

RECENT DEVELOPMENT IN CONTROL OF *THEILERIA ANNULATA* IN IRAN

HASHEMI-FESHARKI R.*

Summary :

During 1973-1990 cattle immunization, in Iran, was induced by two strains within an interval of one month: first the milder and then the mild strain. Although this method of vaccination rendered satisfactory results in the field, yet production, maintenance in deep-freezers and transportation in liquid nitrogen particularly to remote areas of the country proved to be uneconomical and time consuming. Therefore, in order to reduce cost and save time, a new method involving only one local and live attenuated vaccine strain was sought.

Reports received from different ecological areas of the country have shown no presence of any significant abnormal side-effects in vaccinated cattle and the immunization results have been highly satisfactory.

KEY WORDS : *Theileria annulata*, cattle protozoa, vaccination.

Résumé : DÉVELOPPEMENTS RÉCENTS DANS LE CONTRÔLE DE *THEILERIA ANNULATA* EN IRAN

Entre 1973 et 1990, l'immunisation du bétail en Iran a été effectuée avec deux souches à un mois d'intervalle, en commençant par la souche la plus atténuée. Bien que cette méthode de vaccination ait produit des résultats satisfaisants sur le terrain, la production, l'entretien en congélateur et le transport dans du nitrogène liquide, notamment vers des régions éloignées du pays, s'est révélée coûteuse et longue. Par conséquent, afin de réduire les coûts et de gagner du temps, une nouvelle méthode ne recourant qu'à une seule souche d'un vaccin vivant atténué a été expérimentée. Les rapports reçus de diverses régions écologiques du pays n'ont pas montré d'effets indésirables significatifs chez le bétail vacciné, et les résultats de l'immunisation ont été très satisfaisants.

MOTS CLÉS : *Theileria annulata*, protozoaires du bétail, vaccination.

Bovine tropical theileriosis due to *Theileria annulata* (Dschunkowsky & Luhus, 1904) is a severe and fatal disease of cattle in Iran. The mortality rate of this threatening disease in pure and cross-bred cattle reached 40-80 % respectively (Hashemi-Fesharki, 1988). Iranian scientists did not pay much attention to the disease because the indigenous cattle having been exposed to infested ticks had developed resistance. But after the importation of exotic cattle (*Bos taurus*) in order to improve the indigenous ones they found that the imported cattle were highly susceptible to the disease. Therefore they began developing some methods to eradicate the disease. At that time a vaccine prepared from the blood infected with attenuated strain was commonly used in Algeria, Tunisia, Israel and old Russia (Adler & Elenbogen, 1934; Brocklessby & Hawking, 1958; Cordier & Manager, 1935, 1936; Donatien, 1936; Markov, 1962; Sergent *et al.*, 1931, 1933, 1940; Sing, 1990). This kind of vaccine was also experimented in Iran indicating that it was not a completely safe vaccine because it could also transmit some other blood born micro-

organisms causing morbidity and mortality (Rafyi & Maghami, 1962). Attempting to immunize cattle against *T. annulata*, inoculation of infected blood invariably resulted in successful infection but the reactions ranged from mild to moderate and severe.

This method of immunization was abandoned due to difficulties in inducing mild uniform *Theileria* reactions and because of transmission of other disease. Since then a basic research has been performed in Razi and other Institutes to satisfactory grown schizont infected lymphoid cells through monolayer and suspension methods with or without feeder cells (Huilliger, 1965; Hashemi-Fesharki & Shad-Del, 1973; Hooshmand-Rad & Hashemi-Fesharki, 1968; Pipano & Tsur, 1966; Tsur-Tchernomoretz, 1945; Tsur-Tchernomoretz *et al.*, 1957; Tsur & Adler, 1962, 1965.)

Our research results indicated that vaccination of pure and cross-bred cattle with schizont tissue culture vaccine (S.T.C. vaccine) induces immunity which could last more than one year (Hashemi-Fesharki & Shad-Del, 1973; Hooshmand-Rad & Hashemi-Fesharki, 1988). The immunization programme started and was continued for 17 years using two vaccine strains within an interval of one month. First milder and second was mild. Although satisfactory results have been obtained by using these strains, it is uneconomical and time consuming. Therefore since 1990 only one vaccine

* Department of Protozoology and Production of Protozoal Vaccines, Razi State Serum & Vaccine Research Institute, P.O. Box 11365-1558, Tehran (Iran).

strain has been used with satisfactory results. This paper presents the results of cattle vaccination with one vaccine strain.

MATERIALS AND METHODS

LABORATORY RESEARCH WORK

Characteristic features of strains and process of vaccine production

Vaccine strain: in this study only the mild strain "the second strain of the ancient protocol" was used while tolerating more *in vitro* sub-passages in order to reduce its virulence without decreasing immunogenicity (Hashemi-Fesharki, 1988).

Challenge strain: it is a very virulent strain which usually causes a mortality rate of 80% in susceptible cattle (Hashemi-Fesharki, 1988) and is only used for controlling the efficacy of the vaccine in the Department and never used in the field.

The process of vaccine production: this new vaccine was also produced by growing schizont infected lymphoid cells with the mild strain of *T. annulata* and maintained at frozen state at -70°C after production until use. The production process included the same steps applied in producing the two-strain vaccine (Hooshmand-Rad & Hashemi-Fesharki, 1968). The cells concentration, however was doubled $2.5-2.8 \times 10^6/\text{ml}$ decreasing the volume of the vaccinated dose from two ml to one ml.

Sterility, safety and efficacy tests

Sterility test: a few vials were randomly removed, thawed and grown in aerobic, anaerobic, mycoplasma and fungal media.

Safety test: 10 pure-bred calves, three-five months old, were chosen. They had been brought from a farm where no case of theileriosis had been reported for the past five years. They were closely observed and examined for a period of one month to make sure they were totally healthy. They received all necessary bacterial and viral vaccines (Anthrax, Pasteurellosis, Food and Mouth disease and Rinder Pest).

Seven out of ten calves received one dose of the vaccine equal to one ml containing $2.5-2.8 \times 10^6$ schizont infected lymphoid cells. General condition, body temperature, size of lymph-nodes near the inoculation site were closely examined for a period of 30 days post-inoculation. During this period the blood and lymph-node smears were also checked three times a week.

Efficacy test: the vaccinated animals together with the three healthy calves, as controls, received the blood infected with challenge strain. General condition, daily temperature, blood and lymph-node biopsy smears of

the animals were strictly checked for a period of 30 days post-inoculation.

Serological test: complement fixation test was also performed before and after vaccination and challenge test (Hooshmand-Rad & Hashemi-Fesharki, 1971).

FIELD RESEARCH WORK

Experiment 1: 12 pure and cross-bred milking cows from a farm near Institute were vaccinated with new vaccine. All vaccinated animals were kept under control for a period of 30 days post-inoculation.

Experiment 2: 60 milking cows from three farms in Ghasvin (100 kilometre away from Institute) received the new vaccine. All vaccinated animals were closely observed for a period of one month.

Experiment 3: 1,500 milking cows from Zandjan province North-East of Iran, were vaccinated. They were kept under control for 30 days post-vaccination.

Experiment 4: 6,000 milking cows from different parts of Arak province, Central Iran, were vaccinated. They were kept under close observation for a period of 30 days.

Experiment 5: 10,000 cattle from Fars province, South Iran, were vaccinated. These animals, which were of various breed, age and task, were kept under close observation for 30 days post-vaccination.

RESULTS

LABORATORY RESEARCH WORK

Sterility tests indicated that the vaccine is free from any contamination and be used in the field (vaccine seed had already been checked to be free from any viruses).

Safety tests: general condition in vaccinated calves was satisfactory. Neither any significant temperature rise and enlargement of lymph-nodes near the site of inoculation nor erythrocytic forms were observed.

Efficacy test: the vaccinated calves came down with a sub-acute theileriosis due to reinoculation of very virulent challenge strain. Slight enlargement of prescapular lymph-nodes near the site of inoculation was observed. Rising of temperature was not more than $39.7-40^{\circ}\text{C}$ and the fever lasted for only 12-24 hours. Lymph-node biopsy smears showed schizont infected lymphoid cells, the number of which came to not more than 1-2 per microscopical field. The vaccinated calves survived and developed resistance against very virulent challenge strain of *T. annulata*. The controls (non-vaccinated calves), however, showed acute theileriosis with clinical symptoms and died to the disease.

Smears prepared from liver, spleen, abomasum's ulcers and mesentric lymph-nodes of the dead animals showed more than 20 schizont infected lymphoid cells

per microscopical field. They had also developed splenomegaly, hepatomegaly, cardiac and kidney petechia and abomasum ulcers.

Serological tests: the serological test showed that the antibody titre could be detected following first infection (usually 20-30 days post infection) and lasted for 100 days. It can be inferred that appearance of C.F. antibodies is due to multiplication of the parasite in the animal body and the resistance of the animals to homologous re-inoculation depends on cellular immunity rather than circulating antibodies. Re-inoculation of vaccinated calves with heterogenous virulent strain is followed by reappearance of complement fixing antibodies without causing significant clinical reactions.

FIELD RESEARCH WORK

Field evaluation of the vaccine indicated no adverse effect in more than 17,000 milking cows vaccinated in different parts of the country.

Neither abortion nor reduction of milk were observed in vaccinated milking cows. They tolerated the vaccine very well and resisted against natural acute theileriosis transmitted by tick vectors.

The non-vaccinated milking and pregnant cows (due to different reasons such as farmer's reluctance against vaccination and others), however, had very high morbidity rate among which the mortality rate reached more than 60 %. Milk reduction was common among these animals and a few cases of abortion were also observed.

DISCUSSION

The results indicated that the vaccine induces perfect immunity and the vaccinated calves were fully protected against lethal challenge with very virulent strain.

Since the vaccinated cattle do not act as a source of infection to tick-vector, therefore, it can be concluded that the S.T.C. vaccine can be safely used as a live vaccine in the field without any risk of spreading the disease.

The humoral responses in recovered cattle play a minimal role in mediating immunity (Irvin, 1985; Musoke & Nene, 1990), and passive transfer of sera from immune cattle with high antibody titre of *T. parva* or *T. annulata* is not protective (Musoke & Nene, 1990).

Hall (1988), Hall & Baylis (1993) and Irvin (1985) indicated that cell-mediated immunity (C.M.I.) is very efficient against theileriosis due to either *T. parva* or *T. annulata*. Based on our experience we also believe

that C.M.I. functions very actively in animals vaccinated against *T. annulata* infection.

Although the molecular techniques open up new possibility to develop recombinant vaccine for the control of theileriosis (Morzaria & Nene, 1990; Musoke & Nene, 1990; Musoke *et al.*, 1992; Nene *et al.*, 1992, 1995). The use of such techniques do not present a solution in short run but this initial success will hopefully result in the production of new and truly effective vaccine in not-too-far future. For the present time, however, in the absence of such molecular vaccine, the S.T.C. vaccine is the only effective, safe and a valuable vaccine to control the disease.

Since 1990 more than 1×10^6 cattle, pure and cross-bred, two months old and older, have been vaccinated with only one vaccine strain. No significant side or adverse effects have been reported. The vaccine possesses high prophylactic efficacy and give full protection against the disease. It should be noted that one strain-vaccination is more economical.

At present, production of S.T.C. vaccine with one strain in large scale plays the main role in our current programme for controlling the disease and the vaccine is routinely used in all parts of the country transported in liquid nitrogen. Regular vaccination has led to the establishment of *Theileria* immune cattle in infected zones of the country. It has also prepared the ground for importation of highly productive animals and a reduction in the usage of drugs and acaricides.

Finally it should be emphasized that the vaccine induces protective immunity for more than one year and has been welcomed by veterinarians and farmers. An investment in mass vaccination in developing countries where there are risks of infection is quite encouraging.

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