Bovine theileriosis in Israel

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Summary: In Israel, Theileria annulata infection is transmitted by the tick Hyalomma detritum. Economic losses have been reduced considerably by using a frozen, pelleted vaccine prepared from culture-derived attenuated schizonts. The pellets are thawed and diluted before inoculation into cattle. Some 3,000 cattle imported into Israel have been protected by vaccination. Research on a sporozoite vaccine is in progress. The infection cannot be controlled effectively by treating cattle with acaricides because the parasites are transmitted shortly after attachment of Hyalomma ticks. Chemotherapy is used only as a last resort.

KEYWORDS: Cattle diseases - Immunisation - Israel - Live vaccines - Protozoal infections - Theileria annulata.

Theileriosis of cattle in Israel is caused by Theileria annulata (9). Theileria infection was described as early as 1924 (11) although T. annulata was only identified a decade later (2). This infection seriously interfered with the cattle improvement programme in Israel during the first half of the century since it caused heavy losses among European breeds imported to improve local stock (46). Research on theileriosis has been carried out in Israel since 1934 (2) and has led to the development of effective control methods (20), which may be applicable to other areas.

EPIZOOTIOLOGY AND TRANSMISSION

Theileriosis is confined to those regions of the country where the two-host tick, Hyalomma detritum, occurs (20). The parasite is transmitted trans-stadially from larva-nymph engorging on cattle carrying erythrocytic merozoites to adult ticks in which the infective stages (sporozoites) develop 1-2 days after the attachment of the tick on cattle (40). Three-host Hyalomma spp. such as H. excavatum, are capable of transmitting T. annulata under experimental conditions (42), but no field outbreaks have been associated with this species. The preimaginal stages of H. excavatum are not found on cattle under natural conditions in Israel. Therefore, the host preference of potential vectors plays an important role in the epizootiology of theileriosis.

Hyalomma ticks are capable of producing an enormous amount of infective sporozoites. A dose containing one hundredth of a single macerated infected tick can produce lethal infection in cattle (35). Laboratory trials have shown that infected adult Hyalomma may become infective for cattle without a blood meal if maintained at high temperature and humidity (39). It appears, therefore, that in some instances the ticks can transmit the infection from the moment of attachment or very shortly thereafter.

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During the last three decades, improvements in cattle housing and management have caused the disappearance of *H. detritum* from cattle sheds, so that this species has remained as a predominantly field tick (20, 32). As a result, theileriosis affects mainly grazing cattle. Sporadic outbreaks are occasionally observed in zero-grazing cattle which seem to be caused by ticks introduced by stray cattle or by hay from fields where theileriosis is enzootic.

The preimaginal stages of *H. detritum* are found on cattle during the cold months of the year from November to March (32). In the past, outbreaks of theileriosis were observed mainly during May-October which is the main period of activity of the adult *H. detritum*. However, sporadic cases were also recorded throughout the year. Nowadays the mass immunisation of cattle at risk using cell culture vaccine has reduced the number of clinical cases of theileriosis for the last three years to 25 for 1987, 23 for 1986 and 58 for 1985.

**DEVELOPMENT OF VACCINE AGAINST THEILERIOSIS**

Immunological studies of *T. annulata* in Israel, accompanied by vaccination trials, were begun in the early 1930's (2, 46, 3, 4). These studies resulted in the elaboration of a two-step vaccination procedure in which cattle were primed with an exotic strain of *T. annulata* of a relatively low virulence and were reinoculated two months later with a local virulent strain (1). Both strains were maintained by subinoculation in young calves. The serial passages in susceptible cattle neither attenuated nor exacerbated the virulence of the strains, but this unnatural transmission resulted in loss of the ability to produce erythrocytic merozoites (14). Although this vaccination procedure was accompanied by a 1.8% mortality due to severe reactions caused by the partially virulent schizonts in cattle (56), the programme was highly beneficial overall for the development of the cattle industry. According to the chief of the clinical veterinary service during this period, in one settlement with about 3,000 cattle, a theileriosis mortality rate of 13% in non-vaccinated cattle declined to 0.03% following vaccination (47). To this should be added the 1-2% mortality associated with vaccination.

The exotic *T. annulata* strain used for vaccination was maintained in the Beit Dagan Institute through 420 passages at which point successive passages did not yield infection. A method for cryopreservation allowing safe long-term storage of *T. annulata* strains was subsequently developed (52, 53).

The loss of the vaccine strain triggered efforts to elaborate an alternative method for antitheilerial vaccination. A new avenue in the study of *T. annulata* was opened by Tsur (49) when he cultivated the schizont stage of the parasite in plasma clot cultures of liver and spleen explants (48, 50). Later the eminent virologist A. Kimron showed that *T. annulata* could multiply in monolayer cultures of cells obtained by trypsinisation of a kidney from a calf dying from theileriosis (unpublished data, 1960). Subsequently, monolayer cultures were obtained from other internal organs and buffy coat cells (51, 55). Initial trials in cattle showed that subcultivation *in vitro* resulted in a gradual attenuation of the virulence of the schizonts (54). At first the schizonts derived from cultures produced clinical theileriosis but with a lower or nil mortality rate. In addition, lymphocytic schizonts and erythrocytic merozoites occurred in the experimental animals (17).
Prolonged cultivation, from several months to about two years, resulted in a complete loss of virulence (26). Such schizonts no longer yielded erythrocytic merozoites in intact or splenectomised cattle (30) and passages in susceptible cattle did not exacerbate their virulence (15). Serological examinations (28) showed that completely attenuated schizonts induced specific antibody in inoculated cattle (17, 27), thus reflecting the cryptic multiplication of schizonts in the animals.

The capacity of schizonts to become established in cattle was similar for both virulent and attenuated schizonts. About $10^5$ schizont-infected cells were needed to cause infection or seroconversion in all inoculated cattle (16, 22). On the other hand, as many as $5 \times 10^9$ completely attenuated schizonts did not provoke clinical theileriosis when inoculated intravenously into susceptible cattle (21).

Cell culture-derived *T. annulata*-infected cells proved to be safe for all types of cattle regardless of age or physiological condition. Assessment of the safety of cell culture vaccine in laboratory trials with hamsters and splenectomised calves and with 3,650 cows in the field (of which about 20% were in various stages of pregnancy) did not reveal any harmful effects that could be attributed to the vaccine (31).

Each of the three main stages of *T. annulata* that are capable of infecting cattle elicit a stage-specific immune response. Erythrocytic merozoites do not confer any protection against the schizonts of this parasite (18). Schizonts do not prevent infection with infective stages derived from ticks (sporozoites), but in most trials they engender a high degree of protection against the later stages and so prevent severe clinical symptoms or death following a tick-transmitted infection (19). In tests for potency of vaccine produced from attenuated schizonts, out of 22 Friesian calves exposed to tick infection, 15 reacted with low parasitaemia and/or mild fever, while 7 remained asymptomatic (24). These findings formed the basis for use of cell culture vaccine in routine large-scale vaccination of cattle (20).

**PREPARATION OF VACCINE**

The production of sufficient vaccine to immunise large numbers of cattle requires mass cultivation of schizonts.

After confirming the non-virulence of the schizonts by preliminary testing, the production of vaccine is started with cells from a single tube of frozen seed schizonts. In the course of sequential subcultivations, the number of culture flasks is increased to provide large quantities of schizont-infected cells. Expanding the number of culture vessels to hundreds and thousands in order to prepare a single large batch of vaccine is not practical in a conventional parasitological laboratory. For this reason, cells are grown in several tens of culture vessels at any single time and vaccine is produced in small batches every 3-4 days. At every harvest of cells about 70% are used for preparing vaccine and the remainder are used for further subcultivation. Consequently, this method implies continuous passaging of cells and schizonts during the production process. Present experience shows that once schizonts become attenuated, no reversal to virulence occurs following further passages *in vitro* (21).

On the other hand, it is not yet clear whether the immunogenic capacity of the schizonts may become altered during prolonged cultivation of hundreds of passages. Consequently, after the cells have been passaged during the production process for 20-30 times, mass cultivation is restarted from a new tube of cryopreserved seed cells (24).
Conventional Roux bottles or roller bottles in a roller system incubator are used for mass production of schizont-infected cells.

In the past, freshly produced vaccine with a shelf life of 4-5 days at 4°C was used (25). Nowadays, only frozen pelleted vaccine is produced. Schizont-infected cells are mixed with dimethyl sulfoxide to obtain a final concentration of 7% which is dispensed in 2 ml plastic cups. The cups are introduced in a -70°C electric freezer and 24 hours later transferred for storage in liquid nitrogen. Each pellet of 2 ml volume contains 10 doses of vaccine and each dose contains $10^7$ schizont-infected cells (23).

Before use, batches are tested for purity according to a procedure recommended by the US Code of Federal Regulations for animal products (5). This testing provides an important control for the quality of the production process. Batches of vaccine that are released for use are stored in liquid nitrogen in the district veterinary service stations.

**Application of the vaccine in the field**

The frozen vaccine is transported to the farms in field liquid nitrogen containers. The vaccine pellets are thawed and diluted in bottles containing isotonic phosphate buffered saline. The bottles have an open-top screw cap with a rubber or silicone septum allowing the aseptic withdrawal of the vaccine. The diluted vaccine is inoculated subcutaneously within 30 min of thawing.

Since live attenuated schizonts induce a marked antibody production in cattle (27) the response to vaccination is assessed, if required, by measuring antitheilerial antibody in vaccinated cattle (31, 29). Blood samples absorbed on blotting paper can be used for determination of the antibody level (10).

**CONTROL OF THEILERIOSIS**

Ixodicides are applied on cattle in Israel with the main goal of controlling the cattle tick *Boophilus annulatus* which transmits *Babesia bovis* and *B. bigemina* (36). Unlike babesiosis, theileriosis cannot be controlled effectively by periodic ixodicidal treatment because infected *Hyalomma* ticks can transmit *T. annulata* within only a few hours to two days after attachment. For this reason, tick control has not been applied as an organised method for prevention of theileriosis in Israel.

Recently synthesised antitheilerial drugs (13) have not yet been evaluated as a practical means for reducing losses from theileriosis. In any case, chemotherapy can hardly be considered a method for planned mass control of theileriosis. Chemotherapy is to be seen as a means of dealing with emergency situations when routinely applied control measures have failed or have not been applied at all.

Long-term experience has shown that vaccination is a successful means for preventing losses from theileriosis. Local and imported cattle have been protected adequately against tick-transmitted theileriosis. The animals are inoculated only once during their lifetime with schizont vaccine, usually in the first months of their life. Dairy cows destined for export to areas where theileriosis is enzootic are vaccinated upon request.

During the last two decades about 3,000 cattle, mainly young bulls, have been imported from Western Europe to Israel. After vaccination, these animals have
survived in theileriosis-enzootic pastures. Non-vaccinated local or imported cattle exposed accidentally on these pastures have invariably contracted theileriosis. Although antigenically different isolates have been detected (33), the vaccine protected cattle in all theileriosis-enzootic areas in Israel. Large field trials in other countries with cultured schizont vaccine prepared from local strains have confirmed the high degree of protection engendered against field infection with *T. annulata* (45).

Concerning the epizootiological implications of vaccination with the schizont vaccine, vaccination is not followed by appearance of erythrocytic stages, thus vaccinated cattle are not infective for ticks (44). On the other hand, vaccinated cattle will develop erythrocytic stages of *Theileria* upon infection with ticks. It follows that vaccination cannot lead to eradication of theileriosis in enzootic areas.

**SPOROZOITE VACCINE**

A technique that involves infecting cattle with tick-derived parasites and then mitigating the subsequent symptoms of theileriosis by chemoprophylaxis was proposed by Brocklesby and Bailey (6) for immunisation against *T. parva* theileriosis. The tremendous subsequent work performed in this field was summarised in several comprehensive treatises (8, 37, 38). This technique has been evaluated also for *T. annulata* theileriosis.

In Israel the method for rearing ticks under laboratory conditions elaborated by Hadani and co-workers (12) was used for mass production of *T. annulata*-infected *Hyalomma* ticks. Several batches of sporozoite suspensions derived from *H. excavatum* were produced (41, 43). The material proved to be highly infective for cattle (35) and Friesian calves have been successfully vaccinated under chemoprophylaxis (34). This method may be considered as possibly useful for reinforcement of immunity conferred by schizont vaccine (24).

**FUTURE PROSPECTS**

During the past several years research on theileriosis in Israel has been reduced because of the great reduction in economic losses caused by this disease due to vaccination of cattle at risk.

However, further studies are recommended to improve results of vaccination in cattle. Culture medium for growing cells is one of the most costly components in vaccine production. Therefore, formulations yielding greater numbers of schizont-infected cells, or which can be produced more cheaply, are being sought.

Antigenic variations of *T. annulata* schizonts during prolonged cultivation should be studied to detect any immunogenic alterations that may occur during long-term subcultivation *in vitro*.

Testing of vaccine is performed in susceptible cattle. A standard method for *in vitro* titration of viability of schizonts is to be desired in order to replace the expensive cattle test. A uniform standard challenge for evaluation of the protecting capacity of the schizonts using stabilate of sporozoites is to be elaborated. The amount of sporozoites for challenge should be based on an *in vitro* titration technique based on infection of bovine lymphocytes in culture (7).
The existence of immunogenically different field isolates in *T. annulata* is well known. A serological method, perhaps based on monoclonal antibody, should be developed to allow the determination of the major antigenic groups of this parasite which give a high degree of cross protection.

A safe and easily applicable procedure for reinforcement of immunity engendered by the schizont vaccine might be needed for protecting highly susceptible cattle in areas heavily infected with theileriosis.

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**LA THEILÉRIOSE BOVINE EN ISRAËL. – E. Pipano.**

*Résumé* : En Israël, l'infection à *Theileria annulata* est transmise par la tique *Hyalomma detritum*. Les pertes économiques qu'elle provoque ont été réduites dans une proportion considérable par l'emploi d'un vaccin congelé, sous forme de granulés, préparé à partir de schizontes atténués provenant d'une culture. Les granulés sont décongelés et dilués avant l'inoculation aux bovins. Environ 3000 bovins importés en Israël ont été protégés par la vaccination. Les recherches sur un vaccin à base de sporozoïtes sont en cours. Le traitement des bovins par des acaricides n'est pas efficace pour contrôler l'infection, car les parasites sont transmis peu de temps après que les tiques *Hyalomma* se sont fixées sur les bovins. La chimiothérapie n'est utilisée qu'en dernier recours.


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**TEILERIASIS BOVINA EN ISRAEL. – E. Pipano.**

*Resumen*: En Israel, la infección por *Theileria annulata* es transmitida por la garrapata *Hyalomma detritum*. Las pérdidas económicas que provoca esta infección han sido reducidas en proporción considerable mediante el empleo de una vacuna congelada en forma de granulados, preparada a partir de esquizontes atenuados procedentes de un cultivo celular. Antes de su inoculación en los bovinos, los granulados se descongelan y diluyen. Con esta vacunación, se protegieron unos 3.000 bovinos importados en Israel. Se están llevando a cabo investigaciones acerca de una vacuna a base de esporozoitos. El tratamiento de los bovinos por acaricidas no es eficaz para controlar la infección pues las garrapatas *Hyalomma* se fijan en los animales y transmiten los parasitos casi de inmediato. La quimioterapia se utiliza solamente como último recurso.

PALABRAS CLAVE: Enfermedades de los bovinos - Infecciones por protozoarios - Inmunización - Israel - *Theileria annulata* - Vacunas atenuadas.
REFERENCES


