

## DEVELOPMENT OF SOME TICK SPECIES UNDER STANDARD LABORATORY CONDITIONS

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**Abstract.** Duration of development of all stages of 11 tick species under standard laboratory conditions (22 °C, 90 % RH) has been described. It has been found that some tick species are capable of such a great adaptation to the feeding on laboratory animals that they have been already cultured in the laboratory for several generations (*I. ricinus*, *I. hexagonus*, *D. marginatus*, *D. reticulatus*, *H. asiaticum*). Other species can feed on laboratory animals, too; however, the number of engorged specimens makes a small part only of the total number of ticks attached to a host (*H. marginatum*, *H. anatomicum excavatum*, *H. punctata*, *R. turanicus*).

Only few authors in our country have been engaged in problems of tick culturing and development under laboratory conditions. Their results referred mostly to separate species and were presented as a part of complex ecological observations. Mačíčka, Rosický, Černý (1955) describe the duration of development of the species *D. marginatus* under laboratory conditions; *D. pictus* (= *D. reticulatus*) was described by Mačíčka, Nosek, Rosický (1956). Nosek, Lichard, Sztankay (1967) describe — except ecological observations — the duration of developmental stages of the species *I. ricinus*, *I. hexagonus*, *D. marginatus*, *H. concinna*, *H. punctata* under laboratory conditions.

### MATERIAL AND METHODS

The development of the following species under standard laboratory conditions (22 °C, 90 % RH) was observed: *Ixodes ricinus* (L., 1758), *Ixodes hexagonus* Leach, 1815, *Dermacentor reticulatus* (Fabr., 1794), *Dermacentor marginatus* (Sulz., 1776), *Hyalomma asiaticum* P. Schl. et E. Schl., 1929, *Hyalomma dromedarii* Koch, 1844, *Hyalomma marginatum marginatum* Koch, 1844, *Hyalomma anatomicum excavatum* Koch, 1844, *Haemaphysalis concinna* Koch 1844, *Haemaphysalis punctata* Can. et Fanz., 1877, *Rhipicephalus turanicus* Pomi, 1940. With the exception of *Hyalomma dromedarii*, which originated from laboratory breeding, all tick species were collected in free nature.

For the feeding of larvae of the species *I. ricinus*, *D. reticulatus* and *D. marginatus* we used the method of a free feeding on a fixed mouse. All larvae of *I. ricinus* attached themselves to the back of the mouse; as far as both species of the genus *Dermacentor* are concerned, only some larvae attached themselves to the back and approximately the same number to ears. Larvae of the other species which had a small final number of engorged specimens with this method, as well as nymphs, were fed on the back of the mouse in small plastic cups (Reháček 1957). Some larvae of *I. hexagonus* were fed on a hedgehog and the duration of feeding on both hosts was compared. Imagines of all species were fed on rabbits according to the method described by Kurchatov (1953), exceptionally couples of imagoes of *D. reticulatus* and *D. marginatus* were fed in caps on white mice. A thermostat with ticks, which were placed in specially adjusted centrifugal test-tubes, was set to +22 °C, humidity was regularly checked and maintained at 90 % RH; all the time the ticks were kept in the dark.

**Table 1.** Duration of development of *Ixodes*, *Dermacentor* and *Haemaphysalis* ticks under standard

	Feeding ♀♀		Period prior to oviposition	Hatching of larvae
	Host	Duration		
<i>Ixodes ricinus</i>	rabbit	6—9	4—7 (V.—VI.) 8—10 (XII.) 10—12 (III.) 22 (IX.—X.)	36—64
<i>Ixodes hexagonus</i>	rabbit	8—13	11—16	30—40
<i>Dermacentor reticulatus</i>	white mouse	7—9 (spring) 10—14 (autumn)	1—7 (spring) 32—50 (autumn)	17—27
	rabbit	11—14		
<i>Dermacentor marginatus</i>	white mouse	7—13	5—10 (spring)	19—37
	rabbit	13—24	9—42 (autumn)	
<i>Haemaphysalis concinna</i>	rabbit	8—12	10—15	53—67
<i>Haemaphysalis punctata</i>	rabbit	9—14	7—11	27—54

**Table 2.** Duration of development of *Hyalomma* and *Rhipicephalus* ticks under standard

	Feeding ♀♀		Period prior to oviposition	Hatching of larvae
	Host	Duration		
<i>Hyalomma asiaticum</i>	rabbit	10—13	4—8 (30—38)	26—39
<i>Hyalomma dromedarii</i>	rabbit	10—13	7—9	43—55
<i>Hyalomma marginatum marginatum</i>	rabbit	9—13	16—18	48—49
<i>Hyalomma anatomicum excavatum</i>	guinea pig	9—11	10—27	47—52
<i>Rhipicephalus turanicus</i>	white mouse rabbit	17—19 10—11	10—28	41—64

\*) Period between the beginning of feeding of larvae and the dropping off of engorged nymphs

## laboratory conditions (in days)

Feeding of larvae		Onset of metamorphosis in engorged larvae	Feeding of nymphs		Onset of metamorphosis in engorged nymphs
Host	Duration		Host	Duration	
white mouse	2—5	31—224	white mouse	3—6	35—55
white mouse hedgehog	4—8 5—18	7—31	white mouse hedgehog	8—9 6—14	32—44
white mouse	2—8	7—8	white mouse	5—8	18—31
white mouse	3—8	9—11	white mouse	5—8	20—23
white mouse	3—6	32—45	white mouse	5—7	24—37
white mouse	5—7	25—28	white mouse	3—5	25—30

## conditions (in days)

Feeding of larvae		Onset of metamorphosis in engorged larvae	Feeding of nymphs		Onset of metamorphosis in engorged nymphs
Host	Duration		Host	Duration	
white mouse	4—6	11—19	white mouse	7—12	22—33
white mouse	3—7	—	white mouse	5—13	54—62
white mouse	4—6	—	white mouse	14—18*)	34—44
white mouse	4—5	—	white mouse	11—25*)	34—38
white mouse	4—5	21—31	white mouse	4—10	35—49

## RESULTS

The results obtained by investigation of individual species only specify or complete the data given in Tables 1 and 2.

*Ixodes ricinus*: Ascertained duration of individual developmental stages under standard conditions is given in Table 1. During our laboratory observations we found out that the duration of feeding of larvae differed according to their age. On the basis of the achieved results it is possible to conclude (Fig. 1) that older larvae feed for a longer time than the younger ones.

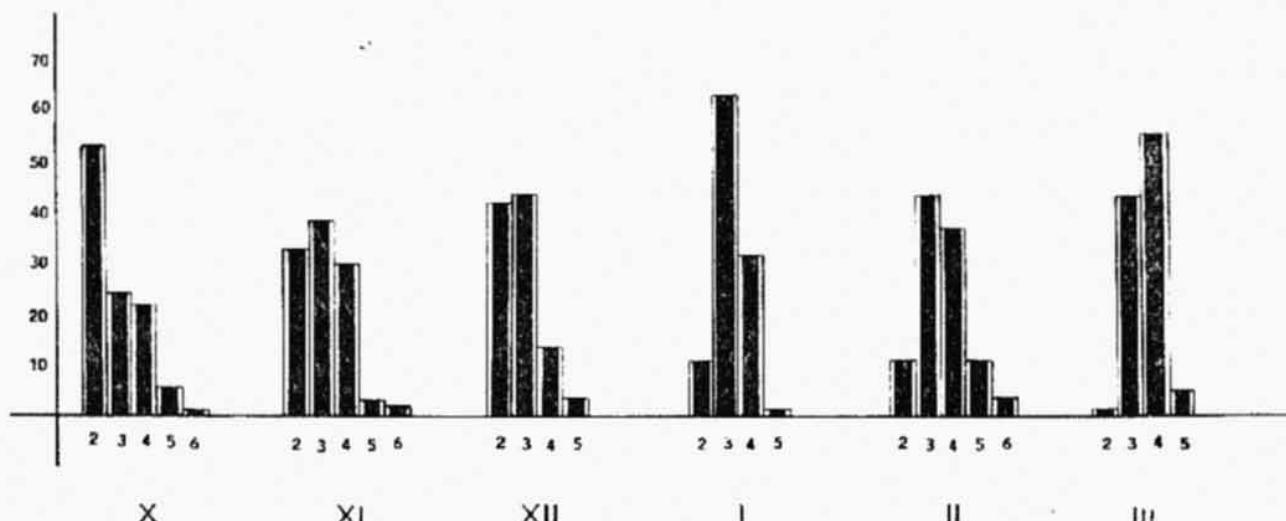


Fig. 1. Duration of feeding of larvae *I. ricinus* of a different age (2—7 months) in different year seasons (X—III). The horizontal axis bears data on the groups of engorged larvae aged 2 months (fed in X) to 7 months (fed in III) and their detachment from host during individual feeding days; the vertical axis shows the percentage of detached larvae.

*Ixodes hexagonus*: Data on the duration of individual developmental stages are given in Table 1. Except the period between the 24th and the 31st day, when the engorged larvae begin to moult, in summer months the moulting was observed as early as in 7 days under standard laboratory conditions.

*Dermacentor reticulatus*: In Table 1 observations of the duration of feeding are given partly on different hosts (rabbit — white mouse), partly in different seasons. The hatched larvae of this species had a limited vitality; if not engorged they dried up in 8 weeks even in an environment of a high relative humidity (90%). Unengorged nymphs as well as larvae had a more limited vitality than larvae and nymphs of *I. ricinus* and *I. hexagonus*; in unengorged state they did not live more than 3 months.

*Dermacentor marginatus*: All information about the duration of feeding and of the resting periods of individual stages is given in Table 1. A short survival period was observed as in *D. reticulatus* in pre-imaginal stages of this species.

*Hyalomma asiaticum*: The duration of development of individual stages is given in Table 1. According to our observations most of the engorged ♀ started to lay eggs in 4—8 days, in isolated cases in 30—38 days. The whole cycle was completed well under laboratory conditions and there were no difficulties with the maintenance of colonies. The ticks underwent a three-host-type of development.

*Hyalomma dromedarii*: Information on the duration of individual stages is given in Table 2. According to our observations the feeding of ♀ which had laid eggs lasted

10—13 days. Females feeding for a longer time (up to 28 days) did not — with some exceptions — lay eggs. During the feeding of larvae some of the engorged larvae, as well as some of unengorged nymphs (approximate ratio 1:1), dropped off. Engorged larvae dropped off in 3—7 days. In 7 days after the beginning of feeding unengorged nymphs appeared on the back of the mouse, and either dropped into the water or attached themselves again and fed until the stage of engorged nymphs. The feeding of the attached nymphs lasted at least 5 days, at most 13 days. The repeated feeding of unengorged nymphs which had dropped off the mouse lasted within the same time period. Thus, some of the larvae developed in a two-host type, the others in the three-host type of development.

*Hyalomma marginatum marginatum*: Time data on development of individual stages are given in Table 2. Unengorged larvae of this species can live at most 175 days. When feeding on a mouse, this species maintains partly the two-host character. Some of the larvae feed and drop off at the stage of engorged larvae in 4—6 days. The remaining larvae feed, moult still on the mouse and change to nymphs which feed and detach themselves. The engorged nymphs drop off in 14—18 days after the beginning of the feeding of larvae. The ratio between engorged larvae and engorged nymphs was 1:3. Being placed on a mouse, the unengorged nymphs which had dropped off were feeding for 6—8 days. Isolated specimens which had moulted from engorged larvae and survived in an unengorged state could live up to 168 days.

*Hyalomma anatomicum excavatum*: Duration of development of individual stages is given in Table 2. During the feeding in laboratory it was observed that some of the larvae underwent a three-host type of development and the others a two-host type of development. As far as the feeding in small cups attached to the skin of white mice is concerned, the situation developed in such a way that some of the engorged larvae dropped off after having finished the feeding (in 4—5 days) and the moulting to nymphs passed away from the host's body. Most of the engorged larvae moulted to nymphs on the mouse back. These unfed nymphs behaved in two ways, too. Some of them moved freely and did not attach themselves, others attached themselves immediately after the metamorphosis and stayed on the host up to the stage of engorged nymphs and then dropped off. In some cases the whole process from the attachment of larvae to the dropping of engorged nymphs lasted only 11 days, in other cases it amounted to 25 days. Nymphs which had left the host in an unengorged state as well as those which had dropped off as engorged larvae and metamorphosed away from the host's body attached themselves again after a short time (7—14 days) to the host and fed. The feeding of nymphs lasted in this case 5—7 days. The ratio between engorged larvae and unfed and engorged nymphs was 1:1:2.

*Haemaphysalis concinna*: The obtained data on the duration of development are given in Table 1. The whole developmental cycle of this species passed under laboratory conditions with many difficulties. Only a low percentage of pre-imaginal developmental stages fed on laboratory animals so that few specimens only passed through the whole development.

*Haemaphysalis punctata*: All information concerning the development of this species is given in Table 1. As to this species, we determined the most convenient time for feeding unengorged larvae. Although we used larvae of different age (from 2 weeks to one year) and employed both methods — free feeding and small pertinax cups, we did not succeed in feeding on white mice. Larvae either refused to attach themselves or were reluctant to do so and only few of them started to feed.

*Rhipicephalus turanicus*: Data on the duration of individual stages are given in Table 2. During observations of the development of this species in laboratory it was found out that feeding of pre-imaginal stages was less difficult than that of ♀♀ on unnatural hosts (laboratory animals).

## DISCUSSION

During the feeding of a large number of ♀♀ *I. ricinus* in laboratory it was observed that ♀♀ which had fed for more than 10 days were not fully engorged and most of them did not lay eggs after having dropped off. It seems to be probable that these ♀♀ were inseminated neither before, nor during the feeding, so that even in the case of having laid eggs these eggs were sterile. As far as this species is concerned, it was also found that the duration of ovogenesis changed during the year. A considerable difference was for instance found in the duration of ovogenesis in May—June (4—7 days) and in September—October (22 days). However, the total extent of the duration of ovogenesis does not essentially differ from Pomerantsev's data (1950), according to which the duration of ovogenesis is 4—27 days.

As to *I. hexagonus*, during the feeding of larvae and nymphs on different hosts it was found that the feeding on a white mouse was shorter than that on a hedgehog. According to the fact that in such a case the hedgehog is a natural host while the mouse is an unnatural one, a contrary could be rather expected. Such behavior can be explained by the acquired immunity of hedgehogs. The employed hedgehogs came from free nature where many ticks could feed on them, provoking an immunity.

The difference in the duration of ovogenesis in accordance to the season was found to be much more apparent in case of *D. reticulatus* and *D. marginatus* than in case of *I. ricinus*. All these species seem, even in laboratory, to retain their capability of a more rapid development in the spring than in the autumn.

As it has been already said in the text concerning *H. dromedarii*, some of the larvae underwent the two-host type of development, while the others the three-host type. Delpy (1946) found that at sufficiently high temperatures *H. dromedarii* had always the three-host character. At low temperatures or on an unusual host (in this case a white mouse) the original three-host type of development changed into the two-host type.

During observations of the development of *H. anatolicum excavatum* our data on the duration of individual stages were found to be different from those of Hoogstraal and Kaiser (1959) who were culturing this species at summer room temperatures in Cairo. Those temperatures were apparently higher than the standard culturing conditions during our observations and accounted for a shorter duration of individual developmental stages. Also our data on the species *R. turanicus* differ a little from those of Petrova-Piontovska (1947) and Ogandjanyan (1948) who used higher temperatures for laboratory culturing.

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