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Bovine Theileriosis : Progress in Immunisation Methods

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Abstract

There are two economically important *Theileria* parasites of cattle, *T. annulata* and *T. parva*, which cause tropical theileriosis and East Coast fever (ECF), respectively. Over 200 million cattle are at risk to the diseases caused by these parasites. At present two methods of immunisation with live parasites are used in the control of these diseases and depend on establishing a limited infection in the animal.

The tissue culture vaccine for *T. annulata* utilises schizont-infected lymphoblastoid cell-lines attenuated through passage *in vitro*. The infection and treatment method, used mainly for *T. parva*, involves the simultaneous inoculation of a lethal dose of sporozoites and treatment with a long-acting oxytetracycline. Both methods of immunisation elicit a protective immunity against tropical theileriosis, however, due to differences in the biology of *T. parva* the only practical and reliable way to immunise against ECF is by the infection and treatment method. Problems associated with the development and application of both immunisation methods are discussed.

In view of the inherent difficulties in using live vaccines, current research is focused on developing recombinant subunit vaccines. This approach is dependent on cloning parasite genes which encode antigens that elicit a protective immunity. *Theileria* antigens which evoke sporozoite neutralising antibody in an *in vitro* assay have been identified. However, the major protective immunity is elicited by cell-mediated immune responses which appear to be directed against schizont-infected cells. Antigens which induce these responses remain to be identified.

It is likely that to achieve effective protection both the humoral and cellular immune responses to *Theileria* antigens will have to be evoked. Therefore, it would be necessary to use a combination of parasite antigens for a subunit vaccine.

Introduction

Theileria are tick-borne protozoan parasites infecting domestic ruminants and wild Bovidae. A majority of the wild Bovidae are asymptomatic carriers of *Theileria*. Cattle, sheep and goats are the most commonly affected ruminants and the diseases that occur in these animals are collectively known as theileriosis. The parasites are widely distributed in tropical and subtropical regions of the world.

Theileria parva and *T. annulata* both infect cattle and are the most important pathogenic *Theileria* species of domestic ruminants. *Bos taurus* cattle, their crosses and improved *Bos indicus*, raised in non-enzootic areas of theileriosis are very susceptible and suffer major losses due to the disease. Consequently, bovine theileriosis is a major constraint to the improvement of the livestock industry in large areas of Africa, the Middle East, India, Southern USSR and China.

Bovine theileriosis and other tick-borne diseases are controlled mainly by the application of acaricides which in many instances has to be performed as frequently as three times a week. The method is expensive and not always reliable.

Recently, two antitheilerial drugs, parvaquone (Clexon, Wellcome, U.K.) and halofuginone lactate (Terit, Hoechst, W. Germany) have been developed, which have effective chemotherapeutic activity against the pathogenic stages of the parasite in the mammalian host (Dolan, 1981; Schein *et al.*, 1979). However, for successful treatment of bovine theileriosis, these drugs need to be used early in the infection. Furthermore, the drugs are expensive and their availability is often erratic.

Alternative approaches to the control of theileriosis are urgently needed. Large scale immunisation against *T. annulata* has been successfully carried out in Israel (Pipano, 1981), Iran (Hashemi-Fesharki, 1988) and Russia (Stepanova *et al.*, 1985) using attenuated parasites grown in tissue-culture. A

method of immunisation called the infection and treatment method was developed in the early 1970's and tested against *T. annulata* and *T. parva* with successful results. This review discusses the currently available methods of immunisation against bovine theileriosis, the problems associated with the use of each of these methods and the approaches towards development of improved vaccines.

The literature on theileriosis has been extensively reviewed (Purnell, 1977; Pipano, 1977; Irvin and Morrison, 1987; Dolan, 1988). This paper will focus on *T. parva* and *T. annulata*, the two most pathogenic*Theileria* spp of cattle.

Bovine *Theileria* spp.

Theileria parva is the causative agent of East Coast fever (ECF). The main vector is the three-host tick, *Rhipicephalus appendiculatus*, although several other Rhipicephalid species are known to transmit the parasite experimentally. The transmission is exclusively transstadial. The parasite is present in parts of Kenya, Uganda, Tanzania (including Zanzibar and Pemba), Rwanda, Burundi, Zaire, Southern Sudan, Malawi, Zambia, Zimbabwe, Mozambique, Angola and South Africa (Dolan, 1988). It has been estimated that in Africa 25 million cattle are at risk and nearly half a million die every year (Miller *et al.*, 1977). As *R. appendiculatus* is the main vector capable of maintaining *T. parva* infection under natural conditions, the parasite is not found in areas where the tick is absent. However, the distribution of the tick is wider than the parasite and the potential for its spread to new areas poses a serious threat (Dolan, 1988).

According to the present classification, *T. parva* is divided into three subspecies, *T.p. parva*, *T. p. lawrencei* and *T.p. bovis*. This trinomial system of nomenclature is based on certain epidemiological and behavioural characteristics of the parasites and is used for convenience rather than zoological correctness (Uilenberg, 1981).

Theileria annulata is the cause of Mediterranean Coast fever or tropical theileriosis. The parasite is transmitted by two - or three-host ticks of the genus *Hyalomma*, principally *H. anatolicum anatolicum, H. asiaticum asiaticum, H. detritum, H. excavatum and H. dromedarii*. The transmission is mainly transstadial. The distribution of the parasite is wider than *T. parva*, covering tropical and subtropical zones from Portugal and Spain through most of the Mediterranean coast of Europe and North Africa extending southward to Northern Sudan. Eastwards, it affects south eastern Europe, the Near and Middle East, India, China, Southern Russia and Siberia, and the Far East. Approximately 200 million cattle are at risk. Africa is the only continent where both *T. parva* and *T. annulata* occur. West Africa has neither of these pathogenic bovine theilerias while Sudan has both pathogenic species (Morzaria, 1981).

Life cycle

The life-cycles of *T. parva* and *T. annulata* are essentially similar. Development takes place in both the vertebrate and invertebrate hosts (Mehlhorn and Schein, 1984). The sporozoites are the infective stages of the organisms which are inoculated by nymphs or adult ticks into cattle where they rapidly attach to and enter lymphocytes. *Theileria annulata* also infects monocytes. Within the lymphocytes, sporozoites are located near the Golgi apparatus where they undergo differentiation to form trophozoites and then multinucleate schizonts. The development of the schizonts induces the host cells to transform and proliferate and the parasites divide synchronously with the host cell. This mode of replication results in clonal expansion of parasitised cells. Between 3-5 days after infection of lymphocytes, some schizonts undergo a process of differentiation termed mergony, resulting in the production of merozoites which are released through rupture of the host cells. The merozoites rapidly enter erythrocytes to develop into piroplasms. The piroplasms may also undergo merogony to produce more merozoites which can infect other erythrocytes. In *T. parva* infections, merogony in erythrocytes is limited (Fawcett *et al.*, 1987) while in *T. annulata* it is an important feature of parasite development causing high piroplasm parasitaemia and anaemia.

Ticks become infected when the larvae or nymphs feed and engorge on an animal containing infected erythrocytes. Within the ticks, the parasites undergo a complicated development cycle with the formation of gametes, zygotes and later motile kinetes which invade E cells of type III acini of the salivary glands. Here they undergo further maturation through a process of sporogony to form large numbers of sporozoites.

Disease

Clinical aspects of ECF and tropical theileriosis have been discussed by several authors (Irvin and Mwamachi, 1983; Jura and Losos, 1980; Hooshmand-Rad, 1976; Gautam and Dhar, 1980). In susceptible exotic (*Bos taurus*) cattle the disease is usually acute and is characterised by pyrexia, anorexia, lachrymation, a drop in milk production and rapid weight loss. Throughout the clinical disease, one or all of the superficial lymph-nodes may be enlarged. As the disease progresses, ocular and nasal discharge may become pronounced and the animals become dyspnoeic. Diarrhoea is common. Terminally, animals become recumbent, cachectic and hypothermic. In subacute to chronic cases corneal opacity in one or both eyes may develop. Nervous signs associated with chronic theileriosis have been reported although the predisposing factors have not been fully elucidated.

In ECF, merogony in erythrocytes is limited and anaemia is uncommon. However, in *T. annulata* infections repeated erythrocytic merogony produces high piroplasm parasitaemia and causes haemolytic anaemia, jaundice, haemoglobinuria, bilirubinaemia and bilirubinuria.

The incubation period, severity and course of ECF and tropical theileriosis depend on several factors including host susceptibility, the dose of sporozoite challenge, and the type and spectrum of strains inoculated. The clinical picture in the field may be further confused by multiple infections with other *Theileria* spp. (e.g. *T. mutans, T. taurotragi*) and other tick-borne parasites (e.g. *Babesia* spp., *Ehrlichia* spp., *Cowdria ruminantium* and *Anaplasma* spp.)

Mortality in susceptible cattle maintained tick free and then introduced into areas endemic for ECF or tropical theileriosis may approach 80%. However, in *Bos indicus* cattle raised in endemic areas the mortality may be quite low.

Immunity

Cattle exposed to natural infection with *T. parva* or *T. annulata* exhibit variation in the severity of disease, which is believed to be dependent on the dose of sporozoites inoculated during tick feeding (Cunningham *et al.*, 1974; Gill *et al.*, 1980).

Cattle that recover from ECF or tropical theileriosis after infection with sporozoites are subsequently resistant to homologous challenge (Neitz, 1957). The severity of the initial disease reaction in animals that recover from a primary challenge does not appear to affect the quality of immunity (Cunningham *et al.*, 1974). The immunity, in the absence of further challenge, lasts for at least 3-5 years with *T. parva* (Burridge *et al.*, 1972; Neitz, 1957) and 3 years with *T. annulata* (Sergent *et al.*, 1945). Cattle immunised artificially exhibit the same quality of immunity as seen in cattle that spontaneously recover from a primary sporozoite infection.

Cattle which recover spontaneously or which have been artifically immunised exhibit high titres of circulating antibodies against schizont and piroplasm stages of the parasite. In the absence of further challenge, these antibodies wane to below detectable levels after about six months (Burridge and Kimber, 1973; Irvin and Morrison, 1987). However, such animals are still immune to lethal challenge (Burridge *et al.*, 1972; Irvin and Morrison, 1987.) The humoral responses in recovered cattle probably play a minimal role in mediating immunity to challenge. Passive transfer of sera from immune cattle with high titres of *T. parva* anti-piroplasm antibodies to naive animals is not protective (Cowan, 1981). The lack of a demonstrable role for antibodies in protection in immune cattle or in cattle immunised with schizont or piroplasm antigens led to the belief that the generation of cell-mediated immune responses was necessary for the expression

of immunity. In agreement with this hypothesis, it has been found that transfer of thoracic duct lymphocytes from a *T.parva* immune calf to its naive twin protects the recipient against a homologous lethal challenge (Emery, 1981). Analyses of cell-mediated responses have indicated that parasite-specific cytotoxic T-lymphocytes restricted by class I major histocompatibility complex (MHC) molecules, are probably the major effectors of protective immunity (Morrison *et al.*, 1986). Similar cell-mediated protective immunity has been observed in *T. annulata* (Preston *et al.*, 1986).

Immunisation methods against bovine theileriosis

Various methods of immunisation against ECF and tropical theileriosis have been developed. All the methods have exploited the fact that a protective immunity is developed only following the establishment of infection with schizonts in the host. Although the host immune responses to both *T. parva* and *T. annulata* are essentially the same, peculiarities in the biology of each parasite have dictated the course and choice of vaccine development and application. Of the many immunisation methods and vaccines which have been tested, only two methods have shown promise; the *T. annulata* tissue-culture vaccine using attenuated parasites in schizont-infected lymphoblastoid cell-lines and the infection and treatment method pioneered originally for ECF and then developed for tropical theileriosis (reviewed by Irvin and Morrison, 1987). The tissue culture vaccine against *T. annulata* has been successfuly used in many parts of the world, while the use of infection and treatment method has been confined to experimental trials. Infection and treatment for *T. parva* has been successfully used in several field trials and many countries are adopting its use on a wider scale. It is important to highlight that both these methods employ live parasites as immunogens.

Infection and treatment method of immunisation

Since the observation that prolonged treatment with chlortetracycline, (Aureomycin, Cyanamid), during the incubation period of ECF, suppressed severe disease reaction and produced immune cattle (Neitz, 1953), the method has been modified and developed by many workers (reviewed by Irvin and Morrison, 1987). The early method of immunisation depended on infection of cattle with sporozoites using infected ticks and a prolonged period of tetracycline treatment. As infectivity of ticks could not be critically evaluated prior to application, the possibility of animals not becoming infected and remaining susceptible existed. Furthermore, as the parasites are inoculated by the ticks over a number of days during feeding, tetracyclines had to be administered for a long period, reducing the practicality of the method.

These problems were overcome when stabilates of sporozoites harvested from infected ticks were prepared (Cunningham *et al.*, 1973). Infection of cattle with a sporozoite stabilate produced uniformly lethal ECF reactions. Brown and his colleagues were the first to use this method of infection (Brown *et al.*, 1977) to immunise 27 cattle against ECF using a potentially lethal dose of sporozoites given on one occasion and treating each animal with 4 daily doses at 5m/kg of tetracycline (N-pyrrolidinomethyl tetracycline, Reverin, Hoechst, W. Germany) starting on the day of inoculation with the stabilate. In order to reduce the need for prolonged drug administration, a series of experiments was performed using different oxytetracycline formulations and regimes (Radley, 1981). This work showed that using a short-acting formulation of an oxytetracycline, animals could be successfully immunised with sporozoites and two doses of 10mg/kg, given on day 0, the day of the sporozoite stabilate inoculation, and day 4. This regime of chemotherapy was further modified by using a long-acting formulation of oxytetracycline (Terramycin LA, Pfizer, USA). Animals could be successfully immunised with a single dose (20 mg/kg) given simultaneously with the stabilate. However, using the infection and treatment protocols described above, it was found that not all stocks of *T. parva* could be controlled with a single treatment of long acting oxytetracycline. With some 'difficult' stocks of *T. parva*, chemoprophylaxis had to be extended to 6 days (days 0 to 5 inclusive) at

5 mg/kg of short-acting oxytetracycline. One of the difficulties in controlling some parasite stocks may be the very high infective dose. Attempts were made to immunise cattle by diluting the original infective dose but on homologous challenge some of the animals were susceptible to ECF and died. It was postulated that the dilution of sporozoites might result in a loss or a reduction of the dose below the threshold of infectivity of some of the parasites within the immunising stock.

The immunity engendered following immunisation is effective against challenge with high doses of the homologous stock (Neitz, 1957; Radley, 1981). The duration of immunity following recovery from ECF and immunisation by the infection and treatment method in the absence of further challenge can last for over 36 months (Burridge *et al.*, 1972). It is expected that in nature immunised cattle would receive a continual parasite challenge which would reinforce the immunity and provide protection throughout the animal's life.

Cross-immunity studies and field challenge of animals immunised by the infection and treatment method showed that different immunogenic types of *T. parva* existed in the field (Young *et al.*, 1977; Radley *et al.*, 1979; Dolan *et al.*, 1980). In an attempt to provide a wide antigenic cover against different stocks the use of a cocktail of *T. parva* stocks was explored (Purnell, 1977, Radley, 1981). In a series of *in vivo* cross-immunity experiments a combination of 3 theilerial 'strains', (*T. parva* Muguga, *T.p. lawrencei* Serengeti transformed, *T. parva* Kiambu 5), termed the 'Muguga cocktail' was found to protect aginst stocks of *T. parva* isolated from widely different locations. It was suggested that such a method of vaccinating cattle could be used on a wide scale in East and Central Africa.

In the last ten years three antitheilerial drugs have been developed all of which have been used in the infection and treatment method of immunisation. A naphthoquinone, parvaquone (Clexon, Wellcome) has been tested in various infection and treatment regimes (Dolan *et al.*, 1984, 1988). It was found that when the drug was given either on day 0 or 4 after *T.p. parva* (Muguga) sporozoite inoculation the animals either did not become infected and were thus fully susceptible to homologous challenge or the prepatent period was extended and the animals developed severe ECF and died. However, if the animals were treated on day 8 when there were low numbers of schizonts (early clinical disease) the animals recovered and were subsequently immune to homologous challenge. If, however, the treatment was delayed and given on days 14 or 16 after sporozoite challenge the animals continued to develop severe ECF and some died. It was further shown that for successful recovery and immunisation the day of treatment with parvaquone varied with the sporozoite stabilate used.

Another drug, halofuginone lactate (Terit, Hoechst AG, W. Germany), was tested by Uilenberg (1984) for its effect on the early developmental stages of *T.parva* and was shown to have no effect at the recommended dose of 1-2mg/kg. As the compound has a narrow margin of safety, higher doses were not examined. However, it has powerful antischizont activity and like parvaquone can be used to treat cattle early in the infection curing the disease and rendering the animal immune.

More recently, a potent new antitheilerial naphthoquinone, buparvaquone (Butalex, Wellcome), was shown to have a suppressive effect on the early developmental stages of *T. parva* (McHardy and Wekesa, 1985). It was further shown that animals given one dose (2.5 mg/kg) of buparvaquone at the same time as the sporozoite inoculation had mild to inapparent ECF. These animals were subsequently immune to challenge. The possibility of using buparvaquone in infection and treatment to replace long-acting oxytetracycline in controlling problematic stocks of *T. parva* was investigated by Morzaria and Dolan (unpublished) and Mutugi *et al.* (1988). They found that 'difficult' stocks of *T. parva* could be contained with one dose of buparvaquone (2.5 mg/kg) given on day 0. Buparvaquone shows great promise in chemoprophylaxis against ECF and may be the drug of choice for use with certain problematic parasite stocks.

Because of the close similarities between *T. parva* and *T. annulata*, many of the technological developments in the field of ECF immunisation were adopted in tropical theileriosis, notably by workers in India. Gill *et al.* (1977) successfully immunised cattle against tropical theileriosis using either one or two doses of a long-acting oxytetracycline depending on the sporozoite stabilate used. Gill *et al.* (1980) demonstrated that stocks of *T. annulata* isolated from different parts of India were cross-protective. Some of these stocks (e.g. Ludhiana and Bangalore) were isolated from regions which are approximately 2000 km apart.

Parvaquone and buparvaquone, both have good therapeutic activity against *T. annulata* and can be used to treat cattle early in the course of the disease following artificial infection with sporozoites. These animals are immune to challenge. It has also been shown that, as with *T. parva*, buparvaquone can be used to treat cattle at the time of *T. annulata* infection and achieve successful immunisation against tropical theileriosis (Dhar *et al.*, 1987).

As can be seen, depending upon the susceptibility of the immunising stock to the different drugs, various infection and treatment regimes are available for immunisation against ECF and tropical theileriosis. Currently the practical options are :

(a) Infection and treatment using tetracyclines

The basic method is to infect cattle with a selected stock of *T. parva* or *T. annulata* and to treat simultaneously with one dose (20 mg/kg) of a long-acting oxytetracycline. If this is not possible with some stocks, two doses (10mg/kg) of short-acting oxytetracycline given on day 0 and 4 is recommended. With certain stocks even this may not suffice when a more expensive option would have to be adopted involving two doses (20mg/kg) of long-acting oxytetracycline given on days 0 and 4.

(b) Infection and treatment using antitheilerial naphthoquinones

i) Cattle are infected with a selected stock of *T. parva* or *T. annulata* and treated simultaneously with one dose of buparvaquone (2.5 mg/kg). This regime may be preferred to using tetracycline when problematic *Theileria* stocks must be used.

ii) Cattle are infected with a selected stock of *T. parva* or *T. annulata* and treated with one or two doses of either buparvaquone (2.5 mg/kg) or parvaquone (20mg/kg) given early in the clinical disease. The optimum time to treatment will vary but general recommendation would be after 2 days of pyrexia and schizont parasitosis.

When any of the above infection and treatment methods of immunisation are performed animals may develop low grade parasitosis and transient pyrexia. The reaction may last for 3-4 days but following recovery the animals are immune to homologous challenge. It has been shown that if oxytetracycline treatment is not given on day 0 the parasite may not be controlled. The main effect of oxytetracyclines appears to be on the development of sporozoites to schizonts, and schizonts which do develop have low numbers of nuclei and undergo limited multiplication. It appears that this low grade infection is sufficient to elicit a protective immune response. Recently, Spooner (1987) showed that oxytetracyclines affected the mitochondrial protein synthesis of the host cell and he speculated that the drug might have a similar effect on the parasite mitochondrion. The biochemical effect of buparvaquone on parasite development has not yet been studied.

The infection and treatment method has been tested extensively for ECF immunisation in the laboratory and in field trials (Robson, 1977; Uilenberg *et al.*, 1977; Paling and Geysen, 1981, Dolan *et al.*, 1985; Morzaria *et al.*, 1985. One of the major factors which has inhibited its large-scale use is the realisation that different immunological strains exist in nature and that the protective immunity is stock specific.

The practical values of using a selected mixture of *T. parva* stocks, which have been characterised and tested against a wide variety of immunogenic types and which can be distributed for use in many

countries were discussed by Radley (1981). However, before new immunogenic types are introduced for immunisation it is important to recognise that these parasites may become established in the local tick population and may break through naturally immunised cattle in an endemic area. Studies have shown that in ECF-immunised cattle, the frequency of acaricide application can be reduced to 1/2 or 1/3 without loss in productivity and economic benefits of such control measures are significant (Morzaria *et al.*, 1988).

It is now known that animals that recover naturally from ECF or following immunisation by the infection and treatment method become carriers (Young *et al.*, 1981; Dolan, 1981). The mechanism of the maintenance of the carrier state is not known but it is speculated that either low numbers of schizont-infected lymphoblasts sequestered in an immunologically privileged site (eg. brain, testis, anterior chamber of the eye) or circulating infected lymphoblasts (Irvin and Morrison, 1987) from ECF recovered animals may provide a source of merozoites and piroplasms. Limited erythrocytic merogony of *T. parva* piroplasms (Fawcett *et al.*, 1987) may also maintain a carrier state in ECF recovered cattle. The existence of a carrier state following chemoprophylactic immunisation has caused concern and has delayed the use of the method on a wide scale. However, several studies have shown that even if animals were immunised by stocks not inducing carrier state, such animals would become carriers of *T. parva* on subsequent natural challenge. Further, studies (Morzaria *et al.*, 1985) have shown that ticks fed on carrier animals have very low infection rates and therefore large numbers of ticks would be required to transmit lethal infections. More research is urgently needed to investigate the long term effect of a carrier state and especially its potential role in introducing new stocks into an area.

Although no studies have been reported on the establishment of the carrier state following infection and treatment immunisation against *T. annulata*, it is likely to occur. Natural carrier states are common and occasionally replapses have been observed.

In a recent workshop (Irvin, 1984) held to discuss the application of the infection and treatment method of immunisation in the ECF region, 11 countries showed their willingness to use the method on a wide scale. Some of these countries opted to use the 'Muguga cocktail' which has now been shown to provide protection against 17 stocks of *T. p. parva* from 4 different countries. Other countries preferred to use their local stocks, reserving their option to use the 'Muguga cocktail' in the future when more information was available. The important points discussed with regard to the wide-scale use of this method of immunisation were safety, standardisation, role of different strains, buffalo and other wildlife theileriosis, importance of carrier states and other tick-borne diseases. In another workshop on ECF (Irvin, 1985), emphasis was placed on careful planning of the immunisation strategies considering the use of well-characterised stabilates, and the target population to be immunised. It was generally agreed that initially immunisation should only be performed in susceptible high producing cattle in risk areas which should include cattle belonging to smallholder dairy farmers.

It is encouraging to see that despite many shortcomings of the immunisation procedure, many countries (e.g. Burundi, Rwanda, Zambia and Kenya) are beginning to use the method as a disease control strategy with promising results. A regional ECF vaccine laboratory in Malawi with facilities to prepare, characterise and safety-test bulk sporozoite stabilates for immunisation has already been established. This should provide an additional incentive for other countries in Africa to use the vaccine.

Schizont culture vaccine

The ability of theilerial schizonts to infect and immunise cattle was recognised soon after the discovery of the parasite. Spreull (1914) attempted to immunise over a quarter of a million cattle against *T. parva* in South Africa by inoculation with schizont-infected cells derived from spleen and lymph-nodes of naturally infected cattle. Sergent (1924) introduced the technique of immunisation against

T. annulata by inoculation of cattle with blood containing schizonts and piroplasms. No attempts were made to quantify the number of parasites inoculated. Following inoculation of cattle with *T. parva* infected cells variable theilerial reactions occurred resulting in death in a number of animals, successful immunisation in some and no immunisation in others. In addition, other diseases present in donor animals were transmitted to the immunised cattle causing morbidity and mortaility. In attempting to immunise cattle against *T. annulata,* inoculation of live parasites invariably resulted in successful infection but the reactions ranged from mild to moderate and severe. Due to the difficulty in inducing uniform theilerial reactions these methods of immunising against ECF and tropical theileriosis were abandoned.

The *in vitro* cultivation of *T. annulata,* first achieved in Israel (Tsur, 1945), was a significant breakthrough in the development of a schizont culture vaccine. This work showed that the *T. annulata*-infected cell-lines could be established from infected animals. *In vitro* isolates of *T. annulata* have been successfully made from Iran, Tunisia, Iraq, USSR, China and India (Brown, 1980) and Sudan (Morzaria and Pedersen, 1983).

The *in vitro* isolates of *T. annulata* are initially pathogenic to susceptible cattle, causing severe disease and often death. However, this virulence was reduced on passage. The mechanism of attenuation is unknown, but it appears that different isolates require different amounts of time, ranging from 4-30 months in culture, for effective attenuation. The attenuated parasites cause no disease and the animals are subsequently resistant to virluent challenge. Pipano (1984) has given details of the mass production of such a vaccine and has described various procedures used to test for safety (free of viruses, bacteria and mycoplasma). The vaccine is prepared in either 'liquid' or 'frozen' form. The former is prepared from cultures previously shown to be attenuated and stored at 4°C. The shelf life of the 'liquid vaccine' at this temperature is between 3 and 4 days. Such a vaccine is usually transported to the field in cool boxes and is used subcutaneously at a dose of $5x10^6$ infected cells per animal. The 'frozen' vaccine consists of concentrated parasitised cells cryopreserved in liquid N_2. It is transported to the field in liquid N_2 and before use, thawed out and diluted appropriately. The dose used for successful immunisation is 10^7 infected cells inoculated subcutaneously.

As different breeds show different susceptibility to infection the vaccine is tested for safety in highly susceptible breeds such as Friesians. Cattle inoculated with the attenuated schizont vaccine do not normally show pyrexia or clinical signs but occasionally have low numbers of schizonts and piroplasms. Such a vaccine used over a period of 10 years in Israel has been shown to be safe for different breeds, and for all age groups including pregnant dairy cows (Pipano *et al.*, 1973). The success of the attenuated schizont-infected cells in vaccinating cattle depends on the establishment of parasites in the recipient host. It appears that *T. annulata* schizonts readily transfer from cell to cell thus requiring only a small number of infected cells to induce immunity (Pipano, 1970).

The immunity engendered by such a vaccine lasts for two to three years (Sergent *et al.*, 1945; Pipano, 1977). Instances have been reported where breakthroughs have occurred following sporozoite challenge possibly indicating occurrence of strain differences. In areas where different virulent strains occur the use of double vaccination has been recommended whereby animals are first immunised by the attenuated schizont vaccine followed by immunisation by the infection and treatment method using *T. annulata* sporozoites and a long-acting oxytetracycline (Pipano, 1981).

The first successful culture of *T. parva* was achieved by Malmquist *et al* . (1970). Many attempts were made to attenuate *T. parva* infected cell lines (Brown, 1981). Brown *et al.* (1978) working with two different stocks of *T. parva* showed that the parasitised cells could be attenuated after many passages in culture. These studies highlighted several problems which inhibited further development of the

technique for use in immunisation against ECF. The first problem was that it was difficult to define accurately the point at which attenuation was achieved without loss of infectivity. Secondly, it was shown that the frequency of schizont-transfer from the donor to the recipient cells (a pre-requisite for immunisation) was very low (at least 1000 fold less than *T. annulata)* and thus for successful immunisation large numbers of schizont-infected cells were required. This represented approximately 100 times the dose of *T. annulata* cell-culture vaccine and this was uneconomical. Further, it was shown that use of large numbers of infected cells occasionally caused severe ECF reactions.

Brown *et al.* (1978) were able to show that large numbers of *T. parva*-infected cells were not necessary for successful transfer provided the cells were first infected *in vitro* and transferred into autologous animals. They suggested that the establishment of schizonts from the donor cells depended on 'acceptance' of the schizont-infected cells by the recipients. This observation has been confirmed and the 'take' and 'rejection' of *T. parva* infected cells is shown to be dependent on the degree of histocompatibility matching between the donor and recipient cells. Thus Dolan *et al.* (1984) were able to establish infection and immunise cattle with low numbers of infected cells by matching the recipients with the immunising cells for polymorphic determinants on class I MHC antigens.

At present it would not be practical to use a cell-culture vaccine to immunise against *T. parva* unless a parasite can be identified which transfers more easily from cell to cell.

Alternative strategies to immunisation

Athough the two existing methods of immunisation against ECF and tropical theileriosis are effective, they are not without problems. Both method utilise live, cryopreserved, potentially lethal parasites. This creates storage and delivery problems and there is always the possibility of an inadvertent transmission of other pathogens. Additionally, sporozoite stabilates need to be well characterised to be used effectively as different stocks of the parasite exhibit different requirement of dose and duration of antibiotic cover. Parasite antigenic diversity is also a problem in *T. parva* infection.

For these reasons alternative methods of immunisation are desirable. Two approaches are being developed. These involve the identification of antigens from the sporozoite and schizont stages of the parasite which evoke protective immunity during parasite infection. The approaches to the deveopment of sporozoite and schizont vaccines are based on trying to neutralise sporozoite infectivity and on inducing T. cell responses against parasitised bovine cells, respectively. The following two sections discuss these approaches.

Develoment of *T.parva* sporozoite vaccine

The approach to designing a sporozoite vaccine is relatively clear. Sera obtained from cattle that are immune to ECF following natural challenge and recovery neutralise sporozoite infectivity *in vitro* (Musoke *et al.*, 1982) and the neutralisation is not stock-specific. Hence there appear to be common epitopes on the sporozoites of *T.parva* which are the targets for neutralising antibodies *in vitro* (Musoke *et al.*, 1984). Sporozoite antigens carrying these epitopes are, therefore, candidate molecules for inclusion in a sporozoite vaccine.

One sporozoite antigen has been identified by the use of murine monoclonal antibodies (MAbs) that neutralise sporozoite infectivity (Dobbelaere *et al.*, 1984). The MAbs bind to peptide epitopes on the surface of the sporozoite and recognise a 67 kDa stage-specific antigen on Western blots (ILRAD Report, 1987). The presence of antibody alone is sufficient for neutralisation, although the role of complement remains to be clarified. Antibodies directed against exposed epitopes of the 67 kDa molecule may, therefore, mediate immunity to sporozoite infection *in vivo*. Efforts are also being directed at identifying other candidate sporozoite vaccine molecules by analysing surface labelled parasite molelcues and by raising murine MAbs to

other sporozoite antigens by diverting the antibody response away from the dominant 67 kDa antigen.

As purification of the 67 kDa molecule from sporozoites for immunisation studies is not practical the methods of recombinant DNA technology have been applied with the aim of producing antigen for vaccination trials. The gene coding for this sporozoite antigen has been cloned (ILRAD Report, 1987) and when the complete gene sequence is available various methods will be used to obtain maximal expression of the recombinant sporozoite antigen. Following this, different antigen presentation methods will have to be tested to determine the potential of the recombinant 67kDa antigen in eliciting neutralising antibody responses and protection.

The study of the mechanism of sporozoite entry into the target cells shows that the sporozoite coat is shed during penetration and that the epitope recognised by a neutralising MAb is transiently localised on the surface of the bovine cell after parasite entry (Webster *et al.*, 1985). Preliminary data suggest that the sporozoite epitope may remain on the lymphocyte surface for as long as 20 hours (Spooner, 1987). It is possible, therefore, that neutralising antibodies may play a dual role, firstly in directly neutralising sporozoite binding and infectivity and secondly in opsonising the early infected cell and marking it for complement or phagocytic cell destruction. The latter aspect warrants further research.

A second strategy within the approach to a sporozoite vaccine is directed at analysing the bovine lymphocyte molecule(s) involved in sporozoite binding and penetration (Kinuthia, 1987). An understanding of the ligand-receptor interaction and the initial events leading to infection may be exploited to neutralise sporozoite infectivity. Several MAbs have been raised which inhibit sporozoite binding to the lymphocyte receptor. An interesting possibility which remains to be tested is whether the anti-sporozoite and the anti-bovine sporozoite receptor MAbs are anti-idiotypes of each other (Black, S.J. personal communication). If this was the case it would strengthen the idea of a specific interaction between sporozoites and bovine surface molecules and raise the possibility of the development of an anti-idiotype based sporozoite vaccine.

Development of *T.parva* schizont vaccine

The approach to the development of a subunit schizont vaccine is to attempt to identify an antigen common to *T.parva* which will evoke a protective cytotoxic T-cell response. The complexity of the mechanisms that generate such a response poses a technical probem in firstly identifying the antigen and secondly in designing an appropriate immunisation regime.

Analysis of parasite stock specific cytotoxic responses *in vivo* and *in vitro* have led to the conclusion that there is diversity of cytotoxic T-cell epitopes. There also appears to be a dominance in the association of MHC class I products with parasite T-cell epitopes (Goddeeris, 1986, Morrison *et al.*, 1987). Hence, the MHC appears to play a major role in imposing a selection on the quality of the immune response.

It has not been possible to detect *T. parva* specific antigens on the surface of infected cells. Such molecules may carry cytotoxic T-cell epitopes. Other candidate antigens incude those localised at the surface of the schizont membrane within the cell and antigens localised in the cytoplasm of infected cells. The assumption here is that these antigens are localised in areas that are more available for processing and hence presentation. MAbs that bind to the surface of the schizont and to antigens in the infected lymphocyte cytoplasm have been identified. Screening for the presence of cytotoxic T-cell epitopes will be performed on purified antigen molecules (or pieces thereof) or on cells transfected with the genes coding for the antigens. The hope is that a common cytotoxic T-cell epitope will be identified which will protect against the majority if not all stocks of *T. parva*. From the *in vivo* cross-immunity data this is perhaps not an unreasonable hope.

Development of *T. annulata* vaccines

Parasite specific cytotoxic T-cell responses are also evoked in *T. annulata* infections (Preston *et al.*, 1983) and serum from *T. annulata* immune cattle will neutralise sporozoite infectivity *in vitro* (Gray and Brown, 1981). Hence most of the experimental approaches discussed for the development of *T. parva* sporozoite and schizont vaccines are also valid for vaccines against tropical theileriosis.

Since the serum neutralisation of sporozoite infectivity *in vitro* is species-specific it would appear that different dominant epitopes are present on the *T. parva* and *T. annulata* sporozoite surface membrane. The cell types infected by *T. annulata* are more varied than those infected by *T. parva* and although the bovine receptor for *T. annulata* remains to be identified it is likely that different ligand/receptor interactions mediate *T. annulata* sporozoite penetration.

In addition to cytotoxic killing of *T. annulata*-infected cells antibodies may play a role in immunity. The identification of infected lymphocyte associated antigens raises the the possibility that antibody mediated clearing of parasitised cells may reduce parasitosis (Shiels *et al.*, 1986; Preston *et al.*, 1986). Certainly, MAbs that recognise surface epitopes mediate complement lysis of infected cells *in vitro*. One MAb also recognises the surface of sporozoites. Cattle immunised with molecules carrying this epitope may be protected against both sporozoites and schizont infected cells. Also, the transfer of *T. annulata* schizonts between cells appears to occur quite readily. Hence, anti-schizont antibodies may limit this mode of parasite spread helping to curb infection. The importance of the humoral response in immunity against tropical theileriosis remains to be established.

Conclusions

The paper has described the two methods of immunisation against ECF and tropical theileriosis. The eventual choice of using the infection and treatment method or using the tissue culture vaccine against tropical theileriosis will depend on several factors including the scale of the problem, standard of infrastructure, costs and overall commitment and policy towards disease control. The schizont-culture vaccine against *T. annulata* has been successfully used in some countries for controlling the disease. The infection and treatment method may be an attractive alternative as a vaccine for tropical theileriosis in view of the fact that large quantities of infective doses can be produced with minimal facilities. However, the cost of drug therapy may be an inhibiting factor. In contrast the only practical option available for immunisation against *T. parva* is the infection and treatment method. Despite certain drawbacks it is being used in many African countries with promising results. Wide-scale application of this method will be limited by the same constraints as for the *T. annulata* vaccines.

Because of the presence of reservoir hosts for the parasites and the ticks, eradication of *T. parva* and *T. annulata* is not feasible. Therefore, current and future vaccination strategies should aim at the establishment and maintenance of endemic stability. Hence transmission blocking vaccines may not be desirable.

In considering modern technological developments in the production of subunit vaccines against both *T. parva* and *T. annulata* it is encouraging that significant advances have been made in characterising antigens that may play a role in evoking protective immune responses. Unlike a malaria sporozoite vaccine, a *Theileria* sporozoite vaccine is aimed at reducing the challenging sporozoite dose thus moderating clinical disease and allowing natural recovery. It is envisaged that a schizont vaccine could be used in conjunction with the sporozoite vaccine to support and protect against an overwhelming challenge which might be uncontrollable by the sporozoite vaccine alone. Natural challenge of immunised cattle is expected to boost protective immunity.

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