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PARALYSIS OF LABORATORY RABBITS BY NYMPHAE OF *IXODES RUBICUNDUS*, NEUMANN 1904 (ACARINA: IXODIDAE) AND SOME EFFECTS ON THE LIFE-CYCLE FOLLOWING FEEDING UNDER DIFFERENT TEMPERATURE CONDITIONS

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ABSTRACT

SPICKETT, A. M., ELLIOTT, E. G. R., HELOISE HEYNE & NESER, J. A., 1989. Paralysis of laboratory rabbits by nymphae of *Ixodes rubicundus* Neumann 1904 (Acarina: Ixodidae) and some effects on the life-cycle following feeding under different temperature conditions. *Onderstepoort Journal of Veterinary Research*, 56, 59–62 (1989)

Feeding under constant cold conditions resulted in a marginal shortening of feeding period and a major shortening of the subsequent developmental phase of all stages of *Ixodes rubicundus* Neumann 1904, the Karoo paralysis tick. Paralysis occurred in laboratory rabbits when nymphae were fed on them under constant warm, constant cold and fluctuating ambient winter conditions. This paralysis was found to be dependant on infestation rate and feeding conditions. Under constant cold conditions paralysis resulted from lower levels of infestation than under warm or under fluctuating winter conditions. Neurological parameters used in assessing the progression of the paresis are discussed.

INTRODUCTION

Past studies on *Ixodes rubicundus* Neumann 1904 have concentrated on its distribution (Theiler, 1950), host-range (Stampa, 1959), biology and ecology (Stampa & Du Toit, 1958; Stampa, 1959) and its laboratory life-cycle (Neitz, Boughton & Walters, 1971). More recent research includes a survey of the paralysis condition attributed to the tick (Spickett & Heyne, 1988) and host preference studies (Horak, Moolman & Fourie, 1987).

The induction of clinical Karoo paralysis in the laboratory remains important in order to quantify the paralysis-inducing potential of the tick (Hamel & Gothe, 1978). Although the adult ticks are responsible for small-stock losses in the Karoo regions of South Africa (Stampa, 1959; Spickett & Heyne, 1988), the experiments described here showed the nymphal stage capable of inducing paralysis in laboratory rabbits. Recent survey results (Spickett & Heyne, 1988) showed a close association of paralysis incidence with cold conditions such as frost and snow and this initiated these experiments under controlled laboratory conditions. Results presented here are regarded as contributory to the attempted induction of Karoo paralysis in sheep, the major domestic host of the adult stage (Spickett & Heyne, 1988), for which it may serve as a model.

MATERIALS AND METHODS

Except for an *Ixodes rubicundus* strain collected at Middelburg in the Cape Province in 1984 and subsequently maintained in the laboratory, all other ticks which were used in this study originated from the farm "Preezfontein", situated near Fauresmith, in the southern Orange Free State. All laboratory studies were conducted at the Veterinary Research Institute, Onderstepoort.

Ticks were fed in containers glued to the backs of 1,5–1,8 kg. laboratory rabbits or on sheep according to the method of Heyne, Elliott & Bezuidenhout (1987).

Tick infestations took place under different simulated and natural temperatures or combinations thereof, i.e. under cold conditions (C) in a cooled incubator at a temperature of under 10 °C; under warm conditions (W) in a heated breeding room at 27 °C; under so-called natural cold conditions (NC), inside a breeding room without temperature control, during winter at temperatures fluctuating between a miniumum of 4 °C at night to a maximum of 15 °C during daytime. Where death of a host occurred before full engorgement of the ticks they were transferred to a fresh host.

Non-parasitic stages were kept in a dark incubator at 20 °C and 90 % relative humidity.

Infestation parameters monitored were pre-infestation number of ticks, post engorgement number of ticks, time taken to complete feeding, period from engorgement to moult of first larvae and nymphae, period from adult engorgement to hatch of first eggs, period from infestation to onset of paralysis and period from infestation to death.

The degree of paralysis was monitored throughout on a daily basis according to a prescribed neurological examination of parameters given in Table 1 (Chrisman, 1982). Hourly monitoring followed the onset of paralysis.

Except for observational parameters, all reflexes, including deep pain and superficial sensation, were monitored on a scale of 0–4 as follows:

0 = no reflex
1 = depressed reflex
2 = normal reflex
3 = hyperactive reflex and
4 = hyperactive reflex with clonus.

Evaluation of gait and strength (stand, walk, hemistand and hemiwalk), postural reactions and the wheelbarrow responses were on a scale of 0–5 as follows:

0 = paralysis without any movement
1 = some movement but cannot support mass
2 = supports mass but cannot take steps
3 = supports mass and can take a few steps before stumbling
4 = supports mass well but stumbles occasionally
5 = normal

RESULTS

The effect of temperature on tick development and paralysis inducement

Adults

The effects of feeding adult ticks on rabbits and sheep under cold and warm conditions are summarised in Table 2.

Feeding periods varied little between cold and warm conditions, the shortest period being found on sheep under warm conditions. A large difference was, however, found between cold and warm feeding in the sub-

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TABLE 1 Parameters monitored in the neurological examination of rabbits during paralysis experiments with *Ixodes rubicundus* under controlled conditions

General	Cranial nerve reflexes	Neck and forelimbs	Rear limbs	Tail and perineum
Behaviour	Menace reflex	Wheelbarrow	Wheelbarrow	Perineal reflex
Head posture	Pupillary reflex	Postural reactions	Postural reactions	Tail reflex
Gait and strength	Ptosis reflex	Extensor strength	Extensor strength	Bladder
Temperature	Facial and corneal reflex	Biceps reflex	Patellar reflex	Descending colon/rectum
	Facial symmetry	Triceps reflex	Cranial tibial reflex	Panniculus reflex
	Hanging	Extensor carpiradialis reflex	Gastrocnemius reflex	Superficial sensation
	Neck muscle atrophy	Flexor reflex	Flexor reflex	Muscle atrophy
		Deep pain	Deep pain	
		Superficial sensation		
		Muscle atrophy		

TABLE 2 *Ixodes rubicundus* adult feeding on rabbits and sheep under cold (C) and warm (W) conditions. Means in brackets. All periods in days

Feeding conditions	Host	Feeding period	Period from engorgement to start of hatch	Combined period from infestation to start of hatch
C	Rabbit	7–11 (9)	99	108
W	Rabbit	10–16 (13)	124	137
C	Sheep	9–11 (10)	88	98
W	Sheep	6–10 (8)	139	147

C = under 10 °C; W = 27 °C

TABLE 3 *Ixodes rubicundus* larval feeding on rabbits under cold (C) and warm (W) conditions. Means in brackets. All periods in days

Feeding conditions	Host	Feeding period	Period from engorgement to moult	Combined period from infestation to the 1st larvae to moult
C	Rabbit	4–6 (5)	23	28
W	Rabbit	4–8 (6)	33	39

C = under 10 °C; W = 27 °C

TABLE 4 *Ixodes rubicundus* nymphal feeding on rabbits and the inducement of paralysis under cold (C), warm (W) and natural cold (NC) conditions. Means in brackets

Infestation rate per rabbit	Feeding conditions	Feeding period (days)	Degree of paralysis attained	Period (h) from infestation to paralysis onset	Death
100	W	6–8 (7)	None	—	—
300	W	6–8 (7)	None	—	—
800	W	5–7 (6)	Full	98	138
150	NC	6–7 (6,6)	None	—	—
200	NC	5–7 (6)	Initial–Reversed	128	—
300	NC–W	6–8 (7)	Initial–Reversed	106	—
600	NC	5–7 (6)	Full	112	156
800	NC	5–6 (5,5)	Full	72	132
100	C	5–7 (6)	Full	120	168
300	C	4–6 (5)	Full	49	58
300	C–W	6–8 (7)	Advanced–Reversed	92	—
700	C	4–6 (5)	Full	46	52/23/47/42
800	C	4–6 (5)	Full	48	60/21/21/41

C = under 10 °C; W = 27 °C; NC = Day 15 °C, Night 4 °C

sequent non-parasitic period, adult engorgement to hatch of first eggs, independant of the host on which the ticks had previously fed. Cold feeding resulted in very much shorter non-parasitic periods being recorded with the shortest combined period (from infestation to the first eggs to hatch) resulting from cold feeding on sheep, ostensibly the preferred domestic host of the adult stage (Spickett & Heyne, 1988).

Larvae

Results of larval feeding under cold and warm conditions are given in Table 3.

Larvae fed for a marginally shorter period under cold conditions and again the major effect was evident on the subsequent developmental phase where the period from engorgement to moult of the first nymphae was 10 days shorter than with feeding under warm conditions.

Nymphae

Results of nymphal feeding are given in Table 4. No developmental information is available as the moulting period of the nymphae is extremely long.

Feeding under constant warm conditions (27 °C) resulted in rabbit paralysis only at a very high infestation rate. Eight hundred nymphae per host caused full paralysis and death of the host while no paresis effect was evident with the lower infestation rates of 300 and 100 nymphae per host. A marginally shorter feeding period was experienced at the high infestation rate.

Under fluctuating day and night temperatures of natural cold, paralysis effect was encountered with a relatively low infestation rate of 200 nymphae. This paralysis effect was within the initial phase with both hind limbs affected at the time the first nymphae had completed

FIG. 1 Advanced paralysis in a rabbit (R), infested with *Ixodes rubicundus* nymphae, as shown by loss of extensor and flexor strength in both the fore and hind limbs

FIG. 2 Onset of paralysis in a lamb, infested with prefed *Ixodes rubicundus* nymphae as shown by its favouring of the left hind leg and voiding of the bladder after handling

engorgement. The paralysis reversed spontaneously, within 48 h, when the majority of nymphae completed their engorgement. Full paralysis and death resulted with infestations of 600 and 800 nymphae per rabbit. There was a marked shortening in the infestation-paralysis onset and infestation-death period the higher the infestation rate. Feeding periods were also shorter at high infestation rates. An infestation of 300 nymphae resulted in initial paralysis within a shorter period than with 200 nymphae, its onset occurring before the first nymphae had completed engorgement. In this case transfer to constant warm conditions resulted in spontaneous reversal of paralysis within 48 h, concurrent with a marginal lengthening of feeding period.

Under conditions of constant cold full paralysis was encountered with an infestation rate of 100 nymphae and higher (Table 4). The period from infestation to the onset of paralysis shortened progressively the higher the infestation rate.

The early death of rabbits infested with both 700 and 800 nymphae occasioned the transfer of these nymphae, which were only in a prefed state at the time, to fresh rabbits. In both cases two further transfers of the nymphae were necessary to attain complete engorgement. Four rabbits died consecutively due to paralysis (Table 4) by this serial transfer of the prefed nymphae from the 700 and 800 nymphal infestation respectively before the nymphae completed engorgement. The 2nd and 3rd rabbits to die in both cases did so after shorter time intervals than the 1st and last rabbits. In all other cases of full paralysis the nymphae were either fully engorged, or had reached an engorged state where transfer to fresh hosts ensured complete engorgement but did not result in paralysis.

Evaluation of paralysis

Initial paralysis

First signs of rabbit paresis are evinced by a hyperactive response of the patellar, and gastrocnemius reflexes. The time lapses at which these occur varied according to infestation rate and tick feeding conditions. Loss of extensor and then flexor strength in either the left or right hind leg follows the hyperactive reflexes whereafter the process is repeated for the other hind limb until both are flaccid. This stage is referred to as initial paralysis.

Advanced paralysis

The process extends to the forelimbs with hyperactive reflexes and loss of flexor and extensor strength until the forelimbs are flaccid (Fig. 1). The rabbit assumes a state of lateral recumbency at this stage of advanced paralysis.

Full paralysis

Loss of the panniculus reflex is accompanied by no food intake, bladder incontinence, loss of the perineal reflex and diarrhoea. This is followed by loss of superficial sensation and loss of the deep pain reflex after which hind muscle atrophy sets in, the rabbit becomes moribund, has great difficulty breathing and dies.

Anal temperature shows very little indication of clinical changes, fluctuating around 39 °C and dropping sharply to 35,5 °C when the moribund stage is reached.

The time lapse from initial paralysis to death varied, depending on circumstances, from 6–60 h. Cases of spontaneous reversal occurred either at a low infestation rate or with changes in feeding conditions (Table 4). In one instance spontaneous reversal took place even when the host was in an advanced stage of paralysis, although full recovery was attained only 4 days after all the nymphae had engorged.

DISCUSSION

It appears that two factors play an important role in the paralysis-inducing ability of the nymphae of this tick species.

Temperatures below 10 °C and higher numbers of nymphae at initial infestation enhance paralysis by quickening its onset and the death of the host. Faster feeding periods under cold conditions could be due to a triggering effect on feeding behaviour resulting in simultaneous attachment. This would have a synchronous toxin release effect with the majority of nymphae entering the toxic phase thus hastening paralysis. High tick numbers during initial infestation also produced shorter feeding periods, irrespective of feeding conditions, suggestive of intraspecific stimulation, possibly a function of pheromonal communication (Gothe, 1987). High initial infestation numbers, coupled with the cold effect, ensured shorter feeding periods, resulting in consecutive

paralyses. The peak of the toxic phase is clearly illustrated by the extremely short periods needed to cause the deaths of the 2nd and 3rd hosts in these cases. The longer periods needed to cause the deaths of the 1st and last hosts reflect entry and exit from the toxic phase, coinciding with initial feeding and final engorgement respectively. Entry into the toxic phase varied from 2 days post-attachment (high numbers, cold conditions) to 3 days (high numbers, fluctuating temperature conditions) to 4 days (high numbers, warm conditions). Entry into the toxic phase, peak toxin release and subsequent exit from the toxic phase is thus a factor of nymphal engorgement. Although the cause of death could not be determined precisely, asphyxia and hypoxia due to progressive paralysis of the heart and respiratory muscles was most probably responsible for the demise of the animals.

No paralysis could be ellicited by adult tick feeding, probably due to a combination of low numbers of adults used, feeding taking place under environmental conditions not condusive to the stimulation of synchronous feeding, and the ticks not being allowed free choice of attachment site. In one experiment, however, 300 prefed nymphae, transferred from a rabbit in the initial phase of paralysis, did cause left hind leg paresis in a lamb (Fig. 2). This condition reversed spontaneously as the nymphae reached full engorgement within 12 h of being placed on the lamb. The prefed nymphae had to be in an advanced stage of the toxic phase upon transfer to produce paresis symptoms in the lamb. However, engorgement was too far advanced to produce more advanced symptoms before completion of feeding.

Paralysis caused by the nymphae of *I. rubicundus* appears similar to that of the adults of *Rhipicephalus e. evertsi* (Hamel & Gothe, 1978; Gothe & Lämmler, 1981), *Ixodes holocyclus* (Doube, 1975) and *Dermacentor andersoni* (Gregson, 1973) in its dependance on the relation of tick burdens to host mass. This dependance is, however, negated to a large extent by feeding under conditions of lowered temperature which would be approximated by the cold winter months of their prevalence (Stampa, 1959), within their distributional area.

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