

**STUDIES ON THE ANTIGENIC VALUE AND
PREMUNITION AGAINST BOVINE THEILERIOSIS
DUE TO *THEILERIA ANNULATA*
(DSCHUNKVSKY AND LUHS 1904) IN IRAN
AND CONSERVATION OF STRAINS
OF *THEILERIA ANNULATA* AT - 70 °C (*)**

By :

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In a previous note (1), we have demonstrated that in Iran, exist some variants in the virulence and antigenic value of different strains of *Theileria annulata*.

The study of this very interesting variant has been followed subsequently in State Razi Institute, and we found again that a bovine premunized with a less virulent strain of *Theileria annulata* is susceptible to a rather short time with a more virulent strain of the same parasite; even this reinfection a second time cannot protect animals against a third, even more a fourth injection of a virulent material.

On the other hand, the homologous strain completely premunizes cattle in regard of the same strain.

Several experiments have been carried out in this way, and we are reporting in this paper 6 of them carried out recently.

MATERIALS AND METHODS

Bovines used in our trials aged 6-12 months, chosen from Holstein breed or our local breed called Sarabi that are more susceptible to Theileriosis. These animals are kept at tick free stables, regularly sprayed with insecticide. The blood of the animals is regularly examined, daily for a week before any experiment, to be sure that no gametocytes of *Theileria* exist.

Laboratory strains of *Theileria annulata* used in our experiments, are kept by passing on susceptible non-infected animals. (In these experiments we have not used materials preserved in frozen state). The virulence of these strains are as follows :

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Strain 3: A virulent strain kept by passage on cattle named Sarabi (local breed); these animals are known in the country for their dairy capacity and generally are free of Theileriosis. The mortality among these animals with strain 3 is 20 percent. The same strain killed some 50 percent of Holstein pure breed.

Strains, 5, 10 and 11: These are strains attenuated by passage on cattle previously premunized with a less virulent heterologous strain, and entertained on cattle of the Holstein breed. The mortality among these animals is 5-10 percent, while Sarabi breed completely resist these strains and the disease is always benign.

For the inoculation of animals, we inject subcutaneously 100 ml citrated blood, taken from cattle infected with *Theileria annulata* and presenting hyperthermia and schizonts in liver and lymph nodes. The rectal temperature of cattle is taken twice a day during the whole period of the experiment. Blood smears are prepared every day and examined after staining by Giemsa. The puncture of liver or prescapular lymph gland is done if animals show hyperthermy.

Experiment 1: A bovine (371) Holstein breed, premunized with strain 11 of *Theileria annulata*, has been inoculated 30 days after his recovery, with the blood of cattle No. 265 infected with a virulent strain of *Theileria annulata* (strain 3).

After a 17 days period of incubation, this animal presented a typical Theileriosis with fever, during 6 days with Koch bodies in the smears made from liver and lymph nodes. The animal suffered severely and attempts were made to help him by injection of some camphor oil, Acaprin and Antimozan during 48 hours. The animal recovered after a long period of convalescence.

Experiment 2: On the cow No. 372, Holstein breed, premunized with strain 11; after 10 days from this recovery two females of *Hyalomma anatolicum* collected from an infected area, are fed. After some 25 days of incubation this animal showed a typical Theileriosis with fever 40-40°6 C during 10 days, with Koch's bodies in liver and lymph nodes. This animal recovered soon, while Holstein breeds are very susceptible to the tick bites and generally succumbed to the disease.

These two trials showed once more that a less virulent strain of *Theileria annulata* cannot protect cattle against reinfection with a heterologous virulent strain, but nevertheless can give a partial premunition.

Experiment 3:

a) *First infection with strain 10:*

A bovine No. 347, Sarabi local breed, has been inoculated by the blood of cow No. 245, infected with an attenuated strain of No. 10 of *Theileria annulata*. After a period of 18 days, cow 347 presented a benign Theileriosis, with a duration of 4 days fever 40-41 °C, Koch's bodies in liver and lymph nodes.

b) *Reinfection with strain 5:*

After 23 days from the recovery, this animal is reinjected with the blood

of cattle 356 (infected with attenuated strain No. 5).

17th day later, the cattle showed a benign Theileriosis, with fever 40°4 C) during 4 days, with schizonts in liver and lymph nodes. The progress of the disease in a control animal (cattle No. 363) inoculated with the same strain was alike.

c) Third reinfection with strain 11:

Nine days after the recovery from the second infection, the cow No. 347 has been inoculated with the blood of cow No. 503 (Infected with strain 11, attenuated strain of *Theileria annulata*). After a period of 17 days, again the animal showed a benign Theileriosis during 3 days, with hyperthermia (39°9 C) and Koch bodies in liver and lymph nodes.

In control animals (bovines No. 357 and 468 Sarabi) the disease was similar, with a duration of 3-4 days, but the temperature was higher (40°-41° C).

Forth injection with strain No. 3:

The same animal (347) after 37 days, recovered from third reinfection (nearly 67 days after the recovery of the first infection) has been injected with the blood of cattle 481, infected with a virulent strain of *Theileria annulata* (No. 3). Seventeen days after, the temperature of the animal raised to 40 °C during 3 days, with Koch bodies in the liver. The disease was also benign, but the control animal (No. 485 Sarabi) showed a typical Theileriosis, rather severe with 41 °C temperature during 8 days with Koch bodies in the liver and lymph nodes. It recovered later on.

Partial conclusion

In these 3 experiments, mainly the third one, we have been able to demonstrate that the antigenic power of these 4 local strains are different. There is no crossed premunition between these strains. It is obvious that like many other protozoa or bacteria there are some antigenic relations between these strains but not enough for an acceptable premunition. Further studies are necessary to find the value of this strain in regard of a campaign of premunition.

COMPORIMENT OF HOMOLOGOUS STRAINS

As in our previous findings, the following experiments showed once more the existence of a solid premunition in regard of homologous strains.

Experiment 4: Bovine No. 475 premunized with a virulent strain No. 3 of *Theileria annulata* has been injected 130 days later with the blood of cattle No. 58 infected with the same strain. This cattle never showed any reaction during 60 days on observation period. Control animal (58) got a severe Theileriosis and died from the disease.

Experiment 5: The bovine No. 259, Sarabi, after 145 days from his recovery

TABLE I

EXPERIMENT No.	CATTLE No.	BREED	FIRST INFECTION WITH STRAIN	PERIOD OF DISEASE (Days)	INTERVAL BETWEEN FIRST AND SECOND INFECTIONS (Days)	SECOND INFECTION STRAIN No.	PERIOD OF THE DISEASE	RESULT
1	371	II(*)	11	7	30	3	7	Severe reaction: recovery
2	372	II(*)	11	5	10	Bitten by ticks	10	Typical Theileriosis (Recovery)
3(*)	347	S	10	4	23	5	1	Benign Id.
3	363	S	10	4	Control	---	---	
4	475	S	3	7	130	3	---	No reaction Theileriosis. Died
4	58	S	2	8	Control	Control	---	
5	259	S	2	5	115	3	---	No reaction Theileriosis. Died
5	281	S	3	9	Control	Control	---	
6	489	S	11	2	351	11	---	No reaction Theileriosis. Died
6	460	II	11	8	Control	Control	---	

II = Holstein, S = Sarabi Breed.

(*) The third and fourth reinfection are not shown on the table.

with strain 3, has been inoculated with the blood of No. 278 infected with strain 3 (homologous strain). This animal never showed any reaction, while control animal (281) became infected on the 18th day, and died on the 9th day from Theileriosis.

Experiment 6: Bovine 489, Sarabi, recovered from a Theileriosis (strain 11) and after 354 days, was infected with the blood of cattle 549, infected with strain 11. This animal resisted reinfection, the control animal (460) Holstein breed, after 16 days of incubation period, became infected, showed fever (41°8 C) and many schizonts and died after 8 days from the Theileriosis.

The experiments are in favour of a solid premunition in regard of homologous strain even after a rather long period (354 days). These animals are always kept in tick free area. Table I shows the result of these experiments.

CONCLUSION

There are a definite difference in antigenic value of strains of *Theileria annulata* isolated in Iran.

These strains while heterologous cannot premunize completely each against others, but there is some common antigenic power. Animals reinfected can generally show a benign Theileriosis. Homologous strains have a good value in premunition and can protect animals for a period, up to 354 days in our experiment. Experiments are on the way to find the longest period of the premunition in homologous strains.

A biochemical, electrophoresis and gel diffusion method can be helpful in the progress of this work.

CONSERVATION OF STRAINS OF THEILERIA ANNULATA AT -70 °C

Regular routine procedure to keep *Theileria annulata* for experimental purpose needs passage of materials, containing schizonts, to susceptible bovine, kept on special premises. This method while expensive, is sometimes difficult due to shortage of susceptible animals and danger of contamination of materials by viral, bacterial or parasitological agents.

TSUR and PIPANO (1962) demonstrated that strains of *Theileria annulata* may be kept at -70 °C for 150 days (2).

We tried this technique with a satisfactory result. For this purpose:

1. Citrated blood containing schizonts in liver and lymph nodes is taken during hyperthermia of the infected animal. Pure glycerin 12 percent of the volume of blood is added.

2. Spleen or liver broyat diluted with saline (pH 7.4) three times of the volume of this material and to the whole mixture 15 percent pure glycerine is added.

These mixtures are divided in ampoules of 20 ml and flasks of 20 and 50 ml, closed carefully and containers are plunged for about one hour in methyl alcohol, containing parcels of dry ice, and put afterwards in cases containing dry

ice at -70 °C. With this technique, we have been able to keep different strains of *Theileria annulata* for 277 days.

Table II shows the results of inoculation of such materials at different periods:

TABLE II

PRESERVATION AT -70 °C				INOCULATION	OBSERVATION
Material	Cattle	Strains	Conser- vation (Days)	Cattle	Results
Spleen	206	3	32	382	Severe Theil. Died
Liver	198	5	32	519	Benign Theil. Recovery
Liver	198	5	86	228	Benign Theil. Recovery
Liver	198	5	150	331	Benign Theil. Recovery
Blood	42	11	101	516	No reaction
Spleen	359	5	160	199	Typical Theil. Recovery
Liver	179	10	176	77	Ben. Theil. Recovery
Spleen	332	3	239	17	Typical Theil. Recovery
Spleen	353	11	250	79	Benign Theil. Recovery
Blood	505	3	277	601	Severe Theil. Died

Table II shows that blood from calf 505, infected with strain 3 was virulent after 277 days and was capable to transmit the disease to animal 601, with no change in the virulence, while attenuated strains 5, 10, 11 were always infective and able to give a benign Theileriosis.

A failure with the blood of cow 42 kept 104 days, with no explanation has been observed.

This technique and liquid nitrogen (3) is recommended for investigation and used for more experimental and perhaps immunological purposes, in laboratories working with *Theileria annulata* and allied parasites.

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