Arch. Inst. Razi, 1973, 25, 89 - 91

LONG TERM MAINTENANCE OF THEILERIA ANNULATA STRAINS BY FREEZING AT - 70°C (*)

By

R. HASHEMI-FESHARKI and F. SHAD-DEL

Tsur and Pipano (1962) preserved *Theileria annulata* in a frozen state for up to five months, using 15 per cent glycerol. Waddel (1963) reported on the maintenance of *Babesia bigemina* in a frozen state using a rapid method of freezing, and Barnett (1964) preserved *B. bigemina, Anaplasma centrale* and *A. marginale* using the gradual freezing method. Rafyi, Maghami and Hooshmand-Rad (1967), could maintain *T. annulata* at - 70° C. for nine months, using 12 and 15 per cent glycerol. Uilenberg (1971) maintained strains of *B. bigemina, A. centrale,* and *A. marginale* that were frozen by both rapid and gradual methods.

Strains of *Theileria annulata* are maintained in three ways at the Protozoology Laboratory of the Razi Institute:—

1. Continuous sub-culture in susceptible calves, *i.e.* Holstein (an exotic breed) and/or Sarabi (a local breed), by blood inoculations from infected animals when these animals show the highest febrile reactions and numerous Koch's Blue Bodies appear in stained smears of the lymph gland and liver.

2. Gametogenetic strains are maintained in vector ticks, and then are transmitted to susceptible animals.

3. Maintenance of strains at - 70° C.

In addition to preserving strains of *T. annulata* in a frozen state, we store large amounts of vaccines produced from different strains of the parasite at - 70° C. until inoculation. Thus we were interested to determine how long the strains could be kept at - 70° C. without decrease in virulence. A blood, liver, spleen, and culture medium containing lymph cells infected with schizonts were

(*) Reprinted from Vet.Rec. (1973), 92, No. 6, 150–151

used and 0.5g. per cent sodium citrate was added as anti-coagulant. The liver tissue and spleen tissue were ground and diluted with three times their volume of phosphate buffered saline (P.B.S.), (*p*H 7.4). The schizont-infected lymph cells were concentrated to $1-2\times10^6$ per ml. in the culture (medium), Eagle+20 per cent. Y.L.H. (yeast extract lactalbumin hydrolystate), containing 10 per cent calf serum (C.S.) in total volume. After adding 13.5 per cent glycerol to the blood, liver and spleen tissues and 10 per cent. glycerol to the concentrated lymph cells, these mixtures were stirred by a magnetic stirrer for three to five minutes. The mixtures were distributed in 20 ml. vials. These were stoppered with elastic pressed-aluminium lids and directly stored in a refrigerator at –70° C. Carrying the strains, especially vaccine materials, to the field, has been successfully accomplished in dry CO^2 in vacuum flasks.

The vaccine was thawed in water at +37°C. prior to vaccination. Subcutaneous inoculation was made at the rate of 80 to 90 ml. blood, 10 to 15 ml. of emulsified spleen-liver tissues, and $2-4\times10^6$ lymph cells.

During the three years of this study, 85 susceptible Holstein and Sarabi calves were inoculated with the infected blood, liver tissue, spleen tissue, and T.C. lymph cells which were maintained at –70° C. for periods of one to 1095.

Thermal and parasitic reactions appeared in all inoculated animals in a similar way to the control animals inoculated with unfrozen materials. Schizonts were seen in lymph cells, in gland and liver biopsies and proved the presence of theilerial infections. Intensities of thermal and parasitic reactions of the inoculated calves were related to the virulence of the inoculated strains.

No changes occurred in the virulence of the different strains of *T. annulata* maintained constantly at –70° C. for 400,545 and 1095 days. Maintenance of *T. annulata* in a frozen state is more practicable than the methods of continuous passage in susceptible animals or transmission by vector ticks.

Strains of certain protozoans can never be maintained free of other blood parasites through the continuous passages. Inoculated calves which seem to be healthy and do not show parasites in their blood may be naturally infected with other protozoans such as *T. mutans, A. marginale, A. centrale, Eperythrozoon wenyoni,* and *Rickettsia bovis.* Consequently, there is no simple way to separate the different parasites, except by using the deeply frozen-kept strain. Maintenance of the endoerythrocytic form of *T. annulata* in vector ticks is not very successful because of certain operational difficulties, such as attachment to the host animal and latent infections of other parasites in the hosts.

Acknowledgments.—The athors wish to thank Dr. G. Maghami, head of the Department and Dr. P. Hooshmand-Rad for their advice. We are also grateful to Dr. M. Kaveh, General Director, Razi State Institute for his support.

References

Barnett, S. F. (1964). *Vet. Rec.* **76.** 4.
Rafyi, A., Maghami, G., & Hooshmand-Rad, P. (1967). *Bull. Off. Int. Epiz.* **68.** 749.
Tsur, I., & Pipano, E. (1962). *Refuah Vet.* **19.** 100.
Uilenberg, G. (1971). *Rev. Elev. Med. Pays Trop.* **24.** 23.
Waddel, A. H. (1963). *Aust. vet. J.* **39.** 400.