CONTROL OF THEILERIA ANNULATA IN IRAN*

R. Hashemi-Fesharki

Tropical theileriosis or Mediterranean Coast Fever — caused by Theileria annulata — *is a disease of cattle widely distributed across southern Europe, north Africa and central Asia. Its distribution broadly corresponds with that of its main ixodid tick vectors* Hyalomma excavatum *and* H. detritum *(Fig. 1).* 'Exotic' cattle *(Bos taurus)* are particularly susceptible with mortalities up to 40—80% in some areas, whereas indigenous cattle *(B. indicus)* generally suffer much lower mortalities (about 10%) confined mainly to calves. But because imported non-immune cattle are so susceptible,* T. annulata *represents a major constraint to livestock improvement programmes in many parts of the Middle East and Asia.*

Cattle that recover from T. annulata *infection generally show a solid, long-lasting immunity. For many years there have been programmes to protect cattle by inoculation with blood from sick animals, and more recently using live attenuated* T. annulata *vaccines prepared from cultured schizont-infected lymphoid cells. This article reviews a 14-year immunization programme against* T. annulata *in Iran.*

Economically, theileriosis due to *T. annulata* is the most important disease of cattle in Iran, representing a major threat to the expansion and improvement of livestock production. Over the past 14 years however, over 100 000 pure and crossbreed cattle have been vaccinated in Iran, using a live attenuated vaccine prepared from local strains of the parasite grown in cultured lymphoid cells. The vaccine has been administered in various provinces of the country, representing different climatic conditions and different livestock management practices, and has proved very successful. Vitually no adverse reactions have been reported and the vaccinated cattle have shown very good resistance to natural infections.

T. annulata is a haemosporidian parasite transmitted by ticks of the genus *Hyalomma*. Infected ticks inject sporozoites which rapidly invade host lymphocytes and mature to multinucleate macroschizonts. The schizonts multiply rapidly and promote synchronous multiplication of the infected host cells so that a large population of parasitized lymphocytes results[1,2]. Hence symptoms of the disease include hypertrophy of the lymphoid tissues accompanied by fever and weight loss. In many cases, particularly with imported *Bos taurus* cattle, the infection is lethal.

In the early 1920s, Sergent and colleagues showed that blood from infected cattle was also infective, and went on to use blood infected with milder strains of the parasite to immunize cattle in Algeria [3-6]. A similar approach was implemented in Israel[7] and in the USSR[8], although up to 2% mortality of immunized cattle was reported. Moreover, use of such blood for vaccination also risks transmission of other blood-borne parasites[9].

These early studies clearly showed that vaccination against *T. annulata* was possible. A further step in the struggle against theileriosis then came with the successful cultivation of schizont-infected lymphocytes from acutely-reacting cattle[10]. Culture methods have since been substantially improved and have shown that maintaining the schizonts in culture tends to cause a loss of virulence[11-13]. We showed in the early 1970s that inoculation of cattle with vaccine prepared from the

cultured schizonts could produce immunity that persisted for more than one year[14]. This 'schizont tissue culture vaccine' (STCV) is the basis for the current programme of vaccination which began in 1973 and has now involved more than 100 000 cattle in Iran.

Box 1. Strains of *Theileria annulata* used in the Iranian
vaccination programme

The theileriosis vaccination programme in Iran is based upon three local strains of *T. annulata* isolated from naturally infected cattle in 1973. Two strains (S11 and S15) are used for the vaccine, while the third (S3, maintained by needle passage in susceptible calves) is used as a defined challenge strain to check the immunogenicity of the vaccine.

Neither of the vaccine strains were particularly mild on first isolation, but have become attenuated by repeated passage in lymphoid tissue culture. Both have lost their ability to produce erythrocytic forms (piroplasms) and so are unable to infect ticks. They therefore represent no risk of spreading the disease when used as live vaccines in the field.

Strain S11, which is used for the first —— of the two inoculations in our vaccine schedule, became very mild by continuous passage. It is used as a vaccine between 60 and 76 passages. At this stage it causes no clinical reactions, but inoculated animals show positive titres of *T. annulata* antibody by complement fixation tests[17]. Strain S15 has become moderately attenuated by continuous passage; it is used for the second of the two inoculations, between 250 and 280 passages. When inoculated into test calves, S15 causes a slight swelling of the prescapular lymph node near the inoculation site, with a transient rising temperature in about 60% of animals which lasts up to two days but does not exceed 40·5°. Lymph node biopsy smears from animals inoculated with S15 typically show 2–3 schizonts in each microscopic field.

In contrast to the two vaccine strains, our S3 challenge strain is highly virulent, even after 146 needle passages of infected blood between susceptible calves. It causes pronounced swelling of the prescapular lymph node to 3–4 times normal size, with temperature rises up to $41^{\circ}C$ lasting 4–5 days. Lymph node biopsies show 15–20 schizonts per field, and up to 80% of susceptible animals succumb to the infection if untreated. We have shown, however, that this strain can also be attenuated by tissue culture passages, and in this way it also loses its ability to produce erythrocytic forms.

After an appropriate number of passages, all three strains are stored by

cryopreservation at -70°C or -196°C, using 9.5% glycerol as cryoprotectant. S3 is stored as blood taken from infected animals and is used only for our laboratory challenges. S11 and S15 are stored as schizont-infected lymphoid cells (about 2×10^6 cells per ml), to be thawed at 37°C before use as vaccine. Our vaccination schedule requires 2 ml of strain S11 to be given first, followed by 2 ml of strain S15 given 30—50 days later.

Vaccine Production

Our procedure for vaccine production is based on three local strains of *T. annulata* isolated from naturally infected animals showing acute symptoms of the infection[14].[15]. Two strains (S11 and S15) form the basis of the vaccine; they are maintained by passage in tissue culture suspension. Neither of these strains was particularly mild nor attenuated when first isolated, but they acquired these characteristics by continuing passage of schizont-infected lymphoid cells in tissue culture (Box 1).

The third strain (S3) is our standard challenge strain which is maintained by repeated injection (needle passage) of infected blood to susceptible calves. This strain is highly virulent and causes about 80% mortality in susceptible pure-breed cattle. It is used to challenge vaccinated calves during quality control tests of vaccine batches.

Schizont-infected lymphoid cells are grown in standard suspension culture[13]. [14], although instead of 10% calf serum we now use 10% horse serum (or more rarely sheep serum), which is examined periodically to check that it is free from potentially pathogenic microorganisms. Cultured cells are harvested and concentrated to about 2×10^6 cells per ml. 12 ml of cell concentrate is placed in 20 ml vials in 9.5% glycerol for cryopreservation until used (cooled at -20°C for 1 hour and then frozen at -70°C).

Each batch of vaccine contains 4000—7000 doses distributed as 6 doses per vial. To check for safety and immunogenicity, 3—4 vials from each batch are taken at random, mixed, and innoculated into 5—7 calves. These calves are brought from farms that have been free of theileriosis for at least 5 years and are maintained in tick proof pens. They are

examined twice-weekly for one month before trials to check that they are free of blood protozoa. Inoculated calves are then checked twice-weekly over 45 days for packed cell volume (PCV), haemoglobin content, and red and white cell count[16]. Immunity is verified by a complement fixation test[17] and by challenge with the S3 virulent strain of *T. annulata* (administered as 45–50 ml of blood from a susceptible calf infected with this strain). Over the 14 years of vaccine production, all vaccinated calves have resisted challenge, whereas 80% of non-vaccinated calves kept under similar conditions have succumbed to acute theileriosis after challenge.
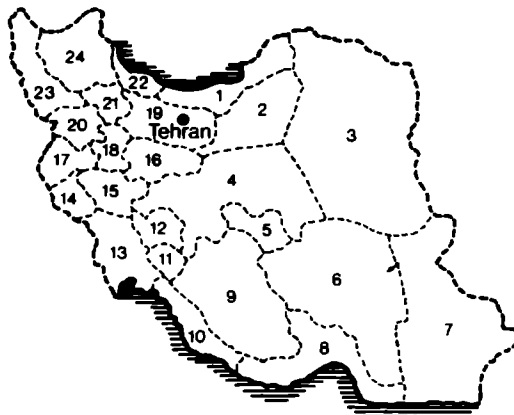
Haematological observations in vaccinated calves show no significant changes in PCV, red cell count or haemoglobin content, although there is typically a small rise in white cell count which returns to normal 20–24 days after vaccination. In contrast, non-vaccinated controls on challenge show acute fever and parasitic lymphocytosis followed by leucopenia, with reduced PCV and haemoglobin content . Around 80% of non-vaccinated animals succumb to acute theileriosis on challenge with our virulent S3 strain.

Cryopreserved vaccine is transported to the field in dry ice or liquid nitrogen. It is distributed by us to provincial livestock officers who are required to administer the vaccine according to our schedules and to report on observed results. Under our schedules, the two vaccine preparations (S11 and S15) should be injected with an interval of 30-60 days; each injection should be 2ml of vaccine (ie. about 4 $\times 10^6$ schizont-infected cells) thawed at 37°C. Since. 1973, over 100 000 cattle, 2 months and older, have been vaccinated in this way. 90% of these were Holstein Friesians (Fig. 2), and the rest were Jersey, Red Danish, Shuitze, Hereford, Angus, Brahman and 'Brangus' (Brahman X Angus). The results are summarized in Table 1. With one exception (report from Hamadan province) the reports from the other 21 provinces have shown that the mortality rate due to *T. annulata* observed in vaccinated animals has been around 0.04%. compared with up to 80% mortality in non-vaccinated animals.

Table I. Provincial reports from the schizont tissue culture vaccine programme against *Theileria annulata* in Iran.

Province	Arpprox. Numbers of Cattle Vaccinated	Provincial Vaccination Report
Mazanderan (1)	10000	"During recent years the result of vaccination was successful. Mortality rate due to theilerioses was very low in some areas and nil in others."
Fars (9)	30000	"We have not received any reports of death due to theilerioses in vaccinated animals in different areas of this province, whereas the non-vaccinated cattle have had a high mortality rate due to the disease."
Khozestan (13)	4000	"The owners of farms in which cattle have been vaccinated are content and report no mortality due to theilerioses in vaccinated animals."
Kohkilovieh (11)	1000	"No positive case of acute theilerioses was recognized in vaccinated cattle."
Khorasan (3)	10000	"No theilerioses has been noticed in animals vaccinated during the past five years."
Ilam (14)	3000	"Vaccination of cattle was successful, and no death due to theilerioses was noticed in vaccinated animals."
Bakhtaran (17)	5000	"5% theilerioses and 1% mortality due to the disease was seen in animals which were vaccinated only with S 11.2% theilerioses and very few deaths were recognized in animals vac cinated with S-11 and S-15 during an interval of 30--40 days."
Hamadan (18)	5000	"The protective effect of vaccine against theilerioses in the field was about 80%."
Zandjan (21)	5000	"The protective effect of vaccine in vaccinated animals was more or less useful."

East Azarbaidjan (24)	10000	"The results obtained from vaccination of animals were successful with the exception of one positive case of acute theilerioses. In this case, the animals received Naphthoquinone together with tonic drugs and recovered."
West Azarbaidjan (23)	10000	"The vaccination was carried out and no undesirable results have been obtained as yet."
Kordestan (20)	4000	"No mortality due to theileriosis was seen in farms in which the vaccination was carried out."
Markazi (16)	25000	"The results of cattle vaccination were effective and no mortality due to the disease was seen in vaccinated animals."
Tehran (19)	2000	
Semnan (2)	500	The vaccination was carried out
Bakhtiari (12)	100	according to schedule in some farms and
Bosher (10)	100	unsatisfactorily in others. No death
Hormozgan (8)	150	report due to theilerioses was seen in
Kerman (6)	3000	animals which received both S 11 and
Gilan (22)	3000	S-15
Esphahan (4)	2000	

Our experience with the schizont tissue culture vaccine parallels that of similar work in the USSR. Russian workers have vaccinated a similar number of cattle since 1976, maintaining them on untreated tick–infested pastures together with non-vaccinated control calves. They report no cases of theileriosis amongst the vaccinated cattle, but that all the non-vaccinated cattle became infected, with a death rate of 27–40%![8]

There is no evidence that schizont tissue culture vaccines have any adverse or depressive effect on the animal's immune system[19, 20] and this accords with our own experience. However, we have found that animals may not be able to tolerate the *Theileria* vaccine if they are already suffering from another infection – especially viral infection. Similarly, if a viral vaccine (e.g. against foot-and-mouth disease) is given during the reaction period of the *Theileria* vaccine, then the immune reaction may be compromised. For these reasons we do not recommend vaccination of sick animals, nor do we recommend concurrent administration of other vaccines (we advise that other vaccines should be given before the *Theileria* vaccine or 25 days after the second *Theileria* inoculation). Also, we have seen a few cases of abortion after vaccination in cows more than five months pregnant; the cause of this is not known but as it may be related to the vaccine we do not recommend vaccination of pregnant cows.

.

Boosting the Vaccine

In the Razi Institute – as also attempted by Pipano *et al.*[21] – we have tried vaccinating calves with killed schizonts (up to 3×10^6) either alone or with Freunds adjuvant. In all cases the animals developed acute theileriosis on challenge with our S3 strain, and around 80% succumbed. In Japan, vaccinations with killed schizonts and propinobacterium or levamisole also failed to produce immunity[22]. It appears that immunity to *Theileria* is mainly cell–mediated rather than humoral[20,23] and that the cytotoxic immune responses depend on the presence of viable infected cells. Further work to improve our understanding of this process and so help to refine our vaccines is clearly vital.

Fig. 1. Distribution of tropical theileriosis (adapted from Purnell[20])

Indigenous cattle (Bos indicus), which are frequently exposed to infected tick vectors in the field, are much more resistant to Theileria than the imported cattle breeds. But even with indigenous cattle, mortality due to theileriosis can reach 15–20% in some parts of Iran, and so we also intend to begin vaccination of these animals in the near future.
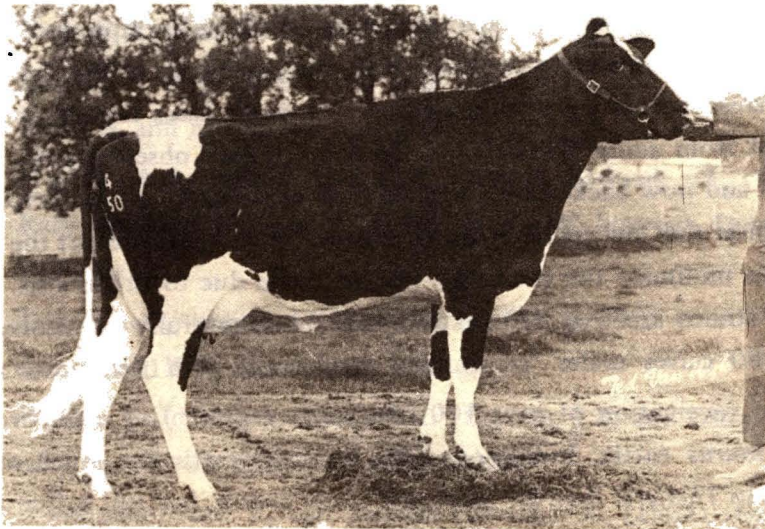

Fig. 2. Holstein/Friesian cows – developed to give both milk and meat – represent the majority of cattle imported to Iran from Europe and North America. Over 100 000 of these cattle have now been vaccinated against Theileria annulata in Iran.

Controversial suggestions that a vaccine produced from stabilated sporozoites or crude piroplasms of *T. annulata* may be more effective than the schizont tissue culture vaccine[24] have yet to be backed by field results. Nevertheless, we do agree that vaccinated cattle should have their immunity boosted with sporozoite stabilates derived from *Theileria* -infected ticks. But the production of stabilated sporozoites is a time-consuming and expensive process. Moreover, many of our livestock centres are not well-kept, the whole country is infested with tick vectors having natural *T. annulata* strains, and so vaccinated cattle are automatically re-exposed to sporozoites from infected ticks. This repeated natural challenge with tick-derived sporozoites appears to reinforce the immunity initiated by the schizont vaccine giving a fully protective effect against acute theileriosis. Acaricides are used in areas where heavy tick infestation is seen, but I have found that spraying cowsheds and dipping or spraving cattle with acaricides once a week, or even more frequently, rarely results in effective control.

Our experience with chemotherapy has also not been encouraging. Cattle infected with *Theileria* can be treated with drugs such as parvaquone (CLEXON, Wellcome) or halofuginone (TERIT, Hoechst), which are most effective when given at the onset of clinical symptoms[25] But these drugs are relatively expensive and our experience shows that successful treatment is not always assured — in some cases treated animals still died from acute disease (Ref. 26 and our own observations). As a result — and now backed by 14 years field experience — I am firmly of the opinion that the schizont tissue culture vaccine is at present the best means to minimize the mortality rate in cattle due to theileriosis. Our vaccine strains are well defined and have been carefully monitored in over 2000 calves under 'laboratory' conditions. with field tests now based on vaccination of over 100 000 cattle representing eight breeds of *Bos taurus* cattle. Indigenous (*Bos indicus*) cattle are much more resistant to *Theileria* than the imported cattle breeds, but still suffer up to 15—20% mortality due to theileriosis in some parts of Iran. We therefore plan to commence vaccination of these cattle in the near future. We believe that this programme is one of the most significant means to pre-

serve our rural livestock and promote the production of milk and meat in this country.

References

1. Bloom, B.R. (1979) *Nature* 279, p. 21

2. Dyer, M. and Tait, A. (1987) *Parasitology Today* 3, 309–311

3. Sergent, E. *et al.* (1927) *Ann. Inst. Pasteur* 41, 721–785

4. Sergent, E. *et al.* (1931) *Ann. Inst. Pasteur* 47, 63–72

5. Sergent, E. *et al.* (1933) *Ann. Inst. Pasteur* 51, 284–285

6. Sergent, E. *et al.* (1940) *Ann. Inst. Pasteur* 65, 199–203

7. Adler, S. and Elenbogen, V. (1934) *Vet. Bull.* 4, 91–93

8. Markov, A.A. (1962) *Bull. Off. Internat. Epizoot.* 58, 165–193.

9. Rafyi, A. and Maghami, G. (1962) *Bull. Off. Internat. Epizoot.* 58, 119–146

10. Tsur-Tchernomoretz, I. (1945) *Nature* 156, p. 391

11. Gill, B.S. (1976) *Nature* 264, 355–356

12. Pipano, E. (1977) in *Theileriosis* (Henson, J.B. and Campbell, M., eds), pp. 55–65, IDRC–Ottawa, Canada

13. Hooshmand-Rad, P. and Hasemi-Fesharki, R. (1968) *Arch. Inst. Razi* 20, 85–89

14. Hashemi-Fesharki, R. and Shad-Del, F. (1973) *Am.g. Vet. Res.* 34, 1465–1467

15. Hashemi-Fesharki, R. (1977) in *Tick-borne Diseases and their Vectors* (Wilde, J.K.H., ed.), pp. 357—364

16. Schalm, D.W. (1975) *Veterinary Hematology*, Lea & Febiger

17. Hooshmand-Rad, P. and Hashemi-Fesharki, R. (1971) *Br. Vet. Med.* 127, 244—250

18. Stepanova, N., Zablotsky, V. and Mutuzkina, Z. (1985) in Proceedings of the VII International Congress of Protozoology, Nairobi, Kenya (abstract 168)

19. Sharpe, S. and Langley, P. (1983) *Br. Vet. J. 139, p. 139, p. 378*

20. Purnell, R.E. (1980) *Symposia Brit. Soc. Parasitol.* 18, 25—55

21. Pipano, E. *et al.* (1977) *Vet. Parasitol.* 3, 11—22

22. Minami, T., Ishihrra, I. and Fujita, J. (1981) in *Advances in the Control of Theileriosis* (Irvin, A.D. *et al.* eds) pp. 94—96 Nairobi, Kenya

23. Pearson, T.W. *et al.* (1979) *Nature* 281, p. 678

24. Irvin, A.D. (1985) *Parasitiology Today* 1, 124—128

25. Uilenberg, G. (1985) *Facus on Bovine Theileriosis,* FAO Report, APHIS-USDA (6505 Belcrest Road, Hyattsville, MD, USA)

26. Uilenberg, G. (1986) *J. Parasitol.* 72, 485—491