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EFFECT OF HUMIDITY ON THE LIFE CYCLE AND REPRODUCTION OF *DERMACENTOR RETICULATUS* (IXODIDAE) IN LABORATORY MICE

ABSTRACT: The reproductive cycle of *Dermacentor reticulatus* (Ixodidae) is described in this paper. Experimental infestation was carried out on immobilized (fixed) RAP mice and those that could move freely. *Dermacentor reticulatus* (*D. pictus*) larvae, nymphs and adults were obtained from eggs placed in glass test tubes in two variants: dry medium ($T^{\circ} = 28-30^{\circ}\text{C}$, R.H. = 49—51%) and wet medium ($T^{\circ} = 28-31^{\circ}\text{C}$, R.H. = 94—95%).

In the mice that were experimentally infested with the eggs incubated in wet medium, there were more fixed larvae that came off after sucking (8—786 larvae) compared with 0—7 larvae that were observed in the mice that could move freely. At the same time, more larvae were attached to the immobilized mice (2—786) compared with the number of larvae observed in the mice that could move freely (0—11). Infestation was not registered in the experimental animals that could move freely when the larvae were incubated in the dry medium. Experimental infestation with *Dermacentor reticulatus* failed in the RAP mice. The infested mice tolerated as much as 786 *Dermacentor reticulatus* larvae, but they died when 12—30 nymphs were fed on them.

KEY WORDS: *Dermacentor reticulatus*, reproductive cycle, experimental infestation, RAP mice, humidity

INTRODUCTION

Dermacentor reticulatus is a three-host tick affecting sheep, horses and, occasionally, cattle and dogs. Larvae and nymphs feed on rodents and insectivores and adults feed on large mammals (Cosoroabă, 1994; Gilota, 1974).

Dermacentor reticulatus is widespread in Europe. In Romania, this species is spread almost in the whole country (Cernăianu, 1957; Dominguez Penafiel, 1999).

Economic losses caused by *Dermacentor reticulatus* are multiple. The initial loss is due to blood sucking, during the biological cycle, at the expense of host animals. Expenses for tick treatment and control should be added to this. *Dermacentor reticulatus* can transmit a series of diseases to humans and animals (Cernăianu, 1957; Cosoroabă, 1994; Rodhain and Perez, 1985).

Studies concerning the biology and ecology of other Ixodidae species are numerous (Aeschlimann, 1967; Cilton et al., 2000; Cosbie and Boyce, 1998). However, studies of *Dermacentor reticulatus* are not many, especially in Romania. Zahler and Gothe (1995) examined the effect of temperature and humidity on *Dermacentor reticulatus* eggs, larvae, nymphs and adults. On the basis of these experiments, they concluded that temperature and humidity affected the hatching, larvae and nymphs molting and longevity, adult longevity as well as the oviposition of *Dermacentor reticulatus* females.

The primary objective of this study was to monitor the biological cycle of ticks (*Dermacentor reticulatus*) in laboratory mice. Another objective was to establish the effect of humidity, under relatively stable temperature, on the egg incubation time and the numbers of *Dermacentor reticulatus* larvae, nymphs and adults that attached themselves and fed on the mice.

MATERIAL AND METHODS

Engorged females of *Dermacentor reticulatus* were collected from parasitized horses from S.C. Izvin S.A. stud. Individual females were stored in glass test tubes in two variants:

a) Metianu test tubes with a 6 cm water column, 2 cotton-wool layers in contact with water, a 0.5 cm lint layer and an air column above; these were considered as wet medium due to high humidity.

b) Ordinary glass test tubes. These were considered as dry medium due to low humidity.

Both types of test tubes were closed with a plug wrapped with lint.

The two mediums, dry and wet, were used to observe the incubation and molting of satiated larvae and nymphs (Tables 3 and 5).

In all cases, temperature and humidity were measured two times a day, in the morning and evening, with the help of an electronic thermohygrometer.

RAP mice were used for experimental infestation with *Dermacentor reticulatus*. Infested forms were obtained from dry medium ($T^{\circ} = 28-30^{\circ}\text{C}$, R.H. = 49—51%) and wet medium ($T^{\circ} = 28-31^{\circ}\text{C}$, R.H. = 94—95%).

The mice to be infested were either left free-moving in cages (non-immobilized) or were immobilized with small leather straps.

The number of larvae used for infesting the mice ranged between 300 and 1000, the number of nymphs ranged between 50 and 56, and the number of adults ranged between 7 and 9. The numbers of fixed and satiated larvae, nymphs and adults taken out from the mice were monitored (Tables 2, 4 and 6).

RESULTS

Table 1 shows the results concerning *Dermacentor reticulatus* egg incubation. In laboratory conditions, under almost identical temperature regimens, the incubation took a longer time when the medium was wet (94—95%).

Table 1. Obtaining *Dermacentor reticulatus* larvae „in vitro”

Incubating eggs	T	R.H.	Incubating time
Dry medium	29—30°C	50%	13—17 days
Wet medium	30—31°C	94—95%	16—20 days

Legend: T — temperature; R. H. — relative humidity

More larvae raised in the wet medium remained attached to mice as compared with those raised in the dry medium (Table 2). Not a single larva from the dry medium, at the same dose per mouse (300 larvae), remained attached to the non-immobilized mice. On the other hand, when the mice were immobilized, 2—7 larvae remained attached and sucked blood.

Table 2. Infestation of RAP mice with *Dermacentor reticulatus* larvae

Source of the larvae	Mice infestation	Larvae per mouse	Mouse number	Attached larvae	Feeding time
Dry medium	Nonimmobilized	300	1	0	0
			2	0	
			3	0	
			4	0	
	Immobilized	300	5	7	
			6	7	
			7	2	
Wet medium	Non-immobilized	300	8	8	4—5 days
		300	9	11	
	Immobilized	300	10	143	5—6 days
		1000	11	786	3—6 days

In the case of the larvae from the wet medium, at the same dose per mouse (300 larvae), 8 to 11 of them remained attached to the non-immobilized mice. Attaching was easier to accomplish with the immobilized mice. At the same dose per mouse (300 larvae), 143 larvae remained attached. When infestation was carried out with 1000 larvae, 786 of them remained attached and fed on the mice.

Dermacentor reticulatus larvae incubated at 28—31°C in dry and wet mediums alike, the molting occurring after 8—10 days. The number of the obtained nymphs is shown in Table 3.

Table 3. Obtaining *Dermacentor reticulatus* nymphs „in vitro”

Incubating larvae	Number of incubating larvae	T	R.H.	Molting time	Nymphs obtained	
					Nr.	%
Wet medium	40	29—30°C	95—96%	9—10 days	22 + 8	75
	40	28—30°C	95—96%	9—10 days	23 + 6	72,5
Dry medium	40	29—30°C	49—51%	8—9 days	28	70
Wet medium	250	30—31°C	94—95%	8 days	103	41,2
Dry medium	400	29—30°C	49%	8—9 days	101	25,25

Dermacentor reticulatus nymphs, irrespective of the type of medium in which they were raised, could not remain attached to the non-immobilized mice (Table 4). Regarding the rate of attachment on the immobilized mice, the nymphs from the wet and dry mediums were attached in almost equal numbers, 11—28 and 12—30, respectively.

Table 4. Infestation of RAP mice with *Dermacentor reticulatus* nymphs

Source of the nymphs	Mice infestation	Nymphs per mouse	Mouse number	Attached nymphs	Feeding time	Observations	
Wet medium	Non-immobilized	56	1	0	—	—	
		56	2	0	—	—	
Dry medium		50	3	0	—	—	
		50	4	0	—	—	
Wet medium		Immobilized	50	5	11	7—8 days	—
			56	6	28	5 days	died on the 5 th day p.i.
Dry medium	50		7	12	5—8 days	died on the 8 th day p.i.	
	56		8	30	6 days	died on the 6 th day p.i.	

Three out of each four infested mice died.

The nymphs incubated in the wet and dry mediums molted at 28—30°C and adults were obtained (Table 5). The period of time necessary for molting varied between 14 and 16 days.

Table 5. Obtaining *Dermacentor reticulatus* „in vitro”

Incubating nymphs	Number of incubated nymphs	T	R.H.	Molting time	Adult ticks obtained	
					Nr	%
Dry medium	14	28—30°C	49—50%	14—15 days	14 (10* + 4**)	100
	21				20 (14* + 6**)	95,23
Wet medium	14	28—29°C	95—96%	15—16 days	14 (9* + 5**)	100
	21				21 (13* + 8**)	100

Legend: * — female, ** — male.

The percentages of the obtained adults ranged between 95.25 and 100. The number of females doubled that of males in all cases.

The results of mice infestation with *Dermacentor reticulatus* are presented in Table 6. Not a single adult tick remained attached to the immobilized and non-immobilized mice alike, no matter if raised in the wet or the dry medium.

Table 6. Infestation of RAP mice with *Dermacentor reticulatus* adults

Source of the adults	Mice infestation	Adults per mouse	Mouse number	Attached adults
Wet medium	Non-immobilized	7	1	0
		9	2	0
7		3	0	
9		4	0	
Wet medium	Immobilized	7	5	0
		9	6	0
7		7	0	
9		8	0	

DISCUSSION

Under laboratory conditions, at 28—31°C and variable relative air humidity, the reproduction cycle of *Dermacentor reticulatus* was successful. Although the incubation of larvae was successful both in the wet medium (R.H. = 94—95%) and the dry medium (R.H. = 50%), the period of incubation was shorter in the later case. However, the larvae obtained from the wet medium proved to be more active and they were more easily attached to mice. Comparing the influence of humidity on the different development phases, it seemed that increased relative air humidity was more necessary for egg incubation than for the incubation of larvae and nymphs. This fact was demonstrated by the higher capacity of the *Dermacentor reticulatus* larvae raised from eggs kept in the wet medium to stay attached and feed on the host.

If infestation by larvae succeeded with both non-immobilized and mobilized mice, the infestation by nymphs succeeded only with immobilized mice. Moreover, the infestation by *Dermacentor reticulatus* adults did not succeed at all. These results are partially contradictory to those of Metianu (cited by Cernăian, 1957) who claimed that the larvae and nymphs were easily attached while adults were more difficult to attach to the host. Perhaps he used another Ixodidae species or his experiments were performed under different conditions.

The mice allowed a large number of larvae to feed on their bodies (up to 786) but the number of nymphs was smaller (11—30). Furthermore, the mice infested by 12—30 nymphs invariably died. No adults were found attached to mouse skin. These results again partially contradict Metianu's report (cited by Cernăian, 1957) stating that a mouse allows 200—500 larvae or 100—250

nymphs or 5—10 adult ticks to feed on it. According to Cosoroabă (2000), however, only the larvae and nymphs feed on rodents while adults attach and feed on large animals.

CONCLUSION

Under the laboratory conditions provided in this study, longer time was needed to obtain *Dermacentor reticulatus* larvae from eggs when the relative air humidity was increased.

The rate of attachment of *Dermacentor reticulatus* larvae to mice was increased when the larvae were raised under higher relative humidity.

The larger the density of *Dermacentor reticulatus* larvae in the incubation medium, the smaller the percentage of nymphs obtained.

Infestation with *Dermacentor reticulatus* nymphs was successful only with the immobilized RAP mice.

The RAP mice tolerated the infestation by *Dermacentor reticulatus* larvae rather well, but it was not the case with nymphs.

The success of raising *Dermacentor reticulatus* adults „in vitro” was not influenced by relative air humidity.

The infestation of RAP mice with *Dermacentor reticulatus* adults failed altogether.

A general conclusion was drawn that under the laboratory conditions provided in this study RAP mice are not suitable hosts for *Dermacentor reticulatus* nymphs and adults.

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УТИЦАЈ ВЛАЖНОСТИ НА РЕПРОДУКТИВНИ ЦИКЛУС *DERMACENTOR RETICULATUS* (IXODIDAE) КОД ЛАБОРАТОРИЈСКИХ МИШЕВА

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Резиме

У раду је описан утицај влажности на репродуктивни циклус *Dermacentor reticulatus* (ixodidae). Експериментална инфестација је спроведена на Р. А. Р. мишевима имобилисаним (фиксирани) и који су се могли слободно кретати по кабезу. Ларва *Dermacentor reticulatus* (*D. pictus*), чауре и одрасле су добијене од јаја која се постављају у стаклене епрувете у две различите средине: у суви медијум ($T^{\circ} = 28-30^{\circ}\text{C}$ R. H. = 49—51%) и у влажни медијум ($T^{\circ} = 28-31^{\circ}\text{C}$; R. H. = 94—96%).

Код мишева који су експериментално заражени јајима инкубираним у влажном медијуму евидентирано је више ларви које су биле фиксирани, које су се након сисања одлепиле (8—786 ларви), у поређењу са 0—7 ларви које су евидентирани када су јаја инкубирана у сувим условима. Истовремено више ларви регистровано је на имобилисаним мишевима (2—786 ларви) у поређењу са бројем ларви које су евидентирани код мишева који су имали слободу кретања (0—11 ларви). Инфестација није забележена код слободно држаних експерименталних животиња када су ларве инкубирани у сувом медијуму. Инфестација ларвама успела је само код имобилизованих мишева, готово у једнаком односу, без обзира на медијум, суви или влажни. Експериментална инфестација *Dermacentor reticulatus* са одраслим ларвама није регистрована код Р. А. Р. мишева. Заражени мишеви су преживљавали инфестацију и до 786 ларви *Dermacentor reticulatus*, међутим мишеви заражени са 12—30 нимфи су угинули.