EAST COAST FEVER AND RELATED DISEASES:

A TECHNICAL CONFERENCE

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THE EAST COAST CONFERENCE IN REVIEW

John J. McKelvey, Jr.

An East Coast Fever (ECF) review meeting was called in order to gain counsel and advice from distinguished immunologists as to progress that has been made during the seventeen months that have passed since the previous technical meeting was held to ascertain how far advanced is the research toward the goal of producing a field vaccine for ECF. This point bears upon the priority of ECF for inclusion among other diseases forming the basic program of an Animal Disease Research Center, presumably to be sited in Kenya. Eighteen technical and administrative people participated. (See attached list for the names and for the institutions these people represented.)

The specific purposes of the meeting were: (a) to produce a concise review of the East Coast Fever situation with appended literature citations, (b) to try to arrive at an estimate of how soon a vaccine for prevention of East Coast Fever will become available, (c) to estimate how much additional research is required to arrive at this end, and (d) to place the East Coast Fever research in perspective with regard to similar research on trypanosomiasis.

Dr. W. I. M. McIntyre, Professor of Clinical Medicine, University of Glasgow, chaired the technical discussions and called upon Dr. M. P. Cunningham, Project Manager, UNDP (EAVRO), concerned with East Coast Fever, to review the life cycle of the Theileria parva (the causal agent of ECF) for the benefit of those unfamiliar with this parasite, then to discuss

his results and those of his colleagues in trying to produce a field vaccine for its control.

Dr. Jack E. Moulton, Professor, Faculty of Veterinary Science, University of Nairobi, commented as the discussions proceeded on the basis of the work that Cunningham presented. In Dr. Malmquist's absence, he reported upon growing the East Coast Fever parasite in tissue culture. (See attached article by Malmquist and colleagues from the Tropical Animal Health and Production publication, and the detailed mimeographed set of experiments embracing Cunningham's work, distributed for participants' review at the meeting.)

Subsequently the meeting was opened for a general discussion of the status of research on East Coast Fever.

Following the meeting Dr. G. M. Urquhart, Professor of Veterinary Parasitology, University of Glasgow, produced a concise review of Recent Progress in Vaccination Against East Coast Fever presented herein. Dr. Ordway Starnes, Director of EAAFRO, served as rappateur of the meeting. His report, approved by an ad hoc technical subcommittee including Drs. Moulton and Cunningham, is appended.

In the course of the meeting certain critical evaluations were expressed. Dr. E. H. Sadun, Chief, Department of Medical Zoology, Walter Reed Army Medical Center, recalled that 17 months ago there was some question whether or not the production of an ECF vaccine was possible; now it seems probable. However, even if sufficient knowledge existed for producing a vaccine by the chemotherapeutic approach, the tissue culture or the irradiation technique, carry-over of research from laboratory

to field tests would take three years at least. More work needs to be done on cross reactions, field strains of the parasite and other specifics. The empirical nature of immunology research on ECF, necessary and important as it is, needs a fundamental component that deals with the rationale of immunological reactions.

Dr. George Urquhart pointed out that inoculation of cattle in large numbers cheaply and satisfactorily could be five to ten years distant.

Dr. Moulton stressed the importance of more research on cellular immunity and on the investigation of drugs, among them antibiotics.

Dr. K. H. Wilde, Senior Research Fellow, Centre for Tropical Veterinary Medicine, Edinburgh, pointed out the lack of information on the most vulnerable stage in the life of the parasite, its passage from salivary glands of ticks to the lymph nodes of cattle. He, too, emphasized chemotherapy as a relatively neglected area of research and he thought opportunities were being missed in not paying more attention to the carcinogenetic activity this parasite produces in the lymphocytes of cattle. He mentioned the need for a better understanding of the erratic pattern of infectivity; why cattle are sometimes readily infected and other times not under an equal challenge. Dr. Wilde also raised the question of the profitability for a commercial drug house to produce vaccine for ECF once the scientific aspects of the problem had been solved, but believed the scientific principles involved warranted full effort in any case. The competitive position of a suitable vaccine was discussed in terms of present methods for control of East Coast Fever which depend upon dipping the cattle to kill the vector, the tick.

Dr. I. E. Muriithi, Director of Veterinary Services, Kenya, elaborated on costs and pointed out that a vaccine for ECF would have to compete with the cost for dipping each animal, which at present is at a level of about four U.S. cents per animal per week.

Dr. H. C. Goodman, Chief, Immunology, WHO, called for more research on the stage in the cycle of the parasite that produced immunity; he stressed the importance of further work in tissue culture and of studies on the nature of immunity. The possibility of a variety of field strains of the parasite that might have a different virulence worried him and he thought five to ten years a conservative estimate for arriving at a practical vaccine.

Dr. Cunningham stated that any one of the several methods employed today for the production of a vaccine would require at least three years' additional investigation before it would prove to be practical.

Dr. William Payne, Consultant for UNDP, announced that he had just received a telegram from Ralph Townley, UNDP in New York, indicating that funds available under the UNDP/SF project governing the research on ECF received an extension of availability from the UNDP governing council from 1972, when they are due to expire, until 1974.

Dr. McIntyre, calling upon Dr. Cunningham in particular but also upon the group as a whole, identified eleven broad lines of research which would help remove the impediments to success in arriving at a vaccine for ECF: 1) irradiation variables, 2) use of drugs and their application at proper dosages, 3) bulk production in vitro of T. parva infected cell lines for vaccines, 4) harvesting the infective stage of T. parva from tick salivary

glands and, associated with this method, grinding up ticks, 5) isolating the parasites from ticks and growing them in tissue culture, 6) field epidemiology - the role of related strains of parasites and related diseases, 7) studies on the acceptance of certain cell lines of parasites in cattle hosts, 8) studies on the mechanism of immunity, 9) preservation of live vaccine, 10) developing other antigens, for example, using dead instead of live parasite material, 11) studies on the virulence of different strains of parasites.

Four to five of these lines of research Dr. Cunningham and his colleagues are already pursuing with great success, but it was the consensus of the meeting that a broader approach to the problems of producing a vaccine would be even more productive.

Dr. Sadun then discussed his own work at Walter Reed Hospital on trypanosomiasis and the success that has been achieved in irradiation techniques to vaccinate animals to combat trypanosomiasis. His work dealt with four species of trypanosomes - brucei, gambiense, rhodesiense, and congolense. Dr. Sadun suggested that the work on East Coast Fever and trypanosomiasis had many points in common; a high-powered group of immunologists working on East Coast Fever could readily apply their skills and knowledge to work on trypanosomiasis and vice versa.

Dr. John McKelvey, The Rockefeller Foundation, introduced a proposition as follows: a) that it was imperative for the Cunningham research program to receive full support, b) that irrespective of whether this research would continue to be located in EAVRO or elsewhere a need does exist for an Animal Disease Research Center of the sort being proposed, c) that one half

or perhaps three fourths of the research toward vaccine production for ECF has been accomplished but to complete the final stages of this research will probably require five to ten years, d) that ECF research as placed in the proposed Center is still of highest priority as a short-term program, and e) that following the solution of ECF, trypanosomiasis, a long-term problem, should then receive highest priority.

Dr. Sadun corrected the statement about trypanosomiasis and indicated research on both diseases should progress simultaneously. As corrected the proposition stood with the consensus of the participants.

Dr. M. Abdussalam, Chief, Veterinary Public Health, WHO, suggested that cystercicosis ought not to be ignored. The participants seemed agreed on this point as well as on Dr. Sadun's above point.

All of the participants expressed their admiration of Dr. Cunningham and his colleagues for the enthusiasm and the skill with which they have worked and for the success of their investigations.

II

PRESENT STATE OF KNOWLEDGE OF EAST COAST FEVER

George Urquhart

Introduction

East Coast Fever, a tick-transmitted disease of cattle in East and Central Africa, is caused by the protozoan parasite Theileria parva. It is one of the major lethal diseases of cattle and has been given top priority in the United Nations assessment of the research needs of East Africa (East Africa Livestock Survey, 1966). In non-enzootic areas (i.e. areas where the tick vector Rhipicephalus appendiculatus occurs only sporadically) mortality may approach 100%; in enzootic areas upgrading of livestock is almost impossible since high-grade cattle cannot be introduced. There is no vaccine against the disease and no cure is available once the disease is established. At present control, in areas where this is feasible, depends on repeated dipping or spraying of livestock at frequent intervals, e.g. initially every three days.

Background to the Current Development of Vaccination Techniques

It is generally agreed that a practical form of immunisation would be the most significant single means of combatting the disease and an examination of the facts, as at present established, gives every indication that such a vaccine would indeed be successful. Thus recovered animals do not become carriers (i.e. support a low-grade blood infection which, although clinically non-apparent, leads to the infection of successive generations of ticks) but possess a sterile immunity which is

life-long. Also there is no evidence as yet that more than one immunological strain of T. parva exists and this if confirmed simplifies immensely the preparation of a vaccine.

Despite these encouraging portents no practical technique of immunisation is yet developed. To appreciate the scientific reasons why this has not been possible it is necessary to outline the essential features of the life-cycle of the disease, some of which have only been established in the last 2 years.

When cattle develop the clinical disease a large proportion of the red blood cells contain East Coast Fever parasites; the latter have a characteristic appearance and are generally referred to as 'gametocytes'.

Immature stages of the tick R. appendiculatus which ingest blood meals during this time become infected with the gametocytes. The tick drops off the infected animal on to the ground, develops to the next stage and subsequently, some weeks later, becomes attached to another bovine for a further blood meal. During the course of this meal large numbers of East Coast Fever organisms derived from the gametocytes develop in the salivary glands. These are structurally different and are called 'sporozoites'. The sporozoites are injected with the saliva into the new host and pass to the nearest lymph node (usually the parotid node since the ear is the preferential site of tick attachment) where they infect mononuclear blood cells called lymphocytes. The parasites, now recognisable as structures called schizonts multiply rapidly and soon reach all the lymph nodes and proliferate rapidly in all the available lymphocytes.

By this time the animal is clinically ill but some days before death a proportion of the schizonts become altered in appearance and give

rise to the gametocytes described earlier. These leave the lymphoid tissue and infect the red cells. The gametocytes do not multiply in the red cells their future development being dependent on being ingested by a suitable tick.

Early work in attempts to develop immunisation procedures have shown that the injection of the gametocytes does not confer immunity. The next most readily available sources of antigen, i.e. materials which possess the potential to stimulate immunity, are the schizonts found in massive numbers in the lymphoid tissues of terminal cases. Inoculation of these to stimulate immunity have been attempted on a number of occasions (see review by Barnett, 1968). The general result of these experiments was that while a variable proportion of animals were successfully immunised, others died of East Coast Fever induced by the immunising inoculation, while the remainder were unaffected by the inoculation but were completely susceptible to subsequent challenge with infected ticks. Probably there are two reasons why this technique was unsuccessful: first, the innate variation in susceptibility of individual cattle to any given dose of schizonts; secondly, the possibility that in a proportion of cattle the lymphocytes were 'rejected' and the parasites unable to develop in their new host.

Current Developments in Vaccine Development

Since this work was completed there have been four major advances which have improved immensely the possibility of immunisation.

In 1969 Jarrett, Crichton and Pirie published a paper dealing with the rate at which schizonts proliferate in infected cattle and the

relationship of this to the size of the infecting dose (i.e. the number of ticks), the onset of clinical signs and the development of immunity. They showed for example that the schizonts typically increased ten-fold every 3 days and that fever occurred when they numbered around 7×10^9 and in their discussion demonstrated how these and other facts might be utilised in immunological studies.

The second advance was the development by Cunningham and his colleagues (private communication at Rome, 1970) of two techniques for the isolation and storage at low temperatures of sporozoites collected from ticks. Until this point immunisation or challenge to cattle with sporozoites had necessitated the use of batches of ticks of varying and unquantifiable degrees of infectivity. As a result of their work it is now possible to store at low temperatures a large volume of material containing infective sporozoites of known potency. Their present work is being directed towards the possibility that the injection of a small number of sporozoites - smaller than the dose injected by one tick - would enable the animal to develop an acquired immunity before its lymphocyte population is seriously depleted and clinical signs and death supervene, i.e. although the schizont population may increase ten-fold every 3 days, the initial dose should be small enough to delay the appearance of clinical signs and allow the immune response to develop. This, according to Jarrett and his colleagues, might require at least 3 weeks.

Cunningham and his colleagues have indicated that variations in the susceptibility of individual cattle may make this objective difficult and that a larger number of sporozoites attenuated by a suitable dose of

cobalt irradiation might prove more efficacious. After irradiation it is possible that the rate of the replication of schizonts might be slowed or even halted at some point after inoculation. At present experiments are in progress on both of these lines but it is premature to assess their ultimate success.

The third significant advance has been recently reported by Malmquist, Nyindo and Brown (1970) who have successfully cultured *in vitro* (*i.e.* in glass vessels) the schizonts of *T. parva* in multiplying cells originally removed from the spleen and lymph nodes of cattle infected with the disease. This has been previously claimed by other workers but it is generally agreed that this is the first unequivocal demonstration of successful culture and *T. parva* infected cell lines have been maintained in their laboratory for 10 months.

Although in the past schizonts have been used for vaccination experiments they have had to be obtained in limited numbers from a variety of diseased animals, any one of whom might also be infected with other diseases transmissable by inoculation. The significance of Malmquist and his colleagues' work is that unlimited numbers of schizonts are now available from a single controllable source for evaluation in vaccination experiments. As with the projected sporozoite vaccine the problems are first, the determination of the right dose and secondly the possibility that some form of attenuation might be necessary. Nevertheless the availability of schizonts in this form is an immensely encouraging advance in these initial stages of vaccine development.

The final development has been the results of the most recent work on the use of tetracyclene drugs to facilitate immunisation. It

was shown by Neitz (1953) and by Barnett (1956) that chlortetracyclene, administered continuously for 30 days after infected ticks were placed on an animal, largely suppressed the overt disease and rendered cattle immune to subsequent challenge. This technique was of course not applicable as a large scale immunisation procedure. Subsequently in 1969 Jarrett, Pirie and Sharp showed that chlortetracyclene was effective even if discontinued as early as the eighth or sixteenth day. Since then Cunningham and his colleagues (private communication at Rome, 1970) have shown that infection introduced by inoculation of sporozoites which had been preserved at low temperatures combined with tetracyclene on two occasions (i.e. the day of inoculation and the subsequent day) induce a very high degree of resistance to challenge. Further developments in this field should be interesting and if inoculation of a given number of preserved sporozoites or perhaps cultured schizonts, combined with a single dose of antibiotic administered on the same occasions, gave an acceptable degree of immunity, a practical method of vaccination could be said to be available. The single reservation might perhaps be the cost of the antibiotic.

Conclusions

There is no doubt that the [four] advances described above, i.e. the development of techniques for quantitating the kinetics of parasite multiplication, the successes in the preservation of sporozoites and the culturing of schizonts, and the advances in chemoprophylaxis have brought forward rapidly the time when a successful method of practical vaccination will be available. The main problems which have still be to surmounted are:

a) As yet none of the present techniques prevent the development of tick-infective forms, i.e. gametocytes in the red cell. This might limit the use of such vaccines under a range of circumstances.

b) The experiments to date have necessarily utilised small groups of cattle. While the death of 1 in a group of 10 immunised bovines is a signal advance in an experiment in which all 10 controls have died, this does not necessarily imply acceptability in the field, where a 10% mortality due to vaccination would be viewed with considerable reservation (probably 1% would be acceptable).

c) The cost of such a vaccine and techniques for distribution are of particular importance in the areas where it would be used and are still to be developed.

d) Field testing of developing vaccines has still to be undertaken and there is little doubt that this will bring new problems.

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III

REPORT OF THE RAPPATEUR
(Dr. O. Starnes)

AS APPROVED BY THE AD HOC TECHNICAL SUBCOMMITTEE
(Drs. Cunningham, Moulton, and Starnes)

SUMMARY AND CONCLUSIONS

The present status of research and development work on East Coast fever under the UNDP/SF Project 300 entitled Immunological Research on Tick-borne Cattle Diseases and Tick Control underway in the EAVRO, the USDA cooperative research on East Coast fever at the same location and work underway in the Faculty of Veterinary Science, University of Nairobi, and related work is reviewed.

Progress is reported in the understanding of the epidemiology of the disease; techniques have been developed for the harvesting of the infective stage of the parasite from ticks; for the viable preservation of the parasite at low temperature; for the production of reproducible infection in cattle by inoculation and for the *in vitro* maintenance of *T. parva* infected cell lines. Using these new techniques, four experimental methods for vaccinating cattle against ECF have been investigated.

The results of field investigations suggest that immunological variants of *T. parva* exist, and that other Theilerial species may be involved in the East Coast fever syndrome.

While results are encouraging the underlying reasons are not well understood.

Priorities for future work include:

1. Irradiation variables.
2. Chemotherapy studies.

3. Bulk production *in vitro* of *T. parva* infected cell lines.
4. Harvesting the infective stage of *T. parva* from infected ticks.
5. The *in vitro* culture of tick tissues and developmental stages of *T. parva*.
6. Field epidemiology - the role of related strains and related diseases.
7. Histocompatability studies.
8. Mechanisms of immunity.
9. The viable preservation of all stages of *T. parva*, using freeze drying and cryopreservation techniques.
10. Antigenic analysis of *T. parva*.
11. Pathogenesis of ECF in cattle.
12. Development of *T. parva* in *R. appendiculatus*.

The effort required on these problems indicates the need for concerted, sustained effort over a period of at least ten years in which time it is considered that a practical solution to East Coast fever will have been found and that substantial progress will have been made in the immunology of trypanosomiasis.

Introductory Comments

The meeting was convened by McKelvey of the Rockefeller Foundation at 0950 on 8 March in the India Room, FAO Headquarters, Rome and Chaired by McIntyre of Glasgow University.

Participants were welcomed by White of FAO who conveyed greetings on behalf of FAO's Director General, Dr. Wells and of the Director of the Operations Division, FAO and expressed on their behalf keen interest in the subject under discussion.

McKelvey expressed his appreciation to participants, conveyed the apologies of Dr. Malmquist, USDA Scientist working on ECF at Muguga who was unable to be present, and recognised Dr. Malmquist's contribution of two papers of interest to participants. He expressed the regrets and apologies of Dr. Pino, Director of Agricultural Sciences, Rockefeller Foundation and conveyed Dr. Pino's wish that conclusions reached at this meeting convey priorities for future work and that the record be appropriately documented.

McKelvey then reviewed previous discussions on this subject and indicated that the objective of this conference is to confirm the present status of research and development work on ECF and to clearly outline what additional research is required to achieve effective control of the disease. He then introduced McIntyre and requested that he Chair the meeting.

McIntyre recognised the principals who would be presenting information on the current status of ECF work. He then introduced Cunningham, Manager of UNDP/SF Project on "Immunological Research on Tick-borne Cattle Diseases and Tick control" and invited Cunningham to present a status report on this project and related work.

Present Status - UNDP/SF Project and Discussion Thereon

Cunningham recognised contributions by Moulton and others of the Faculty of Veterinary Medicine, University of Nairobi, Dr. Malmquist of the USDA and others to the total effort including that of the UNDP team.

The following is taken from Cunningham's notes which he later reproduced and distributed to participants.

East Coast fever is a tick-borne disease of cattle in East and Central Africa caused by Theileria parva. While other theilerial parasites are commonly found in wild and domestic ungulates, T. parva affects only domestic cattle and buffalo. T. parva is transmitted by Rhipicephalus appendiculatus. This is a three host tick: eggs hatch to larvae - nymphs - adults. If either larvae or nymphs feed on an infected cow, the succeeding nymphs or adults will then be infective for cattle. The development of the parasite in the tick is not understood, but in the unfed infected tick, the parasite is found in the acinar cells of the salivary gland. At this stage the parasite is not infective for cattle. When the tick starts feeding the parasite undergoes a cycle of maturation and maximum numbers of mature infective particles (IPs) are excreted in the saliva between the 3rd and 5th days of attachment.

When a susceptible animal is fed on by infected ticks, an indefinite number of IPs are inoculated over an extended period. The macroschizonts of the parasite first appear in lymphoid blast cells from the local drainage lymph node (since the predilection site of attachment of the tick is the head and ears of cattle, the parotid lymph node is regarded as the local

drainage lymph node). The macroschizonts proliferate throughout the lymphoid organ and around the 14th day after tick attachment some of the macroschizonts switch to microschizonts - host cells disrupted, parasites are released and invade red blood cells. This process is continuous, all stages of the parasite continue to increase in numbers (although only the macroschizont is known to multiply) until the animal dies.

Jarrett found that by infecting cattle with increasing numbers of ticks, the prepatent period and time to death was reduced. He found the replication rate of the parasite however to be constant, whether 10 or 1000 ticks produced the infection.

Morbidity and mortality in susceptible animals exposed to infection approaches 100%. No curative drug is available and the only method of controlling the disease is the continuous application of acaricides at very close intervals, as little as three days to prevent attachment of infected ticks. Obviously an effective drug or a vaccine would be of value. The UNDP supported project is investigating the feasibility of vaccinating cattle against ECF. That this should be possible is supported by the observation that in enzootic areas, where calf mortality ascribed to ECF can be as high as 30%, surviving cattle have naturally acquired an immunity against ECF.

Past workers have used three main approaches in attempts to immunise cattle against ECF.

The first method - inoculation of macroschizonts harvested from reacting cattle into susceptible cattle. This method was used on a large scale in the Transkei 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 Whitworth (1930), reported 100% success with suspensions obtained from particular individual cattle. Jarrett, Crichton and Pirie (1969) estimated the numbers of parasites theoretically required to immunise cattle and confirmed their estimates in a small number of cattle. Without doubt cattle can be vaccinated by this method but it is unlikely that progress can be made until large numbers of parasites can be grown in vitro.

The second method - infection and treatment. Neitz (1953) found that when infected ticks were applied to 7 cattle which 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./kilo, starting 24 hours 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, Lambelin and Lateur (1959) in the Congo. Later, Barnett, Bailey and Brocklesby (1965) found that daily oral administration of Aureofac (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, Pirie and Sharp (1969), using Aureomycin orally at a dosage rate of 16 mg./kilogram found that 10 daily treatments markedly

suppressed the reaction, while 18 daily treatments gave complete suppression and subsequent immunity.

Other workers (Robson, Yeoman and Ross, 1961, Roe, 1962 and 1962a, Stobbs, 1964) treated cattle orally with Aureofac while exposed to natural infection. In order to produce immunity, however, continous daily treatment for as long as 3 months was required.

Again, this method can be used to immunise cattle against ECF, but is impractical because of expense. It was considered however that if reproducible ECF infections could be produced in cattle by inoculation, it might be possible to reduce the dose of drug required and the duration of its administration required to immunise.

The third approach - the "Quantum of infection hypothesis" put forward by Wilde et al. (1968). This is based on the observations of Lowe (1932) 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 (1950, 1950a and 1951a) 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 IPs inoculated and the severity of the ensuing reaction but decided that since one infected tick could kill a susceptible animal, the approach had no practicality. 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.

When UNDP started its project all this information was available; the Wellcome and the Glasgow groups afforded every assistance to the UNDP researchers, including unlimited access to unpublished work.

At the outset, we considered that to investigate any or all of the three approaches it was necessary to obtain suspensions of IPs which would regularly infect cattle by inoculation, which could be preserved alive at low temperature, and whose infectivity could be established by titration.

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 on IPs.

In addition we set out to grow the parasite in tissue culture - might be able to substitute test tubes for cows, and tissue culture schizonts might prove better than freshly collected material for immunisation.

The achievement of these objectives was described at the Bellagio Conference held in October 1969. One of the difficulties encountered, which was described in detail at the last conference, and which is relevant to today's presentation, is as follows: When ticks are induced to salivate into whole bovine blood (Purnell and Joyner, 1967, and Purnell et al., 1969) the suspension, containing parasites, will regularly infect the donor animal or its monozygotic twin, but does not infect unrelated homologous

animals. Similarly with the macroschizont from an infected animal. Small numbers of macroschizonts will infect a monozygotic twin, but not unrelated homologous animals. We consider it likely that once the parasite becomes associated with the host cell, the establishment of the parasite in another animal is dependent on the acceptance of the host cell. This phenomenon may explain the divergent results obtained in attempts to immunise cattle by inoculation of macroschizonts.

Following some discussion on problems associated with the production of infected ticks, diluents for the parasites harvested from ticks, the preservation of the parasite at low temperatures, McKelvey referred further to Dr. Pino's request that participants seek to quantify the status and outlook of ECF on the basis of:

1. Citations to pertinent literature.
2. Is a satisfactory solution to ECF (vaccine) now available?
3. How far are we from a solution?
4. What are the prospects?
5. What is required to achieve a satisfactory vaccine?
6. In relation to ECF what emphasis is indicated for trypanosomiasis, i.e. are we one-third, one-half or three-quarter towards a solution to ECF with the expectation that a team if mounted on ECF would continue on trypanosomiasis.

Cunningham then continued his presentation on four methods of vaccination.

1. The Quantum of Infection Hypothesis

He referred to experiment FAO 36 in Appendix 1. Following his presentation and some discussion on results obtained Cunningham concluded that these results support the quantum of infection hypothesis. However, it was considered that this approach is unlikely to be productive of a practical method of vaccination.

2. Effect of Irradiation

Cunningham referred to FAO 40, 45 and 46 Appendix 1 and following discussion observed that a vaccine using the right concentration of parasites exposed to the right radiation dose will immunise cattle.

Moulton enquired as to the effect of radiation on the parasite. Urquhart replied that in his experience with other organisms, radiation stopped the development of the organism.

Moulton considered that parasite replication is inhibited by irradiation. Sadun added that in his experience there is a relationship between numbers of parasites inoculated and the resulting immunity.

Goodman commented that the Cunningham data indicated that replication did continue, although inhibited.

3. Infection and Treatment

Cunningham referred to FAO experiments 48, 50 and 55, Appendix 1, commented on the data and concluded that with the use of Reverin at a dosage rate of 5 mg/kilo intramuscularly, considerable improvement over previous results had been obtained.

Moulton suggested further work with actinomyctin D as this antibiotic was highly effective in inhibiting lymphoblasts in tissue culture and he stated that at the Faculty of Veterinary Science they cured a case of ECF with the drug.

McKelvey posed a question as to the significance of use of drugs in relation to vaccine development. Moulton in response said the drug is to suppress the population of parasites and thus to facilitate employing the vaccine subsequently. Goodman agreed that the use of the drug was complementary.

At this time copies of a reprint entitled "East Coast fever: Cultivation in vitro of Bovine Spleen Cell Lines infected and transformed by T. parva" by Malmquist and others were distributed. Also a multilithoed paper entitled "United States Department of Agriculture - Cooperative Research on East Coast fever" by Malmquist was distributed. Moulton summarised these in relation to previous discussion.

Cunningham considered that there is every reason to believe that the parasite can be grown in large quantities. He then drew the attention of participants to results of tissue culture experiments FAO TC 4 - TC 14.

Goodman enquired as to whether the difficulty in infecting unrelated homologous cattle is a principal issue in relation to the tissue culture derived parasite.

Cunningham said that there is not enough evidence to tell in this particular case.

Goodman then enquired of Cunningham as to what variables now need be resolved to establish the cell line approach to vaccine production. Cunningham replied that the results of each experiment determines the next step to be taken.

Urquhart inquired about differences in results reported in experiments TC 4 and TC 7. Cunningham pointed out that the 15th passage of the C 2 cell line was used in TC 4, while the 58th passage was used in TC 7.

Also, concentration of foetal calf serum (FCS) was reduced in TC 7. He then drew attention to the longevity of immunity of cattle recovered from experimentally induced ECF (Appendix 1).

With consideration to issues yet to be resolved Cunningham noted the possibility of the occurrence of multiple strains of T. parva citing experiments conducted at Aitong, Kenya, where two types of parasites were found, one being similar to T. parva (Muguga), and the second being quite different. Malmquist found that these strains also performed differently in tissue culture. Goodman asked whether it is conceivable that another vector is involved. Cunningham replied that attempts to transmit these strains through R. appendiculatus and through R. evertsi had failed.

Abdussalam enquired as to whether it is known whether there are different strains of T. parva at Muguga. Although not in direct response to the question Cunningham cited other cases in which apparently different strains were encountered. Muriithi added that he considers that there are two types of ECF even in the absence of buffalo.

Following lunch the Chairman charged participants to give clear definition to problems and to the need for further work while continuing the technical discussion.

McKelvey briefly reviewed previous discussions concerning constraints affecting the further development of ECF work within existing institutions. These constraints include limitations on freedom of action, of ability of the institutions to attract and retain top flight scientists, and to respond quickly to new research opportunities. These constraints on existing institutions have led to general agreement on the need for another institution which would be free of such constraints. Envisaged

is a new institution capitalised at perhaps 3.5 million with recurrent costs of approximately one million dollars per annum, it is considered that no one donor agency would seem prepared to support a development of an institute of this magnitude alone. Therefore consideration is being given to possible interest on the part of a consortium of donor agencies who might undertake the project. This concept was well received at the Bellagio meeting of representatives of donor agencies held in April 1970. Following the Bellagio meeting consideration was given to an appropriate base for such an institution. It was deemed desirable that the institution be established near Nairobi in bilateral arrangements with the Government of Kenya. Prior to arriving at this conclusion careful consideration was given to other alternatives including basing such a program in EAVRO at Muguga, and also in the Faculty of Veterinary Medicine, University of Nairobi. In the course of these deliberations two basic questions arose: first, since UNDP has a program at Muguga why not integrate the proposed work into EAVRO, and second, since so much progress has been made on ECF what justification is there to predicate an institute on ECF and related problems?

In response to the first question, the consensus was that a bilateral arrangement with the Kenya Government seemed to be the best approach as conditions obtained in the early planning of the proposed institute. Additionally, it was noted that contact has been established with the East African Community; the Community has been invited to offer the same concesssions promised through a bilateral relationship with the Government of Kenya. Should the EAC be in a position to offer comparable concessions there would be then a basis of discussing the establishment of the proposed institute in the Community.

Following the meeting of the consortium referred to earlier a conference was held with representatives of the French, ODA of the United Kingdom, FAO and USAID. At this meeting a proposal for an international effort on livestock production, and its relationship to this concept of an animal disease institute was discussed. It was considered that there should be a close relationship between the two proposed institutes but that action to mount the Animal Disease Research Institute should not be held in abeyance pending the outcome of further discussions on the parent Livestock Production Institute which is suggested for siting in West Africa.

EAVRO has and will continue to have its own research program on ECF; the development of an Animal Disease Institute in East Africa should not adversely affect this because of the specialised nature of work proposed for the Institute, concluded McKelvey.

Sadun expressed interest in the type of personnel who would be involved in the proposed Animal Disease Research Institute program, and specifically in the possibility of seconding people currently involved in this area of work elsewhere to the Institute's program including personnel from his program.

Abdussalam requested clarification on the bilateral aspects of the proposed institute. McKelvey replied that the institute's international role might be achieved through the involvement of nationals of various countries in the institute's program both as staff and as trainees. With reference to financial support he considered it would be desirable to have as many as four donor agencies underwriting the capital and recurrent costs of the proposed institute over a period of 10 to 14 years.

Griffiths said he was aware of discussions with EAVRO relative to the projected ECF program, but asked whether there had been discussions with EATRO in relation to the site of the proposed institute? McKelvey noted that discussions had been held with Director Rashid of EAVRO and with other Community officials but not specifically with those at EATRO.

Griffiths enquired as to whether it is considered that the establishment of the proposed Animal Disease Institute would mean greater competition for funds. McKelvey cited the favourable effect of the establishment of the International Rice Research Institute on the expansion and strengthening of national rice research institutions. Sadun commended the "built-in redundancy features" of the proposed institute and cautioned against placing complete reliance for such important research matters as ECF and trypanosomiasis on any existing single effort.

Muriithi referred to the lack of understanding behind some of the results reported and noted that it is vital that the reasons behind the experimental results obtained be understood, and that to do so will require concerted sustained effort.

Wilde referred further to the competitive role of the proposed institute and concluded that there will be competition between the proposed and existing institutions for resources and staff, and that the proposed institute would, in his view, hazard the present activities in the present problem areas of ongoing institutes.

McIntyre, Sadun and McKelvey cited additional cases in which the development of an additional institute and program has resulted in the strengthening of the existing institutions.

Moulton enquired as to whether the proposed institute would have a responsibility to train Africans at the postgraduate level. McKelvey replied in the affirmative and indicated that this would be in conjunction with existing educational institutions. Goodman added that the training element would be an important way of strengthening other existing institutions and that the institute should otherwise demonstrate leadership through the offering of conferences, symposia and short-term specialised training programs, all of which would be mechanisms for strengthening other institutions. He suggested also consideration of the possibility of building into the proposed institute a mechanism for grant support of work in other involved institutions.

Ansari observed that participants had discussed two problems, firstly, the technical aspects of ECF and had concluded that certain research and training should be in one institution and, secondly, the possibility of competition between the proposed and existing institutions for staff and other resources. He concluded that a decision should be made as to whether the proposed institute would be concerned primarily with fundamental or problem oriented research which decision will influence the type of institute required.

White enquired into the sort of continuity projected for the proposed institute. He reminded participants that the present UNDP project is only projected through December 1972. He added that it may be very difficult to win a further extension of the project beyond December 1972, unless it is possible to convey to UNDP the likelihood that the program will be continued on a regional or global basis. Responding the Chairman indicated that he considered that the strongest possible justification should be advanced for continuity of the ongoing UNDP/SF project.

The Chairman again requested participants to give further definition to problems and invited certain participants by name to express their views.

Sadun - noted that in the interim since the last meeting much more basic information and data has been generated in support of the general position reported at the earlier meeting. Of the results presented on tissue culture, on chemotherapy and on irradiation it is still not possible to say which approach promises the solution. What really is happening behind the observed results? The quantitative aspects of irradiation need to be worked out and much time will elapse before that information reported here can be employed in the field.

Relapse variance needs more attention. Would a vaccine if available be effective against variants? This aspect needs quantification and qualification. Vaccine development work should not be delayed pending answers but these questions must be answered.

Urquhart - the problem of quantity of antigen need be clarified noting that too frequently the animal dies before developing immunity. What are the dosage safety margins in relation to differential response by animals? He observed that the tissue culture work looks much more promising than at the time of the earlier discussions. He suggested the desirability of going into field trials as a basis for identifying field treatment problems. How practical are the presently available experimental vaccines for field control? What level of mortality associated with vaccination is acceptable? What might be the reasonable cost of producing a vaccine?

Wilde commented that the FAO/UNDP team work is excellent and that they have made more progress in the period than he had expected.

The most important area in which knowledge is required is concerning the period from the beginning of development of the parasite in the tick to the onset of response observed in the lymph nodes. It is in this period that the parasite is vulnerable in the host. It is in this period also that the parasite is vulnerable to chemotherapy. The knowledge that this is the vulnerable period accrued from the UNDP project effort.

Wilde noted that Cunningham has postulated that individual animals may be able to program the disease differently. He concluded by suggesting that the research at Muguga justifies expediting this work in every possible manner, and that he is optimistic as to its outcome.

Goodman - noted that in the results reported solid immunity to ECF is shown. The in vitro culture work is deserving of considerably more emphasis. Tissue culture results open other areas requiring additional work, including: whether or not viruses are present in the lymphoblasts, what do the antibiotics do to the parasite, what is the mechanism of immunity, how much of the immune reactivity is destroyed when the animal becomes immune?

The Aitong field study points toward discovery of different strains of T. parva; other such problems will arise when the work is expanded in the field.

Muriithi - every effort should be made to identify the main immunogenic strains with the objective of developing a broad spectrum vaccine. The solution may be ten years away, but we cannot affort to lose any time.

In response to the earlier question as to mortality to be tolerated following vaccination Muriithi noted that mortality following vaccination of 1 to 2% would be considered tolerable, but the goal is, of course, zero mortality.

Dr. Muriithi - noted that control is now achieved by dipping cattle at a cost of about 25 to 30 E.A. cents per animal per week. This is a lower limit to aim for. Foot and mouth disease vaccine costs are 4 to 5 E.A. shillings per year - a reasonable guide. Muriithi concluded by suggesting that investigators ought to examine certain practices the nomadic people use to treat cattle with local remedies.

Abdussalam - suggested attention to the lag period between laboratory work and field use.

Griffiths - noted that there are now three sub-projects associated with the UNDP/SF project at Muguga, these being the Kenya project which is now active and two projects, one in Tanzania and the other in Uganda, which are to be activated during this year. Each of these projects offer outlets for findings accruing to the Muguga effort.

Moulton added the following to the list of problems for study:

1. A search for a toxin which might be associated with the disease;
2. Investigations on the sudden death of infected lymphoblasts;
3. Humoral versus cellular immunity;
4. Identification of other ECF strains;
5. How the parasite converts the host cell to a neoplastic-like cell;
6. Chemotherapeutics;
7. White blood cell rejection phenomenon.

The Chair then invited Cunningham to respond to the list of problems presented. Cunningham expressed his appreciation to the participants for their discussion of the diversity of problems and issues and noted that these would be taken into consideration in the further development of the UNDP/SF project. He noted that the field program in Kenya is underway, and that immune and susceptible cattle are being exposed to natural challenge at Kiboko. This field activity should lead to the identification of different strains of the parasite if these exist. As an illustration of the possible complications which might arise, he cited a case in which in 1966 a buffalo was captured in the Serengeti and found to be infected with a Theilerial piroplasm. No previous contact by the buffalo with cattle was known. Nymphal ticks were fed on the buffalo. These ticks in becoming adults produced fatal Theilerial infection in cattle, but the disease could not then be transmitted through the tick, R. appendiculatus. Field outbreaks of similar buffalo derived Theileria in cattle have been recorded in East, Central and South Africa. These are likely to complicate any vaccination programme.

The Chairman then expressed appreciation to Cunningham, Moulton and others for making available to participants the wealth of information disclosed at the meeting, much of it pre-publication material. He summarised the priority problems requiring attention as follows, noting that these are not necessarily in order of priority.

1. Irradiation variables
2. Drugs and their dosages

Cunningham noted that with each of the four methods of immunisation described at least a further year's work is required, and he expressed the interest of Pfizer in supporting further work of this nature. He noted that he has only tested one drug, using one dosage level and that there is a need to screen more drugs alone and in combinations and to use multiple treatments.

3. Tissue culture and the production of cells for vaccine

Cunningham noted that variables yet to be examined include age susceptibility of cattle, multiple inoculations and use of killed vaccine material from tissue culture. Quantity production of antigen is not a serious constraint, although quantities of foetal calf serum (FCS) required might be a constraint. A cheaper alternative to FCS would be advantageous.

4. Harvesting of tick saliva

   Collection from ticks, harvesting of parasites for in vitro culture need to be improved.

5. The growing of tick tissues and tick parasites

6. Field epidemiology

   The role of related strains and related diseases such as have been encountered at Aitong.

7. Acceptance of cell lines

8. Mechanism of immunity

9. The preservation of live vaccine

   Concentration and distribution, and methods of field handling.

10. Development of other antigens

11. Pathogenicity

    Strain virulence and pathogenesis in the bovine host.

The Chairman noted that a number of these are receiving the attention of current workers and asked for the identification of those problems which are either not now receiving sufficient attention or those on which efforts should be greatly increased.

Following further discussion it was agreed that each of the above required a substantial increase in effort. McKelvey expressed appreciation for the contributions made by participants noting that we may be perhaps half-way or even three-quarters towards the goal of a useful vaccine for ECF and toward setting the stage for the development of a vaccine for trypanosomiasis.

Sadun agreed but insisted that work on trypanosomiasis should not be delayed pending solution to the above problems of ECF.

Abdussalam suggested an expansion of the proposed institute's program to consider other problems in immunology.

Muriithi suggested restricting the proposed institute's efforts to two problems, ECF and trypanosomiasis.

White reconfirmed his previous statements to the effect that the UNDP/SF project is affected by the proposal for a separate institute, that if there is assurance of an organisation to take up, expand and carry on the present work, that continued support for the current UNDP/SF project might be more assured in the interim.

McKelvey added that he would be hopeful that these discussions might culminate in an institute with program on the ground during the later part of 1971.

Related Work - Trypanosomiasis

The Chairman invited Dr. Sadun to summarise the status of trypanosomiasis research. Sadun noted that the priorities for trypanosomiasis work are identical to those agreed for ECF. He then described briefly the current state of work in Kenya and in Washington on trypanosomiasis.

Ansari distributed to participants a paper entitled "Proposed UNDP/SF Regional Project for the establishment of an African Trypanosomiasis Research Center" which would expand and consolidate the work and facilities of EATRO at Tororo, Uganda.

An announcement was made later by Dr. Payne to the effect that notification of UNDP funding of the project presented by Ansari has been received. It was noted also that the International Institute for Insect Physiology and Ecology, University of Nairobi is to receive UNDP support for its work which includes a project on trypanosomiasis.

The Chairman then conveyed his appreciation to the Convenor, the participants and to our FAO host and adjourned the meeting at 1745 on 8 March 1971.

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of

EAST COAST FEVER REVIEW MEETING

8 March 1971 - Rome, Italy

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