

OBSERVATIONS ON THE RESISTANCE OF CALVES TO THE CATTLE TICK, *Boophilus annulatus* FOLLOWING REPEATED INFESTATIONS*

R. Sreekrishnan¹ and K. Rajamohanam

Department of Parasitology
College of Veterinary and Animal Sciences, Mannuthy
Thrissur - 680 651, Kerala

Tick infestation in domestic animals is responsible for a wide variety of harmful effects both directly and indirectly. *Boophilus annulatus* has been reported to be the most prevalent cattle tick in Kerala acting as the principal vector for babesiosis (Rajamohanam, 1982). It is also responsible for considerable damage to hide, apart from causing severe anaemia in calves.

It is now known that hosts develop resistance to particular species of ticks following sensitization (Willadsen, 1980; 1987). Since *B. annulatus* is the predominant cattle tick in Kerala and literature on the resistance of cattle to this tick is not available, an attempt was made to study the effect of host resistance on certain biological parameters in its life cycle and the host cellular reaction at the tick feeding site.

Materials and methods

Five cross-bred tick-naive female calves aged two months constituted the experimental animals. They were housed in tick-free individual pens and maintained on routine diet of milk, commercial concentrate feed and green grass. They were subjected to thorough clinical examination and dewormed with albendazole (Valbazen - Eskayef) prior to the start of the experiment and repeated at monthly intervals.

The seed ticks were maintained in the laboratory for a pre-release period of seven days to starve. They were then released on the back of each calf at the rate of 3600 per calf. Three weeks after they became tick-free and a second infestation was given which was repeated a third time as before. The calves were totally confined to indoors during the period of tick infestation without grooming or washing.

The engorged female ticks were collected from the body of the hosts as well as from the floor of the pen after 21 days of tick feeding. They were counted, weighed individually, placed in individual vials and incubated at 31°C and 85% relative humidity. The weight of the egg masses laid by individual ticks as well as the corresponding number of eggs were recorded. A representative sample of 100 eggs from individual ticks were placed in separate vials and the number of hatched seed ticks were counted. The data with regard to these parameters were subjected to analysis of variance (ANOVA). The data regarding mean percentage of hatchability were subjected to arcsine transformation prior to analysis of variance.

For histopathological studies, pieces of skin of about 1 sq.cm. were collected from the site of tick-bite on the host during the latter part of

* Part of M.V.Sc. Thesis submitted by the first author to Kerala Agricultural University

1. Present address; Department of Parasitology, Rajiv Gandhi College of Veterinary and Animal Sciences, Kurumbapet, Pondicherry 605 009

engorgement. They were fixed with 10 per cent formalin and stained using standard haematoxylin-eosin technique.

Results

The number of engorged female ticks obtained from each calf in first infestation ranged from 39 to 141. In the second and third infestation these were between 23 to 114 and 4 to 37, respectively. There was significant reduction between the number of female engorged ticks obtained in the first and third exposures ($P < 0.05$), whereas this reduction was not significant between first and second infestations or second and third infestations (Table 1).

The mean engorged weight of ticks obtained in the three infestations ranged between 119.2 ± 7.27 mg to 161.4 ± 4.05 mg in the first infestation. In the second and third infestations these values were 74.8 ± 3.51 mg to 149.7 ± 3.92 mg and 115.0 ± 9.19 mg to 150.6 ± 8.39 mg respectively. The results did not show any significant difference between first and second exposures, but significant differences were observed between the weight of ticks in first and second exposure, and those between second and third exposure at $P < 0.05$ (Table 1).

The mean weight of egg masses laid by the engorged female ticks following first infestation was in the range of 47.5 ± 2.14 mg to 59.6 ± 3.32 mg. During the subsequent infestations, the weight recorded were in between 32.6 ± 2.23 mg and 62.0 ± 2.0 mg, and between 42.2 ± 7.67 mg and 53.4 ± 6.93 mg respectively. There was no significant difference between infestations with regard to the egg mass weight (Table 1).

The mean egg number recorded were 1194 ± 25.26 to 1710 ± 35.29 in the first, 1346 ± 118.73 to 1632 ± 38.92 in the second and 885 ± 86.5 and 1616 ± 118.57 in the third infestation. The difference in this case was not significant (Table 1).

The mean values for the percentage hatchability were in the range of 67.5 ± 4.70 to 99.5 ± 3.16 in the first infestation, 83.4 ± 5.33 to 92.6 ± 3.04 in the second, and 62.0 ± 16.15 to 91.0 ± 1.77 in the third infestations. These values did not differ significantly (Table 1).

Tick-feeding sites on the skin of calves during first exposure revealed a low degree of diffuse infiltration with neutrophils and eosinophils. Focal degenerative changes were observed with progressive necrosis. Degenerative changes in hair follicles, hyalinisation and slight fibrous tissue reaction were observed in the dermis. In the second exposure the reaction was much more pronounced. The prominent cells involved were lymphocytes and to certain extent macrophages and eosinophils. Distinct perivascular lymphocytic infiltration and nodular accumulation of lymphocytes and macrophages were noticed around the blood vessels. Hyperkeratosis and acanthosis were visible with slight dermal fibrosis. The lesion in the third exposure revealed diffuse infiltration of lymphocytes and neutrophils with a predominance of the former. A few eosinophils were also present. Moderate degree of dermal fibrosis with degenerative changes in hair follicles were observed.

Discussion

The reduction in tick yield following repeated exposures were significant enough to show that resistance developed against *B. annulatus* in calves so exposed. This was in conformity with the results obtained by Trager (1939), Allen (1973) and Wikel and Allen (1976) in respect of *Dermacentor andersoni* - Guineapig system. Similar results were obtained with *Boophilus microplus* - Cattle system by Amin - Babjee and Riek (1986) and Miranpuri (1989).

The engorged weight did not significantly differ between first and third infestations,

Table 1 Feeding and Reproductive parameters of ticks following infestations in calves

Experimental Calf Number	Number of engorged female ticks collected			Engorged Weight (mg) Mean ± SE			Egg mass weight (mg) Mean ± SE			Egg number Mean ± SE			Hatchability (%) Mean ± SE		
	First infestation	Second infestation	Third infestation	First infestation	Second infestation	Third infestation	First infestation	Second infestation	Third infestation	First infestation	Second infestation	Third infestation	First infestation	Second infestation	Third infestation
C1	141	58	36	148.0±	101.1±	141.6±	53.87±	61.2±	50.6±	1487±	1604±	1526±	97.0±	83.4±	73.6±
				5.71	10.91	3.81	2.38	4.65	5.99	101.05	44.98	113.23	0.98	5.33	12.58
a	39	23	4	132.5±	113.0±	115.0±	59.6±	58.8±	44.6±	1710±	1545±	885±	67.5±	87.6±	91.0±
				5.40	19.84	9.19	3.32	2.23	4.83	35.29	41.54	86.50	4.70	2.27	1.77
a	78	47	35	119.2±	74.8±	147.8±	47.8±	32.6±	53.4±	1321±	1346±	1616±	86.0±	89.0±	62.0±
				7.27	3.51	10.37	2.39	2.23	6.93	33.05	118.73	118.57	1.17	2.40	16.15
C4	94	98	34	136.0±	100.8±	150.6±	47.5±	56.2±	47.2±	1194±	1591±	1542±	99.5±	92.6±	63.4±
				2.78	3.1±	8.39	2.14	8.13	7.67	25.26	187.53	170.69	3.1±	43.04	15.28
C5	131	114	37	161.4±	149.7±	143.6±	54.26±	62.0±	46.0±	1589±	1632±	1346±	89.5±	89.2±	81.8±
				4.05	3.92	4.65	3.09	2.09	4.41	31.13	38.92	133.56	1.39	2.14	5.21
Treat- ment mean 6SE*	96.6± 18.47	rt. << 16.71	29.20± 6.32	139.42±	107.88±	139.72±	52.61±	54.16 ^{NS} ±	47.36 ^{NS} ±	1460.2±	1543.6 ^{NS} ±	1383 ^{NS} ±	87.90±	88.37 ^{NS} ±	74.36 ^{NS} ±
				7.16	12.18	6.38	2.26	5.48	2.04	92.18	1.36	132.17	5.65	1.48	5.50

*P < 0.05 ~ Non-significant
Means with common letters do not differ significantly

though there was significant difference between the first and second infestations. This phenomenon could be due to a possible tick-induced immunosuppression (Wikel and Whelan, 1986).

The mean egg mass weight and egg number did not differ significantly between infestations and indicated agreement with the observations of Brown *et al.* (1984) and Amin-Babjee and Riek (1986). There was no significant relation between acquired resistance of host and hatchability of tick eggs as illustrated by the insignificant difference between the infestations for the percentage hatchability. This was in agreement with the observations of Fujisaki (1978) and Amin-Babjee and Riek (1986).

The tissue reaction around the site of tick-bite revealed the occurrence of definite inflammatory reaction, which is progressive from an acute **neutrophilic** reaction to a chronic lymphocytic one with repeated infestations. The significant reduction in neutrophilic infiltration in latter infestations is suggestive of a transformation to chronic tissue reaction. Mbemba (1983), Gill and Walker (1985), Gill (1986) and Abdul-Amir and Gray (1987) also reported the predominance of mononuclear cells in the latter stages of tick development and latter infestations.

Summary

An attempt was made to study the resistance of cross-bred calves to the cattle tick, *Boophilus annulatus* following repeated infestations with respect to certain biological parameters in the life cycle of ticks such as the mean number of engorged female ticks obtained, engorged weight, egg mass weight, egg number and percentage hatchability of eggs, and also the cellular reaction around the tick feeding site. There was significant reduction in the number of engorged ticks and to a lesser extent in engorged weight of ticks following repeated

infestations. The sites of tick-bite revealed a progressive inflammatory reaction from an acute neutrophilic one to a chronic **lymphocytic** one.

Acknowledgement

The first author wishes to thank the Indian Council of Agricultural Research, New Delhi for extending financial assistance through awarding a Junior Fellowship during the period of study.

References

- Abdul-Amir, I.M. and Gray, J.S. (1987). Resistance of Sheep to laboratory infestations of the tick, *Ixodes ricinus*. *Rev. Vet. Sci.* 43(2): 266-267
- Allen, J.R. (1973). Tick resistance - **Basophils** in skin reactions of resistant guinea-pigs. *Int. J. Parasitol.* 3: 195-200
- Amin-Babjee, S.M. and Riek, R.F. (1986). Development of resistance to experimental tick (*Boophilus microplus*) infestations in European breed cattle *Kajian Veterinar.* 18(1): 55-63
- Brown, S.J., Shapiro, S.Z. and Askenase, P.W. (1984). Characterisation of tick antigens inducing host immune resistance I. Immunization of guinea-pigs with *Amblyomma americanum* derived salivary gland extracts and identification of an important salivary gland protein antigen with guinea-pig anti-tick antibodies. *J. Immunol.* 133(6): 3319-3325
- Fujisaki, R. (1978). Development of acquired resistance and precipitating antibody in rabbits experimentally infested with females of *Haemaphysalis longicornis* (Ixodoidea: Ixodidae). *National Inst. Anim. Health Oily.* 18(1): 27-38
- Gill, H.S. (1986). Kinetics of mast cell, basophil and eosinophil populations at *Hyalomma anatolicum anatolicum* feeding

- sites on cattle and the acquisition of resistance. *Parasitology*. 93(2): 305-315
- Gill, H.S. and Walker, A.R. (1985). Differential cellular response at *Hyalomma anatolicum anatolicum* feeding sites on susceptible and tick-resistant rabbits. *Parasitology*. 91(3): 591-607
- Mbemba, Z-Z. (1983). Histological study of the local defensive reaction of sheep to infestations with *Rhipicephalus appendiculatus*. Inaugural Dissertation, *Tierärztliche Fakultät der Ludwig Maximilians- Universität München*. 36.
- Miranpuri, G.S. (1989). Relationship between the resistance of cross-bred cattle to ticks *Boophilus microplus* (Canestrini, 1887) and *Hyalomma anatolicum anatolicum* (Koch, 1844). *Vet. Parasitol.* 31(3-4): 289-301
- Rajamohanam, K. (1982). Identification of the vector for Babesiosis of cattle in Kerala. *Proc. Sym. Vectors and Vector-Borne diseases, Trivandrum*. 125-128
- Trager, W. (1939). Acquired immunity to ticks. *J. Parasitol.* 25: 57-81
- Wikel, S.K. and Allen, J.R. (1976). Acquired resistance to ticks. I. Passive transfer of resistance. *Immunology*: 30(3): 311-316
- Wikel, S.K. and Whelan, A.C. (1986). Ixodid-Host Immune interaction. Identification and characterization of relevant antigens and tick-induced host immunosuppression. *Vet. Parasitol.* 20: 149-174
- Willadsen, P. (1980). Immunity to ticks. *Adv. Parasitol.* 18: 293-313
- Willadsen, P. (1987). Immunological approaches to the control of ticks. *Int. J. Parasitol.* 17(2): 671-677