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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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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 (Tsur-Tchernomoretz 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 subcultivation 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 (199 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 1971a; Mutuzkina 1975). Except for Hulliger, the remaining investigators did not use a feeder layer for initiation of culture. However, none of them used a pure suspension of infected cells, and in most cases the infected cells represented only a small portion of the total number of cells in the primary cultures. It is very likely that the remaining noninfected cells played some role in "conditioning" the medium during the first days of cultivation, even if these noninfected cells did not multiply in vitro.

T. annulata-infected lymphoid cells were cultivated in monolayer (Tsur and Adler 1962, 1965; Zablotsky 1967) and also in suspension (Hooshmand-Rad and Hashemi-Fesharki 1968; 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

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 periferal 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 7×10^9, a rise in body temperature is noted; when the number reaches 2.4×10^{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 5×10^9 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 Poluboyarova 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 theileriosis (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 Uzhekistani 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 administrated chlortetracycline 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 5×10^6 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 acaracides 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 acaricidal 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 *Hyalomma*, 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 stabilate of *Theileria* from ticks would be inoculated.

Considerable experience has been accumulated in preparation of stabilate 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.

References

Abramov, J. V., and Zablotsky, V. T. 1972. Veterinariya (Moscow), 49(10), 91-92.

Adler, S., and Ellenbogen, V. 1934. Veterinary Record (England), 14, 91-93.
1935. Archives de l'Institut Pasteur d'Algerie, 13, 451-471.

Agoev, A. A. 1958. Veterinariya (Moscow), 35(12), 42-44.

Askarov, E. M. 1975. Veterinariya (Moscow), no. 6, 74-78.

Bhattacharyulu, Y., Chandhri, R., and Gill, B. 1975a. Parasitology (England), 71, 1-7.
1975b. Annales de Parasitologie Humaine et Comparée, 50, 397-408.

Brocklesby, D. W. 1970. Proceedings of the Second International Congress on Parasitology, 56, 35-36.

Brocklesby, D. W., and Hawking, F. 1958. Transactions of the Royal Society of Tropical Medicine and Hygiene, 52, 414-420.

Cordassis, Y. 1956. Bulletin de l'Academie Veterinaire de France, 29, 66-72.

Daubney, R., and Sami Said, M. 1951. Parasitology (England), 41, 249-260.

Delpy, L. 1937. Archives de l'Institut Pasteur d'Algerie, 15, 225-264.
1949. Bulletin de la Societé de Pathologie Exotique, 42, 285-294.

Ende Van den M., and Edlinger, E. 1971a. Archives de l'Institut Pasteur de Tunis, 45-54.
1971b. Academie des Sciences de Paris, 272, 87-89.

Gilbert, S. J. 1925. Journal of Comparative Pathology (England), 38, 91-93.

Gill, B., Bhattacharyulu, Y., and Kaur, D. 1976. Research in Veterinary Science (England), 21, 146-149.

Gill, B., Kaur, D., and Bhattacharyulu, Y., 1974. Bull. Off. Int. Epiz., 81, 805-811.

Hadani, A. 1955. La Theileriose bovine en

Israël. (A thesis.) Ecole Vétérinaire de Toulouse, Toulouse, France.

Hadani, A., Pipano, E., and Dinur, Y. 1969. Journal of Protozoology (U.S.), 16, suppl. 37.

Hashemi-Fesharki, R., and Shad-Del, F. 1973a. Veterinary Record (England), 92, 150-151.

1973b. American Journal of Veterinary Research (U.S.), 34, 1465-1467.

Hawking, F. 1958. British Journal of Pharmacology, 13, 458-460.

Hooshmand-Rad, P. 1975. Tropical Animal Health and Production, 7, 23-28.

1976. International Conference on Tick-Borne Diseases and their Vectors, Centre for Tropical Veterinary Medicine, University of Edinburgh, 27 Sep-1 Oct 1976.

Hooshmand-Rad, P., and Hashemi-Fesharki, K. 1968. Archives de l'Institut Razi (Iran), 20, 85-90.

Hulliger, L. 1965. Journal of Protozoology (U.S.), 12, 649-655.

Hulliger, L., Wilde, J. K. H., Brown, J. D., and Turner, L. 1964. Nature (London), 203, 728-730.

Jarret, W., Crighton, G., and Pirie, H. 1969. Experimental Parasitology (U.S.), 24, 9-25.

Konyukhov, M. P. 1969. *Progress in protozoology*. Proceedings of the Third International Congress on Protozoology, 268.

Konyukhov, M. P., and Poluboyarova, G. V. 1967. Veterinaryia (Moscow), no. 10, 56-59.

Mahmoud, A., Haiba, M., Zafer, S., and Awed, F. 1956. Zeitschrift fuer Tropenmedizin und Parasitologie (West Germany), 7, 282.

Malmquist, W., Nyindo, M., and Brown, D. 1970. Tropical Animal Health and Production (Scotland), 2, 139-145.

Markov, A., Gildenblat, A., Kurchakov, V., and Petunin, F. 1948. Veterinariya (Moscow), 25, 13-18.

Markov, A., Stepanova, N., Laptev, V., Dubovy, S., and Storozher, U. 1966. Proceedings of the First International Congress on Parasitology, Roma, 1964, 1, 275-276.

Mazlum, Z. 1969. Parasitology (England), 59, 597-600.

Mirzabekov, D., Agaev, A., Mobsum-Zade, A., and Godjaev, A. 1969. Veterinariya (Moscow), 45(3), 46-47.

Moulton, J., Krauss, H., and Malmquist, W. 1971. American Journal of Veterinary Research (U.S.), 32, 1365-1370.

Mutuzkina, Z. 1975. Veterinariya (Moscow), no. 4, 56-57.

Neitz, W. 1951. South African Biological Society Pamphlet N, 15, 50-51.

1956. Onderstepoort Journal of Veterinary Research (S. Afr.), 27, 115-163.

Pipano, E. 1964. Refuah Veterinarith (Israel), 21, 247-255.

1969. Journal of Protozoology (U.S.), 16, Suppl. 37.

1970. Journal of Protozoology (U.S.), 17, Suppl. 31.

1972. Journal of Protozoology (U.S.), 19, Suppl. 54-55.

Pipano, E., and Cahana, M. 1968. Journal of Protozoology (U.S.), 15, Suppl. 45.

1969. Journal of Parasitology (U.S.), 55, 765.

Pipano, E., Cahana, M., Feller, B., Shabat, Y., and David, E. 1969. Refuah Veterinarith (Israel), 26, 145-148.

Pipano, E., Goldman, M., Samish M., and Friedhoff, K. T. 1976. Veterinary Parasitology. (Submitted for publication.)

Pipano, E., and Hadani, A. 1974. Proceedings of the Third International Congress on Parasitology, Munchen, 1974, 1, 140-141.

Pipano, E., and Israel, V. 1971. Journal of Protozoology (U.S.), 18, Suppl. 37.

Pipano, E., Kloppfer, U., and Cohen, R. 1973.

Research in Veterinary Science (England), 15, 388-389.

Pipano, E., and Tsur, I. 1966. Refuah Veterinarith (Israel), 23, 186-194.

Pipano, E., Weisman, Y., and Benado, A. 1974. Refuah Veterinarith (Israel), 31, 59-63.

Rafyi, A., Maghami, G., and Houshmand-Rad, P. 1965. Bull. Off. Int. Epiz., 64, 431-446.
1967. Bull. Off. Int. Epiz., 68, 749-755.

Rasulov, I. 1963. Veterinariya (Moscow), 40(6), 52-53.

Samish, M., and Pipano, E. 1976. International Conference on Tick-Borne Diseases and Their Vectors, Center for Tropical Veterinary Medicine, University of Edinburgh, 27 Sep-1 Oct 1976.

Schein, E. 1975. Zeitschrift fuer Parasitenkunde, 47, 165-167.

Schein, E., Buscher, G., and Friedhoff, K. 1975. Zeitschrift fuer Parasitenkunde, 48, 123-136.

Schindler, R., and Wokatch, K. 1965. Zeitschrift fuer Tropenmedizin und Parasitologie (West Germany), 16, 17-23.

Sergent, E., Donatien, A., Parrot, L., and Lestoguard, F. 1931. Archives de l'Institut Pasteur d'Algerie, 9, 527-595.
1932. Comptes Rendu de l'Academie des Sciences de Paris, 195, 1054-1056.
1945. Etudes sur les Piroplasmoses Bovines. Institut Pasteur d'Algérie, Alger, 1945.

Stepanova, N. I. 1968. Veterinariya (Moscow), no. 1, 55-56.
1973. Immunitet sel'skokhozyaistvennykn zhivotnykh. 348-350.

Stepanova, N. I., Gorbatov, V. A., and Petrovskii, V. V. 1969. Veterinariya (Moscow), no. 6, 45-47.

Tsur-Tchernomoretz, I. 1945. Nature (London), 156, 391.
1947. Refuah Veterinarith (Israel), 4, 86.

Tsur, I. 1949. Refuah Veterinarith (Israel), 5, 69.

Tsur, I., and Adler, Sh. 1962. Refuah Veterinarith (Israel), 19, 224-225.
1965. Refuah Veterinarith (Israel), 22, 60-62.

Tsur, I., Adler, Sh., Pipano, E., and Senft, Z. 1964. Proceedings of the First International Congress on Parasitology, Rome, 1, 266-267.

Tsur, I., and Pipano, E. 1962. Refuah Veterinarith (Israel), 19, 110.
1963. Journal of Protozoology (U.S.), 10, Suppl. 35.

Tutushin, M. I. 1966. Veterinariya (Moscow), 43(6), 46-48.
1967. Veterinariya (Moscow), no. 3, 60.
1969. Veterinariya (Moscow), no. 6, 47-48.

Zablotsky, V. T. 1967. Veterinariya (Moscow), no. 9, 66-69.