause an infection in some susceptible animals from which a proportion would recover and be immune and others would die, whereas some would not be infected at all and would remain susceptible (Brown et al. 1971). Further work along the lines initiated by Brown and his colleagues might well result in the development of a measurable dose of suitably attenuated parasites that will bring about immunity while maintaining an acceptable level of safety. Thus, the use of tissue culture parasites could become a valuable weapon in artificial immunization and one would hope that work along these lines will continue and that attention will be paid to all possible means of producing an acceptable tissue culture vaccine. A point that springs to mind in this context is the desirability of exploring the concept of lyophilization of such infective material without unacceptable loss of viability.

(c) The use of a measurable dose of infective particles as an immunizing agent, visualized some years ago, has been shelved for the simple reason that, hitherto, it has not been possible to produce a suspension of the particles known to be sufficiently homogeneous to be capable of titration. The variations in "strain" pose a problem and the method suffers from the disadvantage met by Brown et al. (1971) when using measured doses of tissue-cultured schizonts, namely, that no reliance can be placed on a standard reaction to a given dose by individual animals, so that in response to a dose, measured as accurately as possible, some animals might not react, some might react and recover, and others might react and die. However, with the development of effective chemotherapeutic compounds, this method might be re-examined with advantage.

(d) The adaptation of theilerial schizonts to athymic nude mice by Irvin and his co-workers has opened up a possible new line of immunogenesis (Irvin et al. 1975). Many attempts have been made over the years to adapt *T. parva* to a host other than the bovine and all these have met with failure. It became accepted that the parasite was completely and specifically limited to the bovine host. The preliminary success achieved by Irvin et al. (1975) has shown that adaptation to a small animal host is not straightforward since the limited results already obtained have demanded very drastic methods. It is possible that further success in the adaptation of parasites to small animals by this means could lead to new approaches, most probably through the medium of tissue culture. At present, however, there is no indication of early practical application of this technique.

(3) Chemotherapy — The use of tissue culture of schizont-infected lymphoblasts as a preliminary screen for compounds of potential therapeutic value against *T. parva* has brought chemotherapy back into the field of theileriosis in a very significant way. When the successful cultivation of *T. parva* in vitro led to routine production of the macroschizonts and the ready isolation of field strains in tissue culture, it appeared to me that this success could provide a system for primary screening of candidate therapeutic compounds that would be faster, cheaper, and much more elegant and humanitarian than the use of large numbers of cattle that had hitherto been necessary in the search for a cure. I must admit that having had many years of experience of screening compounds against *T. parva* I would never have expected success for such a method so soon. McHardy’s work in developing a technique for assessing the effect of candidate compounds in vitro (McHardy et al. 1976) has been rewarded by early significant results. He has shown that a schizonticidal effect against *T. parva* can be detected when some compounds are tested in the tissue culture system, and Dolan (1977), applying the test compounds to infected cattle in the field, has shown that the effect
can be translated to the animal in vivo. This discovery has been made possible by the success of Brown et al. (1971) and stems from the first really satisfactory isolation in vitro of *T. parva* by Malmquist et al. (1970). This is a breakthrough in the true sense of the word and must rank high in the chemotherapeutic essays into protozoal diseases.

The WRL(EA) team that screened so many compounds and spent many years on attempts by different regimens of treatment to probe the defences of the *T. parva* macroschizont found that some compounds could have a suppressive effect on the schizont but they were unsuccessful in bringing this effect to a predictable cure. Of the compounds that showed this effect, the most promising were pyrimethamine in combination with sulfa drugs and chloroquine. Their effects were believed to be due to action as end-product inhibitors. Unfortunately, any dose that had an adverse effect on the parasite also had a toxic effect on the hemopoietic system of the host animal with, usually, fatal termination. McHardy et al. (1976), in their primary screening work, found that a naphthoquinone called menoctone (Sterling Winthrop) has a schizonticidal effect against *T. parva* in vitro. He attributed this by analogy with the work of Peters (1974) on plasmodial infection to the blocking of the synthesis of coenzyme Q. Dolan (1977) has shown that this substance menoctone can be used in cattle at the time when schizonts have appeared in the lymph node local to the administration of theilerial infective particles with the suppression of the schizonts and the recovery of the host animal. This is indeed an important finding, not so much because of the discovery of this substance specifically, but because it has shown that the schizont of *T. parva* invested in host cytoplasm can be attacked and destroyed without serious concomitant damage to the infected bovine animal. Further, it has now been shown that with suitable techniques, this effect can be detected in vitro.

Much more work must be done to take advantage of this effect, not only on menoctone but also on other related and unrelated candidate compounds. It is possible that menoctone will prove to be unsuitable for practical exploitation for various reasons associated with cost and difficulties in manufacture and/or formulation and other compounds will have to be sought and thoroughly tested. When a really suitable and effective compound for the chemotherapy of theileriosis is found, and I feel confident that one will be found, and if such a compound can be made readily available at an economic price, then, I believe that it will be the main weapon in the control of ECF. It is my opinion that such a compound could be used to contribute very significantly to the eradication of ECF from the geographically limited area in which the disease occurs, provided that the difficulties of administration can be overcome. The advantages of satisfactory, successful chemotherapy are many:

(1) It is most probable that an effective compound would not be subject to varying activity against different antigenic strains of *T. parva*. Indeed it is to be expected that its effect would bridge even specific differences and act on *T. lawrencei* and *T. mutans* as well. There is evidence already in the work of Dolan to show that menoctone acts against *T. annulata*.

(2) If used against the infection already established in cattle, it might be expected to bring about immunity in recovered animals. Indeed, the first cattle experiment by Dolan has shown that cured animals are resistant to infection with the homologous strain. Thus strategically used it could produce a high level of immunity in any area without any serious risk of producing a reservoir of infection and without fostering the introduction and possible persistence of other strains of the parasite in the area.

(3) With the use of the drug in the area, the level of sterile immunity would have the effect of reducing the pool of parasites to be picked up by ticks and hence the
percentage of infection in the ticks would be reduced progressively until the possibility of sustaining an endemic situation would diminish.

(4) This situation would make possible a policy of reduced concentration of acaricidal attack so that, with time, short interval dipping could be replaced by longer and longer interval dipping until a situation could be attained wherein the regimen was governed only by the necessity for the control of tick worry and other tick-borne diseases and not, as is now the case, by the necessity to control ECF.

(5) It is probable, as suggested above, that a drug effective against *T. parva* either per se or as a closely related analog, would be effective against *T. annulata*. Thus, chemotherapeutic methods could be combined with artificial immunization in those vast areas where *T. annulata* presents a problem and it could be of special value in the protection of exotic or highly susceptible animals introduced into the endemic areas.

It is possible that much time could elapse before this really suitable drug might be found but we do know now that its emergence is not beyond the bounds of possibility or even probability. Furthermore, it must be borne in mind that the use of a drug could be faced with problems of drug fastness and it would be necessary to make continual efforts to discover other effective and preferably chemically unrelated compounds. Nevertheless, I would regard this as a minor obstacle, particularly in the control of ECF, with somewhat more risk in the chemotherapy of *T. annulata* infection. It appears, therefore, that the possibilities of control and even eradication of ECF fever are greatly enhanced.

The immediate weapon on hand is the use of infection by stabilates and concomitant chemotherapy with long-acting tetracyclines. Although this method could be put into practice now, albeit tentatively and with great care, there is the need for considerable refinement of the technique and a systematic matching up of strains of *T. parva* and *T. lawrencei*.

I think that it should be possible, before long, to produce a method of immunization using strains attenuated in vitro, with or without the use of chemotherapy an adjuvant. This method, of course, will be subject to the same constraints as the infection and treatment method mentioned above, on account of strain differences and because of certain logistic problems in administration.

Eventually, satisfactory therapy will be developed and this will be capable of playing perhaps the most important role either alone or in conjunction with other methods in making control of ECF a practical possibility.

Turning to the control of *T. annulata*, the cause of Mediterranean fever or malignant theileriosis, one is faced with different problems because of the different biology of this parasite. The two outstanding differences between *T. parva* and *T. annulata* that affect the epidemiology of the diseases caused by these parasites are the persistence of the parasite *T. annulata* in the bovine host after the recovery of the animal and the ready transmissibility of the parasite by blood inoculation and by several species of two- and three-host ticks. Thus, the disease caused by *T. annulata* is much more widespread geographically than is the ECF complex so that its effect is important over a vast area of the tropical belt of the world. *T. annulata* occurs from the western end of the Mediterranean to the Far East involving huge populations of cattle and also the very important water buffalo (*Bubulus bubalis*). In most endemic areas cattle become infected as calves in which the losses can be very variable but in these young animals there is a marked recovery rate so that an immune carrier population of indigenous cattle is built up. There has hitherto been a general acceptance that this endemic situation must be tolerated as any attempts to control the disease would be very difficult and expensive and could, if inadequately carried out, lead to disastrous losses. Recently with the developments in many areas, exotic or improved stock are being brought into
endemic areas and these are highly susceptible to *T. annulata* and so serious losses can result. There has, therefore, been an increased awareness of the importance of control of *T. annulata* infection and work is now in progress in the field of artificial immunization.

The control of theileriosis due to *T. annulata* has been considered fairly fully by Pipano (1977) and Hooshmand-Rad (1977) who have described the methods of husbandry employed in areas where *T. annulata* exists as a problem. These fall mainly into two broad systems:

1. **The barn-kept animals on a system of zero grazing** — Pipano has shown that this system is vulnerable to outbreaks of varying significance dependent on the numbers of vector ticks that persist in the houses. Due to this variation, levels of susceptibility, at times, can become quite high and therefore can lead to serious losses or can be fairly low so that the losses can be minimal.

2. **The grazed or range-raised cattle** — In this situation, all cattle can be expected to become infected in early life and lead to a population of immune animals that will also, incidentally, provide a large reservoir of infection. Hooshmand-Rad has carried out experimental vaccination with one of the attenuated tissue culture strains in cattle kept in barns and yards on zero-grazing and there is circumstantial evidence that this has had a salutory effect on the disease pattern in the vaccinated cattle (Hooshmand-Rad 1973).

It is obvious that in the areas where *T. annulata* is present at significant levels, the manner in which vaccination methods can be of value is in the protection of introduced exotic cattle and similarly in the protection of improved cattle specially bred in well-supervised conditions in which tick control and cattle movement control are exercised rigorously.

With *T. annulata*, therefore, future disease control appears to be dependent on: (a) control of ticks combined with control of cattle movement; (b) the strategic use of vaccines according to the demands made by any particular forms of animal husbandry; and (c) the eventual use of specific chemotherapy where this can be effectively applied.

**Future Work**

On the basis of views I have expressed above, my recommendations for the broad lines of future research in the field of theileriosis are:

1. Concentrated attempts should be made to discover new compounds of chemotherapeutic potential, and success in tissue culture screens should be exploited as soon as possible in the field, so that effective compounds can be brought to the point of acceptability and satisfactory formulation as rapidly as possible. This can be an exceptionally long and tedious process.

2. Isolation of strains of *T. parva* from as many areas as possible should be made and these should be compared antigenically so that a study of the changes in strains, which I am sure can be brought about in different environments and in different vector/host situations, could be carried out aiming at making it possible for strategic attack on the disease to be made in the most satisfactory way.

3. Methods of comparing strains and species (e.g., *T. parva*, *T. lawrencei*, *T. mutans*, and *T. annulata*) by laboratory techniques should be improved and new methods should be sought. In this field studies might be made of iso-enzymes and the characteristics of the DNA of different species of *Theileria*.

4. Various combinations of strains should be investigated immunogenetically with the object of discovering the optimum combinations for use as immunogens in different areas.

5. Efforts should be made to refine the production of infective particles and to make suspensions of these to conform as nearly as possible with the features desirable in a practically applicable vaccine. In this connection, some means of preservation for field use, which could include
lyophilization, should be explored. Investigations should be made into the applicability of the infection and treatment method of immunization to *T. annulata* infection.

(6) While the work is being pursued, advantage should be taken of the availability of material to continue the studies initiated by the EAVRO team on *T. parva* and workers in the Middle East and India on *T. annulata* on the immunogenic characters of attenuated tissue culture lines of *T. parva* and *T. annulata* macroschizont-infected lymphoblastoid cells. Also, investigations should be carried out to determine, definitely, the details of the cycle of the parasites from the moment of inoculation by the tick to the appearance of the very early schizont. This study should take into account the possibility of replication of the parasite with spread in the lymphatic system before the development of schizonts.

(7) In connection with *T. annulata* there are several anomalies that have been recorded by various workers but that have not been studied specifically. The information given in the papers read at this conference has brought some of these anomalies into prominence and there is a need for the problems raised to be clarified. Among them I would mention the apparent suppressive effect of the colostrum from premune dams on the use of vaccine in calves, which has been reported. This could well deserve consideration in future research. The unexpected reports that *T. annulata* in the salivary glands of the tick *H. dromedarii* can be transmitted from the unfed tick could provide an intriguing investigation, and more knowledge should be sought concerning the transstadial transmission of *T. annulata* in two- and three-host species of *Hyalomma*. Variations in this process when compared with the transmission of *T. parva* by nymphs and adults of *Rhipicephalus appendiculatus* suggest anomalies that might be investigated with fruitful results.

A point of great importance that I would like to stress in connection with future work is that banks of stabilates that have so laboriously been built up over the years by workers in Kenya, Israel, and Iran should be carefully preserved and be made available for all bona fide workers in the field of theileriosis.

Finally, as a result of the work of the last 10 yr, I am encouraged to think that we are on the threshold of exciting and important developments that, if carried out with vigour and constant collaboration, could lead to most valuable advances in the control of theileriosis.

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Studies on *Rhipicephalus appendiculatus* at the International Centre of Insect Physiology and Ecology, Nairobi, Kenya

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The International Centre of Insect Physiology and Ecology (ICIPE) is an autonomous research institute with its main laboratories in Nairobi. The centre was founded in 1970 under the present Director, Professor Thomas R. Odhiambo, and scientific research started in 1971 and now comprises six programs, including one on ticks. Each target insect is of agricultural, medical, or veterinary importance in East Africa.

The research scientists are internationally recruited at the postdoctoral level and currently number 22 with 15 graduate scientific officers assisting them. The complete staff numbers approximately 120.

The scope of the work at the ICIPE and the progress achieved can be most readily seen by referring to our first three annual reports (1973-75).

**Tick Program**

Initial work centred on the physiology of soft ticks (*Argasidae*, in particular *Ornithodoros moubata*) but beginning in 1973 increased emphasis was placed on ixodid ticks and a cooperative project was set up with the East African Veterinary Research Organization (EAVRO) at Muguga, Kenya, on *R. appendiculatus*. The objectives of the ICIPE work were to complement the disease studies being carried out by EAVRO and the UNDP/FAO group who have been working toward the development of a vaccine against East Coast Fever (ECF) since 1967. Our areas of concentration have been on the ecology, physiology, and behaviour of the tick in order to lay the foundation for a better understanding of its function as a vector and to develop improved control methods, including those that do not employ substances of high general toxicity.

ICIPE appointed one experimental officer in 1973 to the tick program and two research scientists in 1974 (one ecologist based at EAVRO, one physiologist at ICIPE) and supporting staff. A small laboratory, tick-rearing rooms, and animal accommodation facilities were built on a site provided at EAVRO. To date, the ICIPE input has totaled 7½ man-years at the professional level (including the work of a consultant and active participation by the two research directors) and 16 man-years at the technical and subordinate staff level. In addition, four other ICIPE staff members have been involved in the tick program to varying degrees, and virtually every member of the EAVRO/FAO group has been involved at one time or another.

Following the appointment of a replacement research scientist (physiologist) in 1976, the ICIPE program includes histological and physiological studies on the pathobiology of theilerial transmission by ticks, in order to determine the possible roles of pinocytotic tissues (Obenchain and Oliver 1973, 1976) as secondary barriers to the invasion and multiplication of *Theileria* species.

As an immediate goal, the ICIPE tick physiology program will concentrate on the further elucidation of tick endocrine mechanisms involved in processes of
growth, development, and reproduction. The results of these studies may potentiate the development of two proposed strategies for the control of tick vectors of disease (Galun 1976a, b). The first approach involves the identification of the natural tick hormones and the subsequent development of synthetic analogs that could be used to control tick growth and development. Such developments could also lead to the formulation of broad-spectrum growth regulators that would be effective in the control of most external and internal arthropod and helminth parasites of cattle. The second approach involves the immunization of cattle against natural tick hormones. Galun points out that specific antibodies have been obtained for the ecdysones and juvenile hormones of insects and speculates that immunization against tick hormones might be especially effective since (1) many tick species feed on cattle during all three parasitic stages, (2) they take blood meals of up to 100 times their unfed body weights, and (3) considerable amounts of unchanged host proteins (such as gamma globulin) pass through the gut wall in a serologically unchanged form. Under favourable ecological conditions the application of either of these control strategies for the control of R. appendiculatus could contribute to the suppression or eradication of ECF.

Past Research

In October 1973, a double-fenced paddock at Muguga was infested with R. appendiculatus ticks as they dropped from cattle artificially infected with Theileria parva. ECF was cycled and the tick population built up over the next 15 mo by introducing successive groups of two to five susceptible cattle and replacing them as they died. The progress of the tick population was monitored by counts on the cattle and by regular samples from the vegetation (Purnell et al. 1975). The paddock was then used in two experiments to challenge under field conditions cattle immunized by the vaccine project. The results of the first study have been published (Radley et al. 1975).

The apparent attraction of male R. appendiculatus males to partially fed females was investigated by bioassay and chemical analysis. It was found (Leahy et al. 1976) that phenol and p-cresol can be obtained from females, but only after 3 days of feeding on the host. Males are attracted to the females for mating, but only after a similar period of feeding. The same situation was demonstrated in R. pulchellus. Amblyomma variegatum, A. americanum, and Hyalomma truncatum were investigated chemically and found to contain salicylaldehyde and 2,6-dichlorophenol in addition to the other compounds (Wood et al. 1975).

A series of behavioural studies suggested three other possible pheromone systems at work on the unfed tick, one that inhibits the characteristic climbing behaviour in the vegetation during the day, and another that ensures that ticks attaching to the host do so near a member of the opposite sex. This work was cut short by the untimely death of the investigator and has not been resumed.

The clustering behaviour of the unfed adults of R. appendiculatus and R. pulchellus on the heads of grasses was investigated. It was concluded that prior aggregation at favoured sites on the ground could explain the phenomenon (Browning 1976).

A 3-yr study of an R. appendiculatus population on the Kenyan coast revealed an atypical life cycle in this rather hot situation with two peaks of adult activity per year, but only one effective breeding period (Newson, in press).

The tick-infested paddock was divided into two and used to determine the persistence of T. parva infection in a tick population offered only ECF-immune cattle as hosts. In 10 mo the initial virulent challenge had been eliminated, leaving only a mild, serologically distinct form of Theileria, whereas susceptible cattle placed in the control area still died of acute ECF (Radley et al., in press).
Current Research

The tick-infested paddock is being used for experiments on tick activity on the vegetation, daily rhythm of tick pickup by cattle, and timing of detachment of engorged stages. The basic population monitoring continues.

Another paddock at EAVRO has been divided into double-fenced plots of 1000 m², 4000 m², and 12 000 m², and seeded with disease-free, unfed nymphs and larvae of R. appendiculatus at a uniform rate and are now stocked with one steer each. The effects of the different host-stocking densities and grazing intensities on the tick populations are being studied.

A study of R. appendiculatus was started in 1975 in Kajiado District at a site where conditions are ecologically marginal for this species, but where there was recent clinical and serological evidence of ECF and also of Theileria mutans. The opportunity is being taken to compare the ecology of four other species, including R. pulchellus, which is dominant. Blood slides and serum samples are also obtained from the cattle when the ticks are collected. A preliminary report (Newson and Punyua, in press) shows that the R. appendiculatus population is at present very precarious and only a few of the calves have detectable antibodies to ECF. On the other hand Theileria are commonly seen in blood films and many animals have shown positive antibody titres to T. mutans. The only proven vectors of T. mutans, A. variegatum, and A. cohaerens, are absent but A. gemma is common.

A study by a collaborator at ICIPE of the factors that stimulate the rapid feeding that occurs in female hard ticks after mating is nearly complete.

The dehydration and mortality of R. appendiculatus and R. pulchellus at different temperature and relative humidity regimes is being studied by an FAO fellow.

Rechav (personal communication) demonstrated the existence of an extractable pheromone in male A. hebraeum that induces unfed females of A. hebraeum immediately to attach close to the male. We find that this extract does not attract either sex of A. variegatum or A. gemma, but that males of A. variegatum, at least, produce a substance that elicits similar behaviour in the female of the species.

Studies on the neurosecretory systems of ixodid and argasid ticks (Obenchain 1974; Obenchain and Oliver 1975) are continuing at ICIPE. Initial work shows that applications of the two major groups of insect hormones, the ecdysones and juvenile hormones, have profound effects on tick growth and molting (Mango et al. 1976) and on vitellogenesis (Obenchain et al. 1976).

Future Research

The current ecological/population studies will be continued in the Muguga paddocks, which are stocked at different densities. Observations will be made in the laboratory, at favourable temperatures and humidities, on the survival of all stages of R. appendiculatus, and these data will be related to empirical determinations of survival in the field. Observations on the percentage of successfully engorging ticks will be made on confined hosts. The long-term objective of this work is to develop a population model for R. appendiculatus. Experiments on the levels of tick activity on the ground and rates of pickup and drop-off from cattle will also be completed.

The field study at Kajiado District on several species of Rhipicephalus and A. gemma will be continued. A comparable field study will be initiated, if possible, in the vicinity of a planned ICIPE field station in western Kenya, where the climate is markedly different from any other study site so far used and where R. appendiculatus is abundant.

Interactions between theilerial parasites and their tick vectors will concentrate on the possible involvement of fat body "nephrocytes," pericardial tissues, and hemocytes. The physiological effects of theilerial infection on tick survival, growth, and reproduction will also be determined.
Histological studies on the neurosecretory-neuroendocrine system of *R. appendiculatus* will be supplemented with fine structural observations on probable sites of endocrine synthesis and/or release. The possible existence and natural concentrations of ecdysones and juvenile hormones in ticks will be examined with immunoassay and other techniques. Physiological effects of these hormones on engorgement behaviour, growth and molting, pheromone synthesis and release, reproductive maturation and embryogenesis, some aspects of which have already been studied in other ticks (Obenchain et al., unpublished) will be investigated in *R. appendiculatus*. Simultaneous biochemical investigations will be designed to isolate active components from candidate tick tissues to test the possibility that the natural molting and juvenoid hormones of ticks might be chemically different from those of insects.

Experiments will be designed and tests will be made of the feasibility of immunizing hosts against ecdysones and juvenile hormones.

**References**


Pathology and Pathogenesis of East Coast Fever (ECF)

Gross lesions in natural bovine East Coast Fever (ECF) found at necropsy were pooled together and described to give an indication of the variety of lesions and their incidence in various organs. Of particular importance was the discovery of macroscopic lymphoid aggregates (pseudoinfarcts) in the gallbladder, heart, and small intestines. Such foci of lymphoid hyperplasia had been reported by previous workers to form in the kidney, abomasum, liver, and lungs in that descending order of frequency (1).

The pathological lesions in bovine experimentally infected with ECF have been described. At autopsy the most common findings were lung edema, abomasal ulcers, and straw-coloured fluids in the body cavities. Enlargement of the lymph nodes and the spleen was also observed in all those cases that had short courses of the disease.

Histological examinations of the organs showed aggregates of lymphocytic cells especially in the liver, kidneys, lungs, and the glandular mucosae of the abomasum.

In the majority of cases the lymphocytic cell infiltration was seen especially around the blood vessels. However, in the lungs and in the kidneys the cells were also seen around the walls of the bronchioles and glomeruli, respectively. They were also seen around hepatic triads in the liver. In a few cases the blood vessels of the brain were infiltrated with lymphocytes. Degenerative changes were also observed in the liver and kidneys and some of the lymphocytic aggregates in the body organs were necrotic (2).

A study of the pathogenesis and pathology of ECF induced by irradiated Theileria parva-infected ticks has been described. The infected adult Rhipicephalus appendiculatus ticks were subjected to radiation from a 60Co source at the rate of 3.59 krads per minute. Ticks were irradiated at dose levels of 0, 10, 20, 30, 50, and 70 krads, and then attached to ECF-susceptible steers. Irradiation at 10 krads had no effect on the parasites whereas at 20 and 30 krads the pathogenicity of T. parva was reduced and the course of the disease in the steers altered to such an extent that it could only be detected by the presence of agglutinating antibodies revealed by a C.A. Test. The steers were not affected by 10-tick challenge. Three steers infected with ticks irradiated at 50 krads died after a 10-tick challenge infection and at 70 krads ticks did not attach on the steers (3).

Salivary gland acini of adult R. appendiculatus ticks and bovine tissue infected with T. parva (Muguga) have been studied by electron microscope. Different forms of the organisms, representing different stages of the developmental cycle in the ticks and bovine tissue, are described. The mode of division at different stages of development is also described (4).

The sequence of ultrastructural changes of bovine platelets during the fatal course
of ECF was swelling, formation of vacuoles, pseudopodia, and indentations; then thrombocytorrhexis and degranulation; and finally, thrombocytolysis. These changes lead to thrombocytopenia and release of serotonin, resulting in petechiations and pulmonary edema (5).

Immunology

A study was made of the effects of serum and α-globulins from vaccinated cattle (donor cattle) given to cattle (principals) exposed to *T. parva* infection (ECF). The principals were treated with normal serum, with serum containing high antibody titre against *T. parva* (as evaluated by Indirect Fluorescent Antibody (IFA) technique), or with globulins prepared from normal and from immune serums. For exposure, the principals were inoculated with standardized suspension of the tick *R. appendiculatus* containing *T. parva* infective particles. Comparison was made between treatments with normal serum and globulin and treatments with immune serum and globulin, using observations of time lapse after exposure to febrile response (39.5 °C) to appearance of the *T. parva* macrogamonts and to death. In addition hematologic changes were determined. Neither establishment of infection nor the clinical and hematologic changes in the principals were influenced by their treatment with standardized suspension of the tick *R. appendiculatus* containing *T. parva* infective particles. Comparison was made between treatments with normal serum and globulin and treatments with immune serum and globulin, using observations of time lapse after exposure to febrile response (39.5 °C) to appearance of the *T. parva* macrogamonts and to death. In addition hematologic changes were determined. Neither establishment of infection nor the clinical and hematologic changes in the principals were influenced by their treatment with immune serum or concentrated globulin; all died of ECF. Humoral antibodies did not protect cattle against fatal ECF nor was enhancement of *T. parva* infection observed (6).

Bovine peripheral leukocytes were used in a migration inhibition test to detect sensitization to *T. parva* antigen. Of animals exposed to *T. parva* antigens, 76% yielded sensitive cells. The reaction was most efficiently induced by use of living *T. parva*. Migration of cells from control animals was not inhibited. In some animals the presence of sensitive cells in the peripheral circulation was shown to persist for up to 8 mo after exposure to experimental *T. parva* infection. The significance of cells that are sensitive to *T. parva* antigens in ECF is not known (7).

Treatment

The calves were infected with ECF parasites and after development of the disease, evidenced by fever, lymph node enlargement, and demonstration of macrogamonts in the lymph node smears, they were treated with Actinomycin D.

Treatment for 5 consecutive days at a dosage level of 0.1-4.8 µg per kg body weight, and for 2 days with 9.0 µg per kg, failed to suppress the disease and all died. One calf treated with Actinomycin D for 4 days at a level of 9.0 µg per kg completely recovered from the disease. After recovery this animal had high antibody titre and failed to react to ECF challenge (8).

Present Research

Physiochemical heterogenicity of gamma globulin in cattle experimentally infected with ECF (*T. parva* infection) is being studied, including:

(a) isolation and characterization of the two serum globulin population IgG and IgM where antibody activity is usually localized; and

(b) sequence of occurrence and persistence of each molecular species of the globulin as the disease progresses.

Chemotherapy studies on the treatment of cattle infected with *T. parva* using three antimitotic and anticancer drugs isolated from plants in Kenya are also being done, as well as a study of the development of *T. parva* at the point of injection on cattle using an electron microscope.

There are five professional staff and four technical staff involved in research on theileriosis.

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Research Projections on Theileriosis at the East African Veterinary Research Organization

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The major research activities at the East African Veterinary Research Organization (EAVRO) on theileriosis have been directed toward the development of a method of immunizing cattle against East Coast Fever (ECF) and a better understanding of mechanisms of infection and immune response of the host. One such method of immunization that has already been developed is chemoprophylaxis, i.e., infection with a combination of theilerial strains and concurrent administration of long-acting oxytetracycline; this method is very promising and, although it cannot yet be used on a large scale, its immediate application lies in the prevention of catastrophes that might occur, for example, in cases where tick control measures break down. Cell culture vaccine has produced satisfactory results when used in a homologous system and is thus not quite ready yet for field use.

The projected research activities outlined below are intended to improve on the methods already developed as well as to elucidate the complicating factors and to explore new fields of controlling theileriosis.

Cell Culture

(a) Bovine Cell Culture: studies on the route of inoculation, on the role of adjuvants, and on the dose of inoculum as influenced by route and adjuvants; continuation of work on incorporation of different strains; continuation of studies on methods for releasing and purifying free schizonts from infected cells: (i) studies on the viability of free schizonts in culture and (ii) work on the efficacy of free schizonts as vaccine material; in vitro infection of cell culture by material derived from ticks; studies on the possible induction of a carrier state with cell cultures.

(b) Tick Cell Culture: Development of tick cell culture as a source of antigens and a possible vaccine material.

Chemotherapy

To try and identify drugs with potential antitheilerial activity; to identify possible methods of altering the course of Theileria spp. infection by immunotherapy.

Immunology

(a) Antigens of Theileria: (i) continuation of the characterization of schizont and piroplasm antigens by antibody affinity chromatography; (ii) studies on methods of isolation of infective particles from infected ticks: comparison of antigens of these with schizonts and piroplasms; and (iii) further work on circulating antigen during ECF infection.

(b) Humoral Responses: (i) further fractionations of various immune sera to determine the role of the various immunoglobulin classes in immunity; (ii) examination of various serological assays for the differentiation of strains of Theileria; and (iii) continuation of work on serum protein changes during ECF infections, including complement, conglutinin, and immunoconglutinin.

(c) Cellular Responses: (i) continuation of work on the properties of lymphocytes from cattle during ECF infection, i-
duction of immunity, and resistance to challenge.

**Epizootiology**

(a) *Theileria parva* (the causative organism of ECF): (i) the epizootiology of ECF will be studied in an experimental paddock and elsewhere; (ii) method of immunization against ECF developed will be evaluated in the above paddock and elsewhere.

(b) *Theileria lawrencei* (the causative organism of Corridor Disease): (i) investigation will be made into the adaption of *T. lawrencei* from buffalo to cattle: (1) to establish whether antigenic types evident in buffalo are maintained after the adaption of *T. lawrencei* to cattle; and (2) cattle-adapted strains of *T. lawrencei* will be used to infect buffalo to determine whether the parasite reverts to *T. lawrencei* type and whether the parasite alters its antigenic type; (ii) determine whether antigen variants are produced in carrier buffalo; (iii) study the epizootiology of *T. lawrencei* in a paddock containing buffalo; (iv) evaluate the method of immunizing cattle against *T. lawrencei* by experimental challenge and exposure to natural *T. lawrencei* challenge in the buffalo paddock.

(c) *Theileria mutans* (the causative organism of anemic Theileriosis): (i) the importance of *T. mutans* as a cattle pathogen and its tick vector will be investigated; (ii) control methods against *T. mutans* such as immunization and chemotherapy should be developed.

**Pathology and Pathogenesis**

Further research on theileriosis is to concentrate on extending the present ongoing study of the pathology and pathogenesis of experimental syndromes. Further studies are to focus on defining the major difference in pathology of the disease caused by various theilerial isolates. The work is to investigate the mechanisms that cause the vascular lesions in the acute syndromes, and also on the different organ tropisms that characterize the more chronic forms of the disease. The studies will attempt to define the mechanisms of cellular injury and also give a broad look at the differences in pathogenesis of the various syndromes that are caused by the various isolates of *Theileria*.
The International Laboratory for Research on Animal Diseases

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The International Laboratory for Research on Animal Diseases (ILRAD) was established in Nairobi, Kenya, to conduct research on internationally important animal diseases that constrain livestock production in the developing countries. ILRAD's mandate is to find effective solutions to trypanosomiasis and theileriosis, two important diseases that limit livestock production in vast areas of Africa and other parts of the world.

ILRAD is one of nine international agricultural research centres (IARCs) supported by the Consultative Group on International Agricultural Research (CGIAR), which is a consortium of over 30 donor agencies and countries. Seven of the IARCs are involved in plant research and production improvement and have been very successful in increasing productivity of various food plants including rice, maize, potatoes, and others. Two of the international centres have been established to focus their research activities on livestock: ILRAD is working on livestock diseases, while the International Livestock Centre for Africa (ILCA), headquartered in Addis Ababa, Ethiopia, is working on livestock production systems.

The IARCs are international in operation with their various headquarters located in different developing countries depending on the commodity being researched.

Funding of the IARCs goes directly from the donors to the centres without the encumbrances associated with the administrative and other operational details of many other organizations. Within this context, the IARCs are able to focus intense research efforts at an international level on solving specific food production constraints. This makes the process more effective in the utilization of available resources. ILRAD, like the other IARCs, is governed by an international board of trustees with five members from Africa. The board meets annually to determine the broad outline of ILRAD operations and program and has several committees that examine specific functions and operational details including the research program.

The research approaches of ILRAD are directed to finding effective solutions to trypanosomiasis and theileriosis. Within this approach, the diseases are the main considerations with research projects designed to elucidate data required to understand and control these diseases. The research approaches are multidisciplinary with input from both the basic and applied sciences.

The scientific staff is recruited internationally with the following disciplines represented: pathology, biochemistry, cell culture, immunology, parasitology including entomology, ultrastructure and epidemiology, and clinical medicine. The staff is divided into core scientists, visiting scientists, and postdoctorals. When staffing is completed by late 1978, there will be approximately equal numbers in each category with the total being 45. At the present time, there are 13 scientists working at the institution with the number to increase to 17 in 1977.

The main facilities are located at Kabete, just outside Nairobi, Kenya. Included are animal facilities, laboratories, laboratory
support areas, library, conference and training area, administration, vector facilities including both tsetse and tick, and housing for graduate students, post-doctorals, and visiting scientists. The major facilities are to be completed by January 1978. When the total facilities become available in early 1978, ILRAD will have a modern research complex that is comparable to those in the developing or developed countries. These facilities and equipment will make it possible to conduct a wide variety of research activities relevant to the two diseases under investigation.

In addition to the research outlined below, ILRAD has developed and will continue to develop cooperative research with institutions in the developed and developing countries. These activities are directed at expanding the scope and capability of the ILRAD program by utilizing expertise and capabilities beyond those resident at ILRAD. In addition, there is certain research that must be carried out in locations where the diseases occur or where there are specific requirements that cannot be met by either laboratory manipulations or in the vicinity of the main facilities.

Training is an important component of ILRAD's mandate. Included under training are research training, conferences, short courses, and related activities. Graduate students are included in ILRAD and receive degrees from other institutions while doing research and training at ILRAD. At the present time, the number of graduate students, like the total staff, is limited, but will be expanded in 1978.

The ILRAD theileriosis research program is directed to the development of a vaccine that can effectively control the disease. The program is divided into several aspects that are given below. The scope and magnitude of theileriosis research will expand in 1977 and increase considerably in 1978 to coincide with the completion of the major facilities and the arrival of additional staff. In 1977, a total of five scientific staff will be working on different aspects of the problem. The number of staff will increase to 15 in 1978. Some additional staff will be involved in training.

**ILRAD Research**

ILRAD research places emphasis on cell culture propagation of the parasite and utilization of cell culture material as antigens for vaccines. Present work utilizes both bovine lymphoid and tick cell cultures. The former is the procedure developed at EAVRO with current activities directed at standardizing the procedures for ILRAD use, improving methods for lymphoid cell culture of the parasite, infection of "normal" bovine cells with infectious particles and with infected cells, and attenuation of *Theileria* by serial passage. The tick cell culture work is directed at attempts to propagate the parasite in these cultures and potential utilization of the cultures for immunizing cattle. Cell cultures from *Rhipicephalus appendiculatus* ticks have been established and studies are underway to determine whether these cultures will support propagation of *Theileria*.

Research is also currently underway at ILRAD to utilize biochemical approaches for the purification of infectious particles that are obtained from infected salivary glands of ticks. Also, approaches to obtain macroshizont material as free from cell contamination as possible are being undertaken.

Cooperative research with staff of the Freie University of Berlin is being carried out to develop a hyper-susceptible strain of *R. appendiculatus* ticks. This strain would improve the production of infectious particles, which, in turn, would influence the amount of infectious material that could be obtained from ticks and also would potentially supply a strain of ticks whose tissues might be more capable of propagating *Theileria* in cell culture.

The pathogenesis of the disease will be investigated in 1977 with emphasis on the pulmonary and lympho-reticular systems. Also to begin in 1977 will be studies on immunity to theileriosis involving both cellular and humoral immune systems.
Efforts will be made to isolate and define the protective antigens from both infectious particles and from macroschizont infected cells. Also, as indicated above, studies will be carried out to infect tick and bovine cell cultures to attempt to propagate protective antigens. Experimental vaccination approaches based on the information generated by the theileriosis research at EAVRO will also be carried out.

A great need in theileriosis research is a serologic or diagnostic technique that can differentiate antigenic types of *Theileria*. This will be investigated at ILRAD utilizing antibody as well as cell-mediated immunologic approaches. Such a procedure will be invaluable in determining the epidemiology of the disease and in determining the antigenic types that must be included in a vaccine approach.

It is anticipated that by late 1978 ILRAD will have 15 scientific staff working on various aspects of theileriosis. As indicated above, the overall approach will be to develop an effective vaccine that is safe, does not produce a carrier state, incorporates the spectrum of antigens for optimal protection, and is as stable as possible. A number of approaches will be used for achieving this final goal of the program.

**Cooperative Research and Training**

Cooperative research on theileriosis is limited at present. Discussions have been held with the staff of EAVRO and the Kenya Veterinary Laboratories and other institutions and it is anticipated that similar discussions will be held with other individuals and institutions in Africa and elsewhere. We will attempt to develop meaningful cooperative research with representatives from countries where the disease is a problem and will try to assist research and control in these countries as much as possible. Included will be training for various levels of staff and visiting investigators who come to ILRAD for training in specific research techniques and for the conduct of specific research aspects.
Committee Recommendations
Committee on *T. parva*, *T. lawrencei*, and *T. mutans*

**Control of Vector**

**(a) Acaricides**

Acaricide control will continue to be the first line of defence against tick-borne diseases and every assistance must be given to implement acaricide control where required. Problems associated with implementation are:

1. **Financial** — constraints range from provision of materials for dip construction to provision of acaricide, and problems vary from country to country.
2. **Dip management** — Training facilities are required for dip attendants and other staff, and a publication is required on all aspects of dip management. Control facilities must be available for determining acaricide concentration. In deciding on sites for dips and choice of acaricides, pollution problems must be taken into account.
3. **Acaricide resistance** — A monitoring system should be established to investigate the development of acaricide resistance in the important tick vectors, and information should be disseminated to interested countries.

**(b) Cattle Movement**

Control of movement of cattle is an integral part of all disease control measures and where possible international cooperation should be encouraged.

**(c) Biological Methods**

Research on tick ecology and the biological control of ticks should be supported.

**(d) Alternative Hosts**

The importance of wild animals in maintaining tick populations should be recognized.

**Control of the Parasite**

**(a) Immunization**

1. **Infection and treatment** — This is the only method presently available to immunize cattle against ECF and constitutes a very important research tool. It is felt that this method has application in particular circumstances, and facilities and materials should continue to be made available to meet requests from

**See addendum.**
individual countries to investigate the efficacy of this method of immunizing cattle against ECF.

(2) Tissue culture — Attenuation of *T. parva* schizont-infected lymphoblastoid cells has been achieved by extended cell culture. Research on this and other potential methods of immunization must be continued.

(b) Chemotherapy

Recent promising advances in the chemotherapy of ECF are noted and investigations should be continued and supported.

Research

(a) Vaccine Development

Emphasis should be placed on production of all stages of the parasite in tissue culture for use in vaccinating cattle. Adjuvants should be used to improve all available methods of vaccination.

(b) Strain Differentiation

Development of an in vitro test to identify antigenic types would accelerate the field implementation of available methods of vaccination.

(c) Epizootiological Research

Such research should continue and theilerial parasites involved in the ECF syndrome should be identified and characterized.

Training

There is a great need for training personnel in all aspects of tick-borne disease control and it is strongly recommended that such a training program be set up.

Addendum

The meeting noted and welcomed the information that a joint action by FAO/OAU is under way to set up in Africa, initially, three regional centres for training, research, and monitoring of problems related to the use of acaricides.
Committee on *T. annulata*  

**Epidemiology**  

(a) **Tick Survey**  

(1) To study the incidence of species of ticks that are potential vectors of *T. annulata*.

(2) Investigate the ecology of the genus *Hyalomma*.

(b) **Definition of the Enzootic Areas**  

(1) By parasitological means;

(2) By serological techniques.

(c) **Water Buffalo**  

(1) To study the role played by water buffalo in enzooticality of *T. annulata* infection.

**Immunization**  

(1) Comparison of efficacy of immunization methods such as by tissue culture-grown schizonts and infection and treatment. This should be determined by infected tick challenge in carefully controlled field trials.

(2) Define the longevity of production conveyed by various vaccination regimes as determined by field challenge.

(3) Further study on the occurrence of antigenic types of *T. annulata* as tested by in vitro and in vivo methods.

(4) Improvement in methods of preservation and transportation of the vaccine.

(5) Study the importance of colostrum on susceptibility to infection and its possible interference with immunization. This information is essential in determining at what age calves can be immunized.

**Chemotherapy**  

To encourage the demand for screening different chemical compounds for their effect on *T. annulata*.

**Diagnosis**  

Standardization of the existing serodiagnostic techniques, and efforts to develop more sensitive ones.

**Research and Training**  

(1) Demand for establishment of laboratories or modernization of existing ones for diagnosis and research on *T. annulata*.
(2) The identification of the regional and international reference laboratories. These laboratories to be responsible for providing standard antigen, sera, and anti-sera.

(3) ILRAD to participate in supporting the above proposed research and training projects on *T. annulata*.

The Committee recommends a careful serological research for identification and differentiation of the so-called *T. mutans* in the Middle East, North Africa, India, and Pakistan. It also recommends further studies on *T. hirci* parasitologically as well as epizootiologically.
Committee on Cooperative Research and Control

The need for cooperative research is paramount and has been amply exemplified by the Conference called by ILRAD / IDRC. It is recommended that a Standing Committee be formed in Nairobi under the auspices of ILRAD. The terms of reference of the Standing Committee should be as follows:

1. consider what research is required, keeping in mind the demands of those countries affected by theileriosis;
2. monitor research in theileriosis and related problems, for example tick biology, acaricide applications, etc.;
3. define lines of research necessary and desirable and suggest cooperative projects and areas where these can be done;
4. advise or make recommendations to funding bodies;
5. call scientific conferences on theileriosis at specified intervals, e.g., every 2-3 years.

Membership: (1) ILRAD; (2) EAVRO; (3) ICIPE; (4) OAU(IBAR); (5) co-opted representatives of institutions working on theileriosis and related problems if and when necessary; (6) invited consultants in related fields either to attend the meeting or submit memoranda.

The committee should meet at 2-3-year intervals. Such meetings could be held after a scientific conference on theileriosis called by the committee. ILRAD should apply for special funds for the committee activities.
Committee on Information Development and Exchange

While noting the availability of relevant information on many aspects of theileriosis from EAVRO/EAAFRO Library and elsewhere, this committee feels that there is need for the compilation and dissemination of information specific to theileriosis. Hence it recommends that there be created:

an information bulletin on theileriosis, which would list current topics on theileriosis and related subjects and would be sent to all laboratories known to be working on theileriosis and to other centres and individuals on request; as well, the possibility of an abstract should be worked out.

The committee feels there is need for cooperation among all centres or libraries that may have theileriosis information. ILRAD could take the initiative in coordinating this.
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