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SURVIVAL OF TRYPANOSOMA EVANSI, THEILERIA MUTANS, BABESIA BIGEMINA AND ANAPLASMA MARGINALE FOLLOWING LONG TERM MAINTENANCE AT - 70°C.

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SUMMARAY

Blood infected with locally isolated strains of Trypanosoma evansi, Theileria mutans, Babesia bigemina and Anaplasma marginale, using an uncontroled freezing method, were maintained at - 70°C for a period of four to eight years.

Although the uncontroled freezing method is as practicable as the gradual freezing method for storing the blood protozoa of the camel and the cattle, nevertheless under unsuitable technical conditions the uncontroled freezing method seems to be more convenient.

INTRODUCTION

Weinman and MacAllister (1947) suggested that many important protozoa in tropical medicine may be stored at low temperature without losing thier original characteristics. Waddel (1963) maintained Babesia bigeming in frozen state, using a rapid freezing method, and Barnett (1964) stored Babesia bigemina, Anaplasma marginale and Anaplasma centrale, using the slow cooling method to - 70°C. Tsur and Pipano (1962), Rafyi et al (1967) and Hashemi - Fesharki and Shad - Del (1973) stored different strains of Theileria annulata at - 70°C, using the uncontroled freezing method.

This paper describes the method through which , and the period during which Trypanosoma evansi, Theileria mutans, Babesia bigemina and Anaplasma marginale were stored in frozen state at the Razi Institute.

MATERIALS AND METHODS

Organisms: The strains of protozoa which locally isolated from infected animals, and maintained by syringe passage in susceptible animals or kept in a frozen state at - 70°C are as follows:

1 - . **Trypanosoma evansi** : This is a virulent strain which has been isolated from infected camel and its mortality rate for the inoculated rats is above 80%.

2 - . **Theileria mutans** : This organism, which has been isolated from an infected calf, is non - pathogenic. The schizonts could not be detected with certainty in lymph-nodes biopsy smears, but the piroplasms were seen in the peripheral blood smears. The parasite was considered to be **Theileria mutans** on the basis of its behaviour and its piroplasms morphology.

3 - . **Babesia bigemina** : It is a virulent strain. It has been isloated from a sick calf which had a parasitaemia of approximately 30%.

4 - . **Anaplasma marginale** : This is a mild strain which has been isolated from an indigenous calf and its parasitaemia reaches to a level of 14% in Holstein Freisian calves.

Animals : Twelve Holstein Fresian calves aged 4-6 months and five rats aged 3-4 months were used for this study. One month before the study, the peripheral blood smears of calves and rats were examined microscopically three times per week to make sure that they were free from any kind of blood protozoa.

After inoculation of organisms, the rectal temperature of the experimental calves was taken daily. The blood smears of inoculated calves and rats, After being fixed with metanol and stained with Giemsa's stain, were thoroughly examined under a microscope .

Methods : In addition to sodium citrate which was used as an anticoa - gulent at a level of 0.5g per 100 ml of blood, glycerol was employed as a cryoprotectant. The level of glycerol was 8% for infected blood of rats and 13.5 % for that of calves.

The methods of freezing, storing and thawing of infected blood were performed as described by Hashemi - Fesharki and Shad-Del (1973). The inoculation route was subcutaneous in calves and interaperitoneal in rats.

EXPERIMENTS AND RESULTS

Experiment No, 1 : The blood infceted with **Trypanosoma evansi** stored eight years in a deep freeze, was inoculated into five rats, one ml per rat. The parasitaemia appeared on the 7th post - inoculation day and reached its maximum

level after three days. The animals died due to an acute **Trypanosoma evensi** infection.

Experiment No. 2 : Eight vials' contents of 128ml infected blood with **Theileria mutans,** kept in a deep freeze for eight years, were thawed and inoculated into four calves, each 32 ml. The reactions of the inoculated calves were varied. The parasitaemia was observed on the 25 th to 27 th post- inoculation days. First it reached a level of 12 % and then it decreased to 0.5% .

Experiment No. 3: Eight vials' contents of 128ml infected blood with **Babesia bigemina,** frozen and stored for five years, were thawed and inoculated into four calves, each 30ml, The calves revealed parasitaemia on the 7th to 8th post-inoculation days and it reached 20 to 30%, showing typical symptoms of piroplasmosis. They were treated with 1mg/kg of imidocard dihydrochloride.

Experiment No. 4: Ten large vials' contents of 160 ml infected blood with **Anaplasma marginale**, stored in a deep freeze for four years, thawed and inoculated into five calves, each 32ml. The parasitaemia appeared on the 30th to 32nd post-inocluation days. Parasitaemia reached a maximum level of 14% then it gradually decreased and reached a leves of 1-2% in two months, during which the experiment lasted.

DISCUSSION AND CONCLUSION

The study showed that the strains of **Trvpanosoma evansi, Theillria mutans, Anaplasma marginale and Babesia bigemina** using the uncontroled freezing method, survived after they had been stored at - 70 °C for a long period of time; longest survival period that has been attained so far is eight years for **T. evansi** and **T. mutans** but four and five years for **A. marginale** and **B. bigemina** respectively .

The uncontroled freezing method as compared with gradual freezing method, does not possess more harmful effect on the pathogenicity of the strains, and it does not cause any biological and characteristic changes of the parasites studied. The method can also be deemed a practical way to maintain the blood pathogen protozoa for a long period of time and is more useful than their maintenance in susceptible animals.

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