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VACCINATION OF CALVES AND MILKING COWS WITH DIFFERENT STRAINS OF *THEILERIA ANNULATA* (*)

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SUMMARY

A vaccine prepared from strains of *Theileria annulata* in cell culture suspension was inoculated in 454 calves and 88 milking cows during 2 years. Appearance of the organism and febrile response in the calves and cows vaccinated under laboratory and field conditions occurred with less frequency than in animals infected with virulent organisms. Immunity in vaccinated animals persisted for more than 1 year.

Sergent et al. [10-13] certain workers [2-4,10-14] attempted to vaccinate calves and milking cows against theileriasis by inoculation of blood collected from sick animals (at peak of reaction) infected with some milder strains of *T. annulata*. The same method of vaccination was used in Israel, by Adler and Elenbogena,[a] and in the U.S.S.R., by Markov. [7]

Rafyi and Maghami [9] referred to the possibility of transmission of other parasitic protozoa with the blood used for the anti-theileriasis vaccination.

Tsur-Tchernomoretz [15] and Tsur-Tchernomoretz et al., [16,17,19] Hulliger,[9] Zablothskii,[b,c] and others [1,8,d] obtained growth of *T. annulata* schizonts in vitro, thus facilitating the production of a cell-culture vaccine against theileriasis. Hooshmand-Rad and Hashemi-Fesharki [5] obtained growth of *T. annulata* schizonts in suspension and reported that this method was more practical than the existing method of monolayer cultural growth for mass-producing vaccine.

In the present report, vaccination of calves and milking cows against *T. annulata* with a cell-suspension culture is described.

Materials and Methods

Animals. In the laboratory, the selection of *T. annulata* strains for the preparation of vaccine was based upon trials made with Holstein calves between 5 and 12 months of age. This category is regarded as being the most susceptible

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to *T. annulata* infections. The history of each of these calves was carefully checked, and blood films and gland biopsies examined before consignment to trial. There is thus reasonable certainty that none of these trial animals had previously been exposed to *T. annulata* infection.

In the field vaccination experiments, however, pure or cross-bred Holsteins, Red-Danish, Swiss, and Rotvieh were involved. Though the history of these cattle could not be as carefully ascertained as in the case of the trial cattle, the epidemiologic background and other circumstances made it highly unlikely that any had previously contracted a *T. annulata* infection.

Organisms.–Locally isolated strains of *T. annulata* used for vaccine preparation and challenge inoculation tests in the experiments were (1) vaccine strain 15 (S–15), a moderately virulent strain which has lost its erythrocytic form through several passages in vivo or in vitro (or both); (2) vaccine strain 11 (S–11), a mild strain which has lost its erythrocytic form in a manner similar to that of S–15; and (3) strain 3 (S–3), a virulent strain which was used to challenge inoculate the animals vaccinated with S–15. The last-named strain caused approximately 80% mortality in susceptible Holstein calves.

Vaccine Preparation.–Eagle's balanced salt solution with added yeast extract lactalbumin hydrolysate (YLH; 20%) was used as the culture medium. Schizont-infected lymphoid cells originally obtained by biopsy technique from the prescapular gland of an infected calf were inoculated in the culture medium.[5] These lymphoid cells were then successively subcultured at 4-day intervals in the form of a suspension and were kept in an incubator (37°C). Batches of the suspension cultures at various passages were preserved at –70°C. In the cultures, almost all of the lymphoid cells were infected with schizonts.

To prepare anti-theileriasis vaccine, large Roux flasks containing 80 to 100 ml. of medium and 6 to 7 ml. of suspension culture (4.2 to 4.9 $\times$ 10^6 schizont-infected lymphoid cells) were used. After cells had completed growth, the material was harvested and concentrated to 1.5 to 2.0 $\times$ 10^6 infected cells. Glycerol (10%) was added, and the final suspension (15–ml. quantities) was distributed into small 20–ml. vials. The vials were transferred to a deep freezer at – 70°C. until required.

Vaccination Methods.–The vials of vaccine, kept frozen with solid carbon dioxide, were transported to the field experimental site. The vaccine was thawed–either in warm water (37°C.) or in the hand–immediately before use. Each animal was inoculated subcutaneously with 2 ml. of the vaccine (containing 3 to 4 $\times$ 10^6 schizont-infected cells).

One injection of S–15 was used to vaccinate calves of 4 to 12 months old, and bulls of more than 12 months. Nonpregnant milking cows were given 2 injections at 30- to 60-day intervals–the 1st, S–11, and the 2nd, S–15.

TABLE 1—Procedure of Laboratory Experiments, (Groups A and B); Response to Vaccination and Challenge Inoculations

Group	Vaccination and subsequent challenge inoculum		No. of calves showing response		Febrile response			Parasitic response		
	No. of Calves	Inoculum	Febrile	Parasitic	Days of incubation (No. of calves)	Days persisting (No. of calves)	Maximum rectal temperature (No. of calves)	Day of incubation (No. of calves)	Days persisting (No. of calves)	Maximum degree of infection-schizonts per microscopic field (No. of calves)
A-Principles	21	Vaccinal S-15	18	15	11 (10); 15 (8)	1 (9); 3 (8); 6 (1)	40.5 to 41 (15)	14 (8); 18 (7)	2 (7); 5 (7); 6 (1)	BB (12); +BB (3)*
	18**	Challenge S-3	16	9	15 (11); 19 (5)	1 (9); 3 (6); 6 (1)	39.5 to 40.5 (15); 41 (1)	18 (5); 22 (4)	2 (4), 5 (5)	BB (7); +BB (1)
A-Control	3	Challenge S-3 only	3	3	14 to 15 (3)	7 to 8 to 10 (3)	41.6 (1); Died (2)	15-16-18 (3)	6-7-8 (3)	++BB (1); +++BB (2)
B-Principles	12	Vaccinal S-11	10	5	11 (4); 15 (6)	1 (10)	39.5 to 40.5 (10)	14 (4); 18 (1)	2 (5)	BB (5)
	11**	Challenge S-15	6	2	11 (6)	1 (6)	39.5 to 40.5 (6)	14 (2)	2 (2)	BB (2)
B-Control	3	Challenge S-15 only	3	3	12 (3)	2 (3)	40.5 to 41 (3)	13 (3)	4 (3)	BB (3)

* BB = Koch's blue bodies, (schizonts), less than 1 schizont per microscopic field, +BB = 1 to 5, ++BB = 5 to 10, +++BB = 10 to 15, and ++++BB = over 15 schizonts per microscopic field. ** = three calves in group A and 1 calf is group B were not challenged due to affliction with nonspecific illness prior to challenge test.

TABLE 2— Procedure of Field Experiments (Groups C and D): Febrile Response to Vaccination

Group	No. and category of animal	Vaccinal inoculum	No. of animals showing febrile response	Febrile response: Days of incubation (No. of animals)	Febrile response: Days persisting (No. of animals)	Febrile response: Maximum rectal temperature (C°) (No. of animals)
C	122 Calves	S-15	106	11 to 14 (60); 15 to 18 (39); 19 to 22 (7)	½ to 3 (66); 4 to 6 (36); 7 to 9 (4)	39.5 to 40.5 (50); 40.5 to 41 (22); 41 to 41.5 (34)
D	70 Milking cows	1st dose S-11	12	11 to 14 (3); 15 to 18 (5); 19 to 22 (4)	½ to 2 (12)	39.5 to 40.5 (10); 41 (1); 41.5 (1)
		2nd dose S-15	19	11 to 14 (15); 15 to 18 (4)	1 to 3 (17); 4 to 7 (2)	39.5 to 40.5 (17); 41 (1): 41.5 (1)

Results

Laboratory Investigation.—Thirty-three susceptible calves were inoculated with vaccine–21 with S-15 and 12with S-11. Eighteen of the calves vaccinated with S-15 were challenged 1 month later with S-3 together with 3 unvaccinated control calves. Of the 12 calves vaccinated with S-11, 11 were challenge inoculated 1 month later with S-15 together with 3 unvaccinated control calves. The results of these trials are given (Table 1).

In group A (Table 1), the 21 calves vaccinated with S-15 had febrile or parasitic reactions, or both. Of the 18 calves in this group that were given challenge inoculations 1 month after vaccination, 2 had neither febrile nor parasitic reactions–the remainder showing one or the other, or both, reactions. All 3 control calves in this group developed acute theileriasis, and only 1 recovered.

In group B, 10 of the 12 calves inoculated with S-11 had febrile or parasitic reactions, or both (Table 1). Of the 11 vaccinated calves given challenge inoculations of S-15, 5 calves did not show any response and the remaining 6 were only mildly affected. However, both febrile and parasitic reactions were seen in the 3 group B control calves which received S-15.

Field Experiments.—Of the 454 vaccinated calves and 88 vaccinated milking cows, 122 and 70, respectively, were examined daily for febrile reactions. The findings are summarized (Table 2).

Discussion

Results of clinical observations and microscopic biopsy examinations of calves vaccinated and challenge inoculated under laboratory control conditons (Table 1) indicated that the group A calves, inoculated with S-15, showed a shorter incubation period, had higher and more persistent fever, and had more schizonts in lymph gland biopsy material and for a longer period. But, on challenge inoculation with S-3, these vaccinated calves presented longer incubation periods and had less febrile and parasitic reactions. Moreover, rectal temperatures in these exposed calves did not increase beyond 40.5°C. and the schizonts were less plentiful, i.e., not more than 1 Koch's blue body per microscopic field. In contrast, the control calves inoculated with challlenge inoculum S-3 developed severe signs of theileriasis. Rectal temperatures rose to 41.6°C., Koch's blue bodies increased to between 10 to 15 and more than 15/ microscopic field, and finally 2 of the calves died of acute theileriasis. It was therefore concluded that the calves vaccinated with a live attenuated strain, such as S-15, developed sufficient immunity to resist the challenge inoculation of the virulent S-3.

Considering the febrile, parasitic reactions in group B calves vaccinated

with S-11 and given challenge inoculum S-15, it was determined that vaccination was generally efficacious against S-15 challenge inoculum.

In the calves vaccinated under field conditions (Table 2), rectal temperatures in 34 out of 122 calves (27.86%) increased to 41 to 41.5°C., and these peaks were transient, i.e., did not last more than a day.

Febrile responses of the doublevaccinated milking cows under field conditions (Table 2) indicated that these cows readily tolerated the mildly virulent S-11 used in the 1st vaccination and acquired sufficient immunity to resist the moderately virulent S-15 used in the 2nd vaccination without showing severe reactions. It might well be that the immunity levels in these cows after the 2nd vaccination would be high enough to resist virulent strains of *Theileria*—at least for a limited period.

Vaccination of cattle in given areas has not been followed by reports of reduced milk production, and no enzootics have occurred since. Seemingly, S-11 and S-15 of *T. annulata* induce an immunity in susceptible cattle which persists for at least 1 year.

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