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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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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 chemotherapy 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 Löhr, 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 schizogenous 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 stabilate 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 antigenic makeups 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 stabilate 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 lyophylization 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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