

## QUANTITATIVE STUDIES OF THREE DIFFERENT STRAINS OF THEILERIA ANNULATA IN EXPERIMENTAL CALVES (\*)

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### SUMMARY

The effect of increasing concentrations of three different strains of *Theileria annulata* is reported. The very mild strain S. 11 was used at dose rates which varied from  $3 \times 10^6$  to  $15 \times 10^6$  schizont-infected lymphoid cells, and the calf which received the maximum dose showed neither parasitic nor thermal reaction when challenged with the moderately virulent S. 15.

Seven calves were infected with doses of S. 15 which varied from  $7.5 \times 10^5$  to  $300 \times 10^6$ . One calf which received  $1.5 \times 10^6$  showed thermal reaction only when challenged with the very virulent S. 3, while another which received  $7.5 \times 10^5$  showed both thermal and parasitic reactions when challenged with S. 3. Both of these animals recovered. The five remaining animals received doses of S. 15 which varied from  $3 \times 10^6$  to  $300 \times 10^6$  showed both parasitic and thermal reactions, and all survived challenge with S. 3.

Five other animals were inoculated with varying doses of whole blood infected with S. 3. One receiving 90 ml died of theileriosis, but the remaining four which received doses varying between 15 and 45 ml showed parasitic and thermal reactions resembling those observed in the fatal case.

Increasing the dose of inoculum was seen to confer a stronger resistance to a more virulent strain, but would provoke thermal and parasitic reaction if carried beyond certain limits with all strains.

Increasing the dose did not alter the prepatent periods for thermal and parasitic reactions for particular strains. Calves inoculated with doses of less than  $7.5 \times 10^5$  did not show parasitic reactions and did not withstand challenge

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## INTRODUCTION

As described by various authors (1), (2), (3), (4), (5), theileriosis caused by *Theileria annulata* and *Theileria parva* present serious disease problems in cattle in many tropical countries. A recent advance in theilerial research techniques was made when Hooshmand-Rad and Hashemi-Fesharki (6) adapted strains of *T. annulata* to a tissue culture system. Vaccines derived from these systems were used in successful trials in calves and milking cows by Hashemi-Fesharki and Shad-Del (7) and by Pipano and Tsur (8).

After Jarrett *et al* (9) had indicated the nature of quantitative replicatory kinetics in *T. parva* infections, Radley *et al* (10) showed that both the onset of the febrile response and the effective rate of macroschizont multiplication were dose-dependent in East Coast fever. In the work described here, varying doses of three different strains of *T. annulata* were used to examine the following aspects –

1. Whether an increased concentration of *T. annulata*-infected lymphoid cells in an inoculum would afford greater protection against more virulent strains;
2. The minimum amount of infective material required to give protection against homologous and heterologous strains;
3. The maximum tolerable dose of infective material; and
4. Whether significant differences exist between the reactions caused by different doses of the various strains.

## MATERIALS AND METHODS

The Holstein-Friesian calves were between four and six months old, and were obtained from tick-free farms. One month prior to experiment, the calves' freedom from blood parasites was confirmed by the examination of blood smears.

Three strains of *T. annulata* were used. Strain No. 3 (S.3) is a very virulent strain and is maintained for laboratory use by the straightforward passage of infective whole blood in susceptible calves. The other two strains - the moderately virulent S. 15 and the very mild S.11 - are maintained *in vitro* by propagating schizont-infected lymphoid cells in suspension cultures. All three strains have lost the ability to produce intra-erythrocytic piroplasms. Infections were conferred by subcutaneous inoculation of infective material - whole blood in the case of S.3, suspension cultures in the cases of S.15 and S.11.

Rectal temperatures were recorded each day, and daily examinations were made of Giemsa-stained lymph-node smears and blood films. The numbers of macroschizonts were recorded as a parasitic index - i.e., expressed as the average number of macroschizonts in 20 examined fields. Clinical signs were closely observed, and detailed *post mortem* examinations were carried out in all fatal cases.

In culture inocula , doses were graduated according to the numbers of schizont-infected lymphoid cells - e.g,  $1.5 \times 10^6$ ,  $3 \times 10^6$  , and so on. For brevity doses are expressed hereafter in these numerical terms . Doses of S .3 were varied by volume - i.e., inocula varied between 15 and 90ml and infectivity in this strain was measured as being between three and five macroschizonts per field .

The experimental plan was simple . Calves vaccinated with varying doses of the very mild S. 11 were subsequently challenged with  $3 \times 10^9$  of the moderately virulent S. 15. Calves vaccinated with varying doses of S. 15 were challenged with 50ml of S. 3. Calves infected with varying doses of S . 3 were not challenged since no more virulent strain was available .

## RESULTS

### *Experiment 1*

The results of vaccination with varying dose of S . 11 and of the subsequent challenge with S . 15 are summarised in Table I . It will be seen that increasing the doses of S . 11 did not cause any significant reduction in the thermal incubation period , nor in the prepatent period . It will also be noted that first appearance of macroschizonts of S . 11 was much later ( 17 -18 days ) than with S . 15 in the unvaccinated control animal No . 43 ( 13 days ). Further, no macroschizonts were detected in the vaccinated animals when they were challenged with S . 15 .

### *Experiment 2*

Seven intact calves were inoculated with varying doses of S . 15 as shown in Table II . It will be seen that no reaction at all was discerned with the lowest dose ( $7.5 \times 10^5$ ), and that only a thermal reaction occurred with the next lowest dose ( $1.5 \times 10^6$ ). Thereafter, increasing the dose from  $3 \times 10^5$  to  $300 \times 10^6$  did not reduce the prepatent period , nor did it produce a progressive shortening of the incubation period . It did , however , produce a greater number of macroschizonts , and tended to prolong the duration of their presence .

The results of challenging these calves with S . 3 are shown in Table III . It will be seen that challenge to No . 37 - the animal which showed no response to vaccination with S . 15 - gave a reaction which was not as severe as that in the unvaccinated control animal ( No . 16 ) Challenge of No . 99 , which had shown only a thermal response to vaccination with S.15, gave an even milder reaction. None of the other calves vaccinated with S. 15 showed any discernible reaction to challenge with S. 3. The control calf No. 16 died on the 24 th day after inoculation with S . 3 and *post mortem* examination showed enlargement of the liver , spleen and prescapular lymph nodes , and also ulcerative abomasitis . Impression smears of liver , spleen and lymph nodes showed the presence of 15 to 20 macroschizonts per field . Non - suppurative inflammation of the apical and cardiac lobes of the lungs was also noted .

### Experiment 3

The thermal and parasitic reactions to varying doses of S . 3 are shown in Table IV , It will be seen that increasing the dose of blood infected with this highly virulent strain did not materially influence the duration of the incubation period and did not reduce the prepatent period. Further , increasing the dose did not appear to alter, materially , the duration of the period over which macroschizonts could be discerned, nor the maximum number of macroschizonts per field. The maximum dose 90 ml of S. 3. did , howere, produc the onlty fatal reaction . On *post mortem* examination , this showed enlargement of liver , spleen and lymph nodes , and numerous « punched » ulcers were seen in the abomasum . Impression smears from liver, spleen, lymph node and simulated lymphomatosis showed the presence of macroschizonts which were typical of those found in reactions caused by S . 3 .

### DISCUSSION

On many previous occasions, transmission studies using infective blood or infected ticks have given inconsistent results , and hence this work was carried out in an attempt to standardise levels of infection so that typical reactions could be reproduced reliably . The procedure adopted at the Razi Institute has been one in which doses are graduated by means of standard concentrations of infected lymphoid cells .

In Expeeriment 1 , no really clear - cut effect on initiat reactions appeared on varying the dose of the very mild S.11. Increasing the initial doses of S . 11 did , however , appear to confer a greater degree of resistance to challenge with the moderately virulent S . 15 .

In Experiment 2 , all calves initiallty inoculated with doses of between  $3 \times 10^6$  and  $300 \times 10^6$  showeed typical thermal and parasitic reactions , and thereafter resisted challenge with the highly virulent S . 3 . When inoculated at the lowest concentration ( $7.5 \times 10^5$ ) , S . 15 apparently failed to establish itself for , on challenge with S . 3 , the animal concerned showed severe thermal and parasitic reactions . On the other hand , the animal which received an initial inoculation of  $1.5 \times 10^6$  of S . 15 showed only a mild thermal reaction when challenged with S . 3 This supports the indication of the results from Experiment 1 i. e. , that increasing the quanta of infective material in a vaccination gives greater protection against a more virulent strain. The results also show that there is an upper limit to this , for increasing the vaccine dose beyond  $3 \times 10^6$  gave no further advantage in protection and had the disadvantage of causing severe thermal and parasitis reactions .

In Expenriment 3, one effect which appeared to be attributable to an increase in infective dose was that the maximum level of parasitosis was achieved more rapidly i.e., in the animal which received 90 ml of blood and in one of the animals

which received 45 ml of blood, this maximum level was achieved two-three days earlier than with the others. Apart from this, there were no significant effects attributable to varying the volume of infective blood administered, but it must be recalled that doses can not be graduated as accurately by this method as they can by tissue culture techniques.

These experiments showed that increasing the quanta of infection of a particular strain did not reduce the incubation or prepatent periods. Though *minimum* quanta of infection were seen to be necessary for the establishment of an infection and for the development of resistance, no protective advantage was gained by increasing dose levels much beyond this required minimum.

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Table I. Increasing doses of schizont-infected lymphoid cells of *T. annulata* strain No. 11 (S-11) and challenge test with  $3 \times 10^6$  schizont-infected lymphoid cells of strain No. 15 (S-15)

Reaction parameters	Doses of inoculum by subcutaneous route										
	$3 \times 10^6$ cells				$4.5 \times 10^6$ cells				$15 \times 10^6$ cells		Control
	No. 38		No. 39		No. 40		No. 41		No. 42		No. 43
	S-11	S-15	S-11	S-15	S-11	S-15	S-11	S-15	S-11	S-15	S-15
Incubation period (days)	48	-	-	13	-	13	17	13	17	-	12
Duration of thermal reaction (days)	1	-	-	2	-	2	1	1	3	-	3
Maximum rectal temperature (C <sup>o</sup> ),	39.7	-	-	39.8	-	40.4	39.8	39.7	39.9	-	39.9
Days after infection on which max. rectal temperature occurred	18	-	-	14	-	14	17	13	19	-	14
Days to the appearance of schizonts in prescapular lymphnode and their number per microscopic field	-	-	17	-	-	-	-	-	18	-	13
No. of days on which schizonts were discerned	-	-	+ <sub>1</sub> <sup>b</sup>	-	-	-	-	-	1	-	3
Maximum No. of schizonts per microscopic field	-	-	1	-	-	-	-	-	2	-	2
Days after infection on which max. no. of schizonts were discerned	-	-	1	-	-	-	-	-	2	-	3
	-	-	17	-	-	-	-	-	19	-	14

b = Very few macroschizont per microscopic field.

Table 2. Increasing doses of schizont-infected lymphoid cells of *T. annulata* strain No, 15 (S-15)

Reaction parameters	Doses of inoculum by subcutaneous route						
	$7.5 \times 10^5$ cells	$1.5 \times 10^6$ cells	$3 \times 10^6$ cells		$30 \times 10^6$ cells		$300 \times 10^6$ cells
	No. 37	No. 99	No. 56	No. 57	No. 58	No. 61	No. 62
Incubation period (days)	-	18	12	12	12	12	12
Duration of thermal reaction (days)	-	1	3	5	4	5	3
Maximum rectal temperature (C <sup>0</sup> )	-	39.8	39.8	39.7	39.6	40.5	41
Days after infection on which max. rectal temperature occurred	-	18	14	13	16	17	14
Days to the appearance of schizonts in prescapular lymphnode	-	-	12	14	12	12	12
and their numbers per microscopic field	-	-	1	1	1	1	1
No. of days on which schizonts were discerned	-	-	3	3	4	7	5
Maximum No. of schizont per microscopic field	-	-	4	5	8	10	15
Days after infection on which max. no. of schizonts were discerned	-	-	14	15	14	14	14

Table 3. Challenge inoculation of calves, vaccinated with various doses of inocula as in Table 2, with *T. annulata* strain No. 3 (S-3)

Reaction parameters	No. 37	No. 99	No. 56	No. 57	No. 58	No. 61	No. 62	Control No. 16
Thermal incubation period (days)	16	19	-	-	-	-	-	16
Duration of thermal reaction (days)	5	2	-	-	-	-	-	7
Maximum rectal temperature (C <sup>o</sup> )	40.9	39.9	-	-	-	-	-	41.3
Days after infection on which max. rectal temperature occurred	18	19	-	-	-	-	-	19
Days to the appearance of schizonts in prescapular lymph-node and their numbers per microscopic field	17	-	-	-	-	-	-	17
No. of days on which schizonts were discerned	1	-	-	-	-	-	-	1
Maximum No. of schizont per microscopic field	4	-	-	-	-	-	-	6
Days after infection on which max. no. of schizonts were discerned	10	-	-	-	-	-	-	20
	18	*	-	-	-	-	-	19,20, 21, 22,23 (died on 24th)



Table 4. Increasing doses of blood infected with *T. annulata* strain No. 3 (S-3)

Reaction parameters	Doses of inoculum by subcutaneous route				
	15 ml No. 71	15 ml No. 72	45 ml No. 73	45 ml No. 74	90 ml No. 75
Thermal incubation period (days)	16	19	16	16	17
Duration of thermal reaction (days)	8	7	8	6	5
Maximum rectal temperature (C <sup>0</sup> )	41.2	41.1	41.4	41.1	41.0
Days after infection on which max. rectal temperature occurred	20	21	19	17	19
Days to the appearance of schizonts in prescapular lymph-node and their numbers per microscopic field	17	17	16	16	16
No. of days on which schizonts were discerned	2	1	8	2	5
Maximum No. of schizont per microscopic field	7	8	7	7	6
Days after infection on which max. no. of schizonts were discerned	15	20	20	10	20
	21	20,21	20,21	18	18,19,20,21(died on 22nd)