METHODS FOR THE COLONISATION OF UNFED NYMPHS OF HYALOMMA* SUBSPECIES FOR TRANSMISSION OF THEILERIA ANNULATA**.

BY

BRIAN DENNIS HOSIE

A Dissertation in partial fulfilment of the requirements for the Degree of Master of Science in the University of Edinburgh, Royal (Dick) School of Veterinary Studies.

Centre for Tropical Veterinary Medicine, Edinburgh.

1978

* *Hyalomma* Koch, 1844 (Ixodoidea, Ixodidae).

** *Theileria annulata* Dschunkowsky and Luhs, 1904 (Piroplasmea, Theileriidae).

CONTENTS

	Page
Summary	1
Introduction	2
Literature Review:	
Nomenclature	3
Distribution, Prevalence and Seasonal Activity	4
Life Cycle *Hyalomma anatolicum anatolicum*	7
Life Cycle *Hyalomma anatolicum excavatum*	11
Life Cycles of Other Species of *Hyalomma*	14
Disease Relations	17
Summary and Conclusions	23
Materials and Methods	24
Experimental Design and Results	
Experiment 1	31
Experiment 2	35
Experiment with Calves	48
Minimum Development Times and the Collection Weights of the Ticks	53
Discussion	
Feeding *Hyalomma anatolicum* subspecies on Rabbits	55
Feeding *Hyalomma anatolicum* subspecies on Calves	64
Conclusions	68
Acknowledgements	69
References	70

SUMMARY

Unfed nymphs of one strain of *Hyalomma anatolicum anatolicum* Koch, 1844 and two strains of *Hyalomma anatolicum excavatum* Koch, 1844 were required for further investigations into the development cycle of *Theileria annulata* Dschunkowsky and Luhs, 1904. On rabbits, these ticks have both two- and three-host cycles and there was evidence of a one-host cycle in one of the *H. a. excavatum* strains. Rabbits were chosen as hosts because of their ability to produce many ticks. Two methods of modifying the feeding pattern were successfully developed: engorged larvae, which were developing to nymphs on the host, were removed for moulting in the laboratory; newly moulted, two-host nymphs were picked off the rabbits before they could attach. In these two ways large numbers of unfed nymphs were produced but they did not store well. Also when applied to two calves suffering acute *T. annulata* infection they fed poorly and many died before moulting to adults. The cause of this poor performance on calves was not determined.

Checks were made on the rabbits' health and other than a small, but steady fall in weight, they suffered no ill effects.

INTRODUCTION

The object of this study into the feeding behaviour of *Hyalomma anatolicum* subspecies on rabbits was to develop a suitable technique for obtaining unfed nymphs for use in the transmission experiments with *Theileria annulata* being conducted at the Centre for Tropical Veterinary Medicine.

As will be shown later, several authors have described two and three-host cycles when using rabbits to feed either subspecies of *H. anatolicum*, i.e. *H. anatolicum anatolicum* and *H. anatolicum excavatum*. Bhattacharyulu, Chaudhuri and Gill (1975) used rabbits to provide all instars of *H. a. anatolicum* for their work with calves and *T. annulata*. Schein (1975) and Mehlhorn and Schein (1977) maintained a colony of *H. a. excavatum* on rabbits for work with *T. annulata* and Mehlhorn and Schein (1976) used it for work with *Theileria parva*. Hooshmand-Rad and Hawa (1973b) used rabbits to maintain *H. a. anatolicum* for transmission of *Theileria hirci* in sheep. None of these authors describe a method for obtaining large numbers of unfed nymphs.

Rabbits were chosen as hosts because of the experience gained at this Centre using them to maintain a colony of *Rhipicephalus appendiculatus* and the consequential ease of supply, feeding, housing and handling. Also their relatively large size in comparison to other laboratory hosts makes them suitable for producing many ticks.

LITERATURE REVIEW

Nomenclature

Throughout this dissertation I have followed one authority for nomenclature, Hoogstraal and Kaiser (1959) and Kaiser and Hoogstraal (1964). The former paper established *Hyalomma anatolicum anatolicum* and *Hyalomma anatolicum excavatum* as separate subspecies in western literature and the latter defended and substantiated this work. However Feldman-Muhsam (1962) considered that these two subspecies are but one polymorphic species, *Hyalomma excavatum* and this view is still held by Israeli workers. A summary of the development of these two schools is presented.

Hoogstraal and Kaiser (1959), after a review of the literature and study of many specimens, including some sent by Soviet workers, concluded by agreeing with the work of Pomerantzev (1946, 1950). Pomerantzev (1946, 1950) recognised two subspecies of *Hyalomma anatolicum*, *anatolicum* and *excavatum*, calling *H. a. anatolicum* what many other contemporary acarologists considered a small variety of *H. excavatum*. Delpy (1949) and Feldman-Muhsam (1954) synonymized *H. anatolicum* with *H. excavatum*. This was accepted by Hoogstraal (1956), although in an appendix he described a *Hyalomma* species No. 2, near *excavatum* which he thought may be considered as "*H. anatolicum* (sensu Schulze)". Then in 1959, Hoogstraal and Kaiser concluded that the *H. excavatum* of Delpy and Feldman-Muhsam was *H. anatolicum anatolicum* (sensu Pomerantzev, not Schulze) and the larger sized subspecies of *H. anatolicum* was *excavatum* (sensu Pomerantzev, not Delpy). Kaiser and Hoogstraal (1964) defended this opinion while accepting the need for more work on the subject.

However until recently Soviet workers have followed the work of Pervomaisky (1954). He regarded H. a. anatolicum and H. a. excavatum as H. anatolicum a very polymorphic species. Feldman-Muhsam (1962) agreed with this opinion but believed that H. excavatum is more correct. Now Soviet workers are again using subspecific names, for example Berdyev (1974) and Kuima and Pak (1975).

At present most western authors accept the opinion of Hoogstraal and Kaiser (1959). Where another name is used I have mentioned it and I have followed the tables of synonyms prepared by Hoogstraal and Kaiser (1959) for earlier references.

Distribution, Prevalence and Seasonal Activity

Both subspecies are largely restricted to steppe lands, semi-desert areas and oases, (Hoogstraal and Kaiser, 1959).

Hyalomma anatolicum anatolicum is common throughout northern Africa and it ranges into south eastern Europe, the Near and Middle East, Asia Minor and southern Russia into India, (Hoogstraal and Kaiser 1959). Kaiser and Hoogstraal (1964) consider it to be the most common tick of the genus Hyalomma in Pakistan and northern India while Chaudhuri, Shrivastava and Naithani (1969) describe it as the most widely distributed Hyalomma in India. It is common in Iraq, often accounting for over 80 per cent of all Hyalomma ticks (Robson and Robb, 1967; Robson, Robb and Al-Wahayyib, 1968; Robson, Robb and Hawa 1968 a, b, 1969; Robson, Robb, Hawa and Al-Wahayyib, 1969 a, b). Liebisch and Zukari (1978) state it is the second most common tick of domestic animals in Syria and in Turkey, Warnecke (1978) finds H.a. anatolicum to be the most numerous tick of cattle.

Hyalomma anatolicum excavatum is less numerous and has a more restricted, less even distribution than H. a. anatolicum (Hoogstraal and Kaiser, 1959). They state H. a. excavatum is found in a belt from the Spanish Sahara across Africa to Israel, southwards along both coasts of the Red Sea to Yemen and through the Sudan to French Somaliland. They also find it in Crete, Greece, parts of Turkey, southern Russia and Iran. More recently it has been recorded in Afghanistan (Kaiser and Hoogstraal, 1963), Iraq (Robson and Robb, 1967), Syria (Liebisch and Zukari, 1978), the Somali Democratic Republic (Pegram, 1976), and Cyprus (Le Riche, Altan, Campbell and Efstathiou, 1974). Mazlum (1971) found H. a. anatolicum in Iran but not H. a. excavatum and questions its existance there.

Hoogstraal and Kaiser (1959) neven found the subspecies excavatum on domestic animals restricted to the cultivated Nile Valley and Delta where H. a. anatolicum is common. In Syria, Liebisch and Zukari (1978) found H. a. excavatum widely distributed through desert and steppe lands and also in irrigated lands and oases, but there less frequently than H. a. anatolicum which is restricted to non desert, irrigated and cultivated lands.

Most authors agree with Hoogstraal and Kaiser (1959) that H. a. excavatum is less numerous than H. a. anatolicum where they occur together (Kaiser and Hoogstraal, 1964; Robson and Robb, 1967; Robson et al, 1968; Robson et al, 1968 a, b, 1969; Robson et al, 1969 a, b; Hoffmann, Horchner, Schein and Gerber, 1971; Warnecke, 1978.).

This is also the case in Syria where both subspecies occur together but H. a. excavatum is the more common tick on domestic animals throughout all Syria (Liebisch and Zukari, 1978). On Cyprus, H. a. excavatum is more common than H. a. anatolicum (Le Riche et al, 1974).

Sonenshine and Ziv (1971) are the only writers to apply the nomenclature of Hoogstraal and Kaiser (1959) and Kaiser and Hoogstraal (1964) to H. anatolicum subspecies in Israel. There they find H. a. excavatum to be one of the most important livestock pests. But their study was limited to a particular field in a semi-desert area in winter, and Hoogstraal and Kaiser (1959) state that in North Africa all instars of H. a. excavatum are active throughout the year, and more so in winter than summer. This is unlike H. a. anatolicum which is rare on animals in the cold season. Thus Sonenshine and Ziv (1971) may be misleading concerning the relative frequency of the two subspecies in Israel. However Feldman-Muhsam and Saturen (1961) state that Hyalomma excavatum accounts for more than 95 per cent of Hyalomma species on cattle in Israel and is common on camels, donkeys, horses, mules and sheep.

The difference in seasonal occurrence of the two subspecies has been noted by writers other than Hoogstraal and Kaiser (1959). Le Riche et al (1974) found H. a. excavatum most common on livestock in Cyprus between August and November, whereas other Hyalomma species are seen mainly between April and July. Robson et al (1968 a, b) found that in Iraq H. a. excavatum formed an approximately constant percentage of the total population of Hyalomma ticks in the summer and winter, unlike *H. a. anatolicum* which formed a much smaller percentage in the winter than it did in the summer. However in Syria, Liebisch and Zukari (1978) found adults of *H. a. excavatum* show a first peak of activity in April-May, a second peak with maximum numbers in September which falls to negligable activity in January. *H. a. anatolicum* is active in the spring and after a break in activity in June, has three secondary rises peaking in July, September and November and maximum numbers in September.

Life Cycle

Hyalomma anatolicum anatolicum.

Hoogstraal and Kaiser (1959) state that the immature stages of this subspecies will only parasitise hares and larger domestic animals and refers to lists of all species of domestic animals parasitised by adults in a previous review (Hoogstraal 1956, as *H. excavatum*). Thus Hoogstraal (1971) finds *H. a. anatolicum* is unique in comparison with other *Hyalomma* species in that immature stages usually feed on the same hosts as adults. I believe that it is difficult to be dogmatic about the host preferences of larvae because their small size makes it very difficult to find them on a host. As *H. excavatum*, Hoogstraal (1956) reported rarely finding larvae on Egyptian cattle and also in Egypt Daubney and Said (1951, as *H.* excavatum) did not find larvae in large numbers on cattle but did find many nymphs and adults. Chaudhuri *et al* (1969) and Avsatthi and Hiregauder (1969) claim to find many larvae, nymphs and adults on domestic animals in India but the former writers report finding larvae on the body of field rats (*Rattus rattus*). On cattle the sites most commonly parasitised by adults are the perineum, scrotum, umbilicus, base of tail, udder, inguinal areas and axillae (Hoogstraal 1956; Daubney and Said, 1951; Avastthi and Hiregauder, 1969; Chaudhuri *et al*, 1969). Chaudhuri *et al* (1969) found larvae attach chiefly to the snout, cheeks, pinnae, dewlap, tail and lower leg but Avastthi and Hiregauder (1969) report finding them on the pinnae and in heavy infestations, on the neck. Nymphs generally feed on the neck especially the crest, and with adults (Daubney and Said, 1951). Chaudhuri *et al* (1969) also mention the pinnae, cheeks, throat, dewlap and tail as sites for nymphal attachment and Avastthi and Hiregauder (1969) find them on the back during heavy infestations.

As mentioned earlier *H. a. anatolicum* is less active in the winter. Then it hibernates as replete nymph and young adult in cracks in walls, crevaces and other natural shelters (Serdyukova 1946 b, in Tadzhikistan as *H. excavatum*; Daubney and Said, 1951, in Egypt; Delpy 1952, in Iran as *H. excavatum*; Hoogstraal 1956, in Egypt). Chaudhuri *et al* (1969) also report finding replete larvae in similar situations in India. The annual pattern of activity is reviewed by Hoogstraal (1956).

On cattle, in the field, *H. a. anatolicum* is normally a three-host tick (Feldman-Muhsam 1948, as *H. savignyi*; Daubney and Said, 1951; Delpy 1952; Avatthi and Hiregauder, 1969; Chaudhuri *et al*,1969). This has been confirmed experimentally by Serdyukova (1946a), Chaudhuri *et al* (1969) and Bhattacharyulu *et al* (1975). However Daubney and Said (1951) mention a single larva moulting on a bovine and Chaudhuri *et al* (1969) note that a few larvae may moult and feed as nymphs on the same bovine host before 'dropping' as engorged nymphs. Chaudhuri *et al* (1969) also report that these larvae may remain at the same site until they are engorged nymphs or change position before the nymphal feed or may leave the host as unfed nymphs. The numbers involved are generally small but Lototsky and Pokrovsky (1946, as H. anatolicum) consider that in Tadzhikistan it is a two-host tick.

The most widely quoted work on this subject is that of Serdyukova (1946 a). Using larvae from the same batch of eggs he put some on calves and some on rabbits. Those on calves all detached when replete but of those on rabbits, some detached as replete larvae and others moulted on the rabbit. The resulting nymphs either fed to repletion and detached, or wandered without feeding. Therefore he concluded that H. a. anatolicum is a three-host tick on normal hosts but that on unusual hosts it may alter this behaviour.

Similar results were found more recently by Hooshmand-Rad and Hawa (1973 b). They found that larvae fed on sheep dropped as engorged larvae while the great majority of those larvae on rabbits stayed on to later drop as engorged nymphs.

When larvae of H. a. anatolicum are fed on a small animal such as a rabbit, two and three-host life cycles are observed but the proportion exhibiting each varies according to the host species. On rabbits, Avastthi and Hiregauder (1969) found 85 percent were two-host ticks and Sardey and Ghafoor (1971) found all were two-host ticks. Thus the majority of larvae are two-host ticks on rabbits. Snow (1969), using guinea-pigs, reported that 18 to 20 percent of larvae moulted on the host and the rest detached replete. Feldman-Muhsam (1948), using a hedgehog, noticed only a few larvae moulted on

TABLE 1

The life cycle of *H. a. anatolicum* according to various authors. (Days)

Author	Host	Conditions	Egg incubation	Larva rests	Larva feeds	Larva moults	Nymph rests	Nymph feeds	Nymph moults	Adult rests	Female feeds	Pre-ovi-position	Ovi-position
Serdyukova (1946b)	Calf	Field	23-26	-	2-4	6-12	-	4½-6	12-20	-	-	7-9	15-19
Feldman-Muhsam(1948)	Hedgehog	32°C and 70% R.H.	21	14	5	6	7	9-12	11-35	14	7	7	-
Daubney *et al* (1951)	Cattle	21-32°C	22-38	-	3-8	7-8	-	5-8	16-19	-	8	5-13	-
Chaudhuri *et al*(1969)	Cattle	25-35°C	17-28	4-11	3-4	6-12	2-7	3-4	7-14	7-14	5-7	4-10	7-15
Snow (1969)	Guinea-pig	28°C and 25% R.H.	3-Host 28	6-10	3	7-8	5-6	4	21-32	6-8	7	10-11	15-19
			2-Host 28	"	3	4	0	4	"	"	"	"	"
Sardey *et al* (1971)	Rabbit	25-30°C and 80%R.H.	2-Host 24-26	3	←—	16-24	——	—→	10-15	6	13	6-7	9-10

the host.

Table 1 shows the duration of each stage as found by various authors.

Hyalomma anatolicum excavatum

Hoogstraal and Kaiser (1959) state that immature stages of this subspecies parasitise only smaller animals such as insectivores, rodents and hares.

By obtaining nymphs from a variety of small animals they were able to rear adults for subsequent confirmation of subspecies and so give a list of species of gerbil, hedgehog and jird which act as hosts for the immatures. Sonenshine and Ziv (1971) found when studying ticks infesting sheep and small mammals on a semi-desert pasture in Israel that larvae and nymphs of H. a. excavatum were found on hedgehogs (Erinaceus spp.) and jirds (Meriones tristrami). Adults were found only on sheep. They did not rear through to adults for confirmation of subspecies.

Camels and cattle are usually the hosts most heavily parasitised, with sheep goats and horses being less important and others including antelope serving as occasional hosts (Hoogstraal and Kaiser, 1959). Liebisch and Zukari (1978) found camels very important hosts in Syria and both they and Le Riche et al (1974) found sheep to be as heavily parasitised as cattle while goats carried very light burdens.

This work would appear to establish H. a. excavatum as a parasite of small mammals during its immature stages and of large domestic animals during the adult stage. However in Turkmenia, U.S.S.R., Berdyev (1973) finds cattle to be the main host of all developmental phases of *H. a. excavatum.* There may be problems of nomenclature.

Sonenshine and Ziv (1971) found adults only in the perineal region of sheep and Le Riche *et al* (1974) describe the same area as for *H. a. anatolicum* not distinguishing between any ticks of the genus *Hyalomma.*

The seasonal activity of the adults was earlier contrasted with *H. a. anatolicum.* Sonenshine and Ziv (1971) found that on small animals the nymphs were active through most of the winter and most abundant in November and December while larvae were most abundant in late November and inactive in December and early January but re-appeared at least until late March. As might be expected these results do not agree with those found by Berdyev (1974) in the Turkmen SSR. He finds that from each wintering population of adults and nymphs (replete and hungry) there are approximately one and a half generations a year. The mass moulting of replete immature instars, the hatching of larvae and their subsequent attachment take place in the summer. Adults were found all year round.

In rearing experiments using rabbits Hoogstraal amd Kaiser (1959) found *H. a. excavatum* can undergo either a two-host or three-host life cycle. Of 292 larvae put on a rabbit's ear, 151 detached and moulted to nymphs and the rest, 141, moulted on the host to detach as engorged nymphs. Berdyev (1974) using rabbits and guinea pigs also observed two-host and three-host life cycles regardless of season or air temperature.

German workers studying the life cycle of *Theileria annulata*

TABLE 2

The life cycle of _H_. _a_. _excavatum_ according to several authors. (Days)

Author	Host	Conditions	Egg incubation	Larva rests	Larva feeds	Larva moults	Nymph rests	Nymph feeds	Nymph moults	Adult rests	Female feeds	Pre-ovi-position	Ovi-position
Hoogstraal _et al_ (1959)	Rabbit (3-Host)	Cairo summer room temp.	27	4	4	5	-	-	-	-	-	9	-
	(2-Host)	"	"	"	←	13	17	→	14-15	-	-	"	-
Berdyev (1974)	Rabbit (3-Host)	27.7-34.9°C	20	8	5	20	4	7	15	8	6	11	-
	(2-Host)	23.0-34.4°C	25	5	←	1	5	→	22	10	7	15	-
Al-Janabi (1976)	Rabbit (3-Host)	25°C;86%RH.	-	14-21*	5-11	6-10	14-21*	6-9	22-28	28+*	8-16	5-8	-
	(2-Host)	"	-	"	←	13	18	→	"	"	"	"	-

* Ticks held.

in the tick (Schein, 1975; Mehlhorn and Schein, 1977) have fed H. a. excavatum on rabbits, gerbils and calves. They have not published their findings on rabbits and gerbils but on calves larvae show a three-host cycle.

Al-Janabi (1976) working at this Centre, used rabbits to feed larvae, nymphs and adults. Of 1000 unfed larvae applied 24.3 percent completed their engorgement to drop as engorged larvae and 21.3 percent dropped as engorged nymphs. The rest were lost. Table 2 shows the duration of each stage of the life cycle as found by various authors.

Israeli works regard H. a. anatolicum and H. a. excavatum as a single polymorphic species H. excavatum. However much of the large amount of work they have done on this species may be applicable to H. a. excavatum. Hadani, Cwilich, Rechav and Dinur (1969) have found the immature instars on naturally infested gerbils (Meriones tristrami) and in only two instances have they found a few engorged nymphs on cattle. In the laboratory, they rear larvae and nymphs on gerbils and adults on rabbits. A three-host cycle is displayed. Larvae and nymphs can be fed on calves with difficulty, using either the ears or putting capsules on the body. Adults can be fed on gerbils if confined by capsules. These findings are very similar to those described by Hoogstraal and Kaiser (1959) for H. a. excavatum and suggests that this is the tick with which they are working. Thus H. a. excavatum may be a three-host tick in the natural state.

Life cycles of other species of Hyalomma

Table 3 compares the life cycles of H. a. anatolicum and

H. a. excavatum with other members of the genus Hyalomma on small animals in the laboratory. The table shows as a percentage, the number of three, two and one-host ticks in several species of Hyalomma.

The genus Hyalomma is exceptional in showing such a variety of host cycles. Hyalomma asiaticum asiaticum has a strict three-host cycle (Mazlum, 1968) while Hyalomma scupense has a one-host cycle (Markov, 1948; cited by Pipano, 1977).

Under natural conditions Hyalomma detritum and Hyalomma marginatum subspecies are constant two-host ticks (Hoogstraal, 1978). This is also true of Hyalomma dromedarii which parasitises burrowing mammals in its immature stages and large animals as adults, although nymphs may be found on large animals occasionally (Hoogstraal, 1956).

TABLE 3

Host cycles of several species of *Hyalomma*.

Species	Host Cycle: Three	Two	One	Reference
H. a. anatolicum				
Guinea-pig	80-82%	18-20%		Snow (1969)
Rabbit	15%	85%		Avatthi *et al* (1969)
Rabbit		100%		Sardey *et al* (1971)
H. a. excavatum				
Rabbit	50%	50%		Hoogstraal *et al* (1959) and Al-Janabi (1976)
H. asiaticum asiaticum				
Rabbit	100%			Mazlum (1968) and Berdyev (1972)
H. detritum				
Rabbit		100%		Hadani *et al* (1969)
H. dromedarii				
Gerbil	100%			Hadani *et al* (1969)
Rabbit			100%	Alfeer (1951) cited by Hoogstraal (1956)
Rabbit	>50%	>50%	a few	Feldman-Muhsam *et al* (1966)
Rabbit	43%	57%		Das *et al* (1972 b)
H. marginatum issaci				
Guinea-pig		100%		Rau (1963)
Guinea-pig		100%		Jagannath *et al* (1972)
Rabbit		100%		Jagannath *et al* (1972)
Rabbit	5-6%	94-95%		Das *et al* (1972 a)

Disease Relations

The disease relations of H. a. anatolicum and H. a. excavatum are shown in Tables 4 and 5 respectively. Vector status depends on both experimental transmission and field evidence of the disease.

It is as vectors of Theileriosis that H. anatolicum subspecies interest us. Although Theileria parva has been transmitted experimentally by H. a. anatolicum (Lewis and Fotheringham, 1941; as H. anatolicum) and H. a. excavatum (Mehlhorn and Schein, 1976), there is no field evidence of either as vectors, and the disease, East Coast fever, has never spread from East Africa to the Sudan or Egypt.

Experimental transmission of T. annulata by H. a. anatolicum has been achieved by Daubney and Said (1951), Markov, Stepanova and Laptev (1966) and Bhattacharyulu et al (1975). Israeli workers have confirmed this work with H. excavatum (Hadani, Tsur-Tchernomoretz, Pipano and Senift, 1963; Samish and Pipano, 1978). All have only shown transstadial transmission. Daubney and Said (1951) attempted to show transovial transmission with three generations of ticks but failed and Delpy (1952) stated there was no evidence of hereditary transmission. However, Ray (1950) in India, reported transovarial transmission with Hyalomma aegypticum (probably H. a. anatolicum). He believed that only adults transmitted the disease and fed all instars on calves which raises the possibility of the adults becoming infected as larvae or nymphs.

Field evidence of H. a. anatolicum acting as a vector of

TABLE 4

Disease relations of *H. a. anatolicum.*

Disease	Reference
Strong field and experimental evidence.	
Theileria annulata	as *H. excavatum*, Daubney and Said (1951), Delpy (1952), Hadani *et al* (1963) Samish and Pipano (1978). as *H. a. anatolicum*, Markov *et al* (1966), Bhattacharyulu *et al* (1975), Warnecke (1978).
Theileria hirci	Hooshmand-Rad and Hawa (1975a, b)
Crimean Congo Haemorrhagic fever virus.	Casals *et al* (1970), Kuima and Pak (1975), Kemp (1978).
Near Eastern equine encephalitis virus.	Daubney and Mahlau (1967).
Experimental evidence only.	
Theileria parva	as *H. anatolicum*, Lewis *et al* (1941).
Babesia bigemina	Chaudhuri *et al* (1975).
Less strong or circumstantial evidence or suspect.	
Babesia spp.	Warnecke (1978).
Babesia caballi and *Babesia equi*	as *H. anatolicum*, Engik (1943) cited by Neitz (1956). as *H. excavatum* Riek (1968).
Rickettsia bovis	as *H. excavatum* Neitz and Jansen (1952) cited by Neitz (1956).

TABLE 5

Disease relations of *H. a. excavatum*.

Disease	Reference
Experimental only.	
Theileria annulata	Schein (1975), Mehlhorn and Schein (1977).
Theileria parva	Mehlhorn and Schein (1976).
Russian spring-summer encephalitis virus.	Hoogstraal and Kaiser (1959).
Less strong or circumstantial evidence or suspect.	
Theileria annulata	Warnecke (1978).
Babesia spp.	Warnecke (1978).

T. annulata is provided by Daubney and Said (1951, as *H. excavatum*). They collected hibernating adult *H. a. anatolicum* from cracks in walls in winter, and by applying them to calves, transmitted clinical theileriosis. Further evidence is provided by Warnecke (1978) who examined ticks collected from domestic animals in Turkey, for piroplasms. Of 177 *H. a. anatolicum* examined, 21 had evidence of *Theileria* species in their salivary glands. This level of infection is within the range of nine to sixty four percent found in the field by Laptev (1967, as *H. anatolicum*; cited by Barnett 1977). Warnecke (1978) also found three *H. a. excavatum* of 32 collected had evidence of *Theileria* species in their salivary glands. However, this is only evidence of infection and not of vector status as these ticks may have become infected on their last host and the next stage need not transmit the infection if it is a new larva or if a different species of host is found.

That *H. a. excavatum* can transmit *T. annulata* transstadially has been shown by Mehlhorn and Schein (1977). They used nymphs to transmit infection to three to six month old calves for studies on the development of *T. annulata* in the tick. However there is no field evidence of it being a vector other than that of Warnecke (1978) which is dubious, particularly in view of the immatures preference for small rodents as hosts. Barnett (1977) states that *H. a. excavatum* adults will not naturally be infected with *T. annulata* because nymphs cannot acquire infection from rodents.

Tropical piroplasmosis is the disease caused by *T. annulata*. Both disease and parasite have been recently reviewed by Barnett (1977) and Wilde (1977). The important epidemiological factors are summarised.

Wilde (1977) regards the persistance of T. annulata in the bovine host after recovery, and its ready transmission by blood innoculation and several species of two- and three-host ticks, as the most important factors affecting the epidemiology. Therefore this disease has a wide geographical range, being found in a belt across the world from the western Mediterranean to the Far East. In endemic areas, indigenous cattle become infected as calves and as most recover, they form a reservoir of infection. Exotic or improved stock suffer heavy losses when introduced to these areas. The water buffalo (Bubulus bubalis) is also susceptable.

Two ecological situations for the disease in Israel are described by Pipano (1977), namely the barn and the field. In some areas, barns contain all stages of the two-host cattle tick, H. detritum. This tick is the most efficient vector of T. annulata (Barnett 1977) because the immature stages feed readily on cattle. It has a biological varient, the one-host cattle tick, H. scupense which is found in similar situations. Engorged nymphs of H. detritum hibernate in crevices during winter, to moult to adults in the spring. After moulting on the host, the unfed adult H. scupense remain on the host until March when they feed (Barnett 1977). However these adults can move to a susceptable host before feeding and transmit the disease (Markov, Gildenblat, Kurchakov and Petunin, 1948, cited by Pipano, 1977). Thus these two species cause disease in late spring and early summer.

Not all barns in an endemic area harbour either H. detritum or H. a. anatolicum. Thus a susceptable population of cattle can arise when confined to barns, whereas in the field all cattle are exposed to infection in the summer. Barnett (1977) states that in addition to H. detritum, H. scupense and H. a. anatolicum, H. asiaticum may convey infection in the field. The latter two will cause disease later in the year.

Control of the disease is through vector control and vaccination. Pipano (1977) suggests treating cattle with acaricides every ten to twelve days to prevent development of engorged nymphs of H. detritum. More frequent dipping is required to control three-host ticks. Barns should be treated with persistent acaricides in spray or powder formulation. Tissue culture vaccines have been used in Israel, Iran and the U.S.S.R. (Barnett 1977). Hooshmand-Rad (1977) notes the significance of vaccination has not been evaluated, as its effectiveness against natural tick challenge has not been reported.

Through experimental transmission and ecological studies Hooshmand-Rad and Hawa (1973 a, b) established H. a. anatolicum as an important vector of Theileria hirci. This pathogen of sheep and goats is very similar to T. annulata in that it has a carrier state and is transmitted by Hyalomma ticks. Mazlum (1970) believes that in addition to H. a. anatolicum, H. m. marginatum and H. detritum could be vectors and eliminates Rhipicephalus bursa as a vector, not being found in south and south-east Iran where the disease occurs.

Summary and Conclusions

Hyalomma a. anatolicum and *H. a. excavatum* are readily found throughout a large part of the tropical world and much of their range is common with that of *T. annulata*. Both have been shown to transmit the parasite (see Tables 4 and 5 respectively) and because immature stages of *H. a. anatolicum* may feed on cattle it has been shown to be an important vector. *Hyalomma a. excavatum* is not a vector because it feeds on animals smaller than hares in the immature stages.

Both have been maintained in the laboratory on rabbits and have shown two- and three-host cycles. No one has reported a method for obtaining unfed nymphs for transmission experiments, nor are there any reports concerning the maintainance of *H. a. excavatum* in the laboratory and only one by Snow (1969) concerning *H. a.* anatolicum.

MATERIALS AND METHODS

Hosts

Rabbits: New Zealand White rabbits, four to six months old were supplied by the Centre for Laboratory Animals, University of Edinburgh. I preferred females being more docile but a male had to be used in Experiment 1. They were used only once for tick feeding as recommended by Bailey (1960) and Branagan (1974).

Calves:* British Friesian calves, five months old were obtained commercially through local markets.

Ticks

i) *Hyalomma anatolicum excavatum* (Razi strain).

This, the original C.T.V.M. stock had been obtained from the Razi Institute, Teheran, as *Hyalomma anatolicum*. Mrs. Susan Hood (a technician at the C.T.V.M.) questioned its identity, and considered it to conform to the features of *H. anatolicum excavatum* as described by Hoogstraal and Kaiser (1959). Al-Janabi (1976) held a similar opinion and had it confirmed by Dr. J. A. Campbell, Department of Zoology, University of Edinburgh.

ii) *Hyalomma anatolicum anatolicum* (Ludhiana strain).

Obtained from Punjab Agricultural University (India) as such, its identity was confirmed by Dr. A.R. Walker using the keys of Hoogstraal and Kaiser (1959).

* Descriptions of methods for calves relate to a transmission experiment performed by C.G.D. Brown and A.R. Walker using ticks colonised by myself.

iii) Hyalomma anatolicum excavatum (Berlin strain).

This stock was recently obtained from the Free University of Berlin (G.D.R.). As H. a. excavatum, it had been bred as a strain highly susceptable to infection with the Ankara strain of T. annulata. It originated in Turkey. Dr. A.R. Walker confirmed its identity.

Storage of Ticks

Specimen tubes: Three types of clear plastic specimen tubes were used. They were of two sizes, 80 x 35 mm and 40 x 15 mm and were fitted with plastic caps. These were ventilated by a central hole, 7 mm in diameter, covered by fine guage wire mesh.

The larger tubes held about 100 engorged nymphs or emerged adults. The smaller ones were of two types. The standard flat bottomed type held a third to one complete egg batch and its larval product, or up to 200 engorged larvae and resultant unfed nymphs. The other type, illustrated in figure 1, when connected to a suction pump, collected engorged larvae or unfed nymphs from the rabbits. They also stored unfed larvae in Experiment 2 and unfed nymphs in all experiments. When used for storage, the nozzle was blocked with a piece of cotton wool.

Storage Environment: Three environments were available.

25^{o}C and 80 percent relative humidity: This environment was provided in a hot room equipped with heater, thermostat and humidifier. It was used for routine storage of unfed nymphs and adults and the moulting of engorged instars.

15^{o}C and 85 percent relative humidity: The temperature was maintained by a cooled incubator (Gallenkamp 1H-270). The humidity was maintained by holding the specimen tubes above a saturated solution of analar potassium chloride in a glass confectionary jar (inside dimensions 10x10x20 cms. high), after the method of Buxton and Mellanby, (1934). This environment was used to store unfed larvae, unfed nymphs after many had died on prolonged storage at 25°C and unfed adults.

34°C and 85 percent relative humidity: A standard laboratory incubator was used (Laboratory Thermal Equipment Ltd.) and to maintain the humidity the jars described earlier were used. This environment was only used in Experiment 2 for the moulting of certain larvae.

Preparation of Hosts

Both the body and ears were used as sites for tick feeding on rabbits. On calves, applications of ticks were made to ears and tail. The ticks were restricted to these areas by the use of bags, or, more correctly, sleeves.

Body bags: The first stage was to clip the rabbit's fur in a band round the body from 1½ cms. behind the forelegs to 1½ cms. in front of the hind legs, using electric small animal clippers (Oster Corp., U.S.A.). The fur on the back and flanks was closely clipped while the undersides were only lightly trimmed as few ticks attached there. Next a collar was put on as described by Bailey (1960) except stout polythene with 2½ cm. wide rubber tubing to protect the rabbit's neck, replaced leather. Hobbles of 2½ cm. wide, white, zinc oxide plaster B.P.C. on the hind legs prevented the rabbits scratching the body bags.

Body bags consist of a piece of white cloth with two elasticated ends and a zip fastener to join the two sides. Its dimensions depend on the size of the rabbit. It is put on by first positioning it unfastened, under the rabbit with the fastener lying parallel to the length of the rabbit. The corners beside the hindlegs are pulled up so the elasticated end lies over the rabbit's fur in front of the hindlegs and is held by engaging the fastener. It is fastened so as to lie along the rabbit's spine and the anterior elasticated end lies on the fur behing the forelegs. The fastener is secured by a safety pin.

Ear bags: Rabbits and calves were equipped with ear bags following the modifications of Branagan (1969) on the method suggested by Bailey (1960). The ear bag consists of a sleeve, one end being secured round the base of the ear with zinc oxide plaster B.P.C. and the other closed with two elastic bands and tied to the collar. The sleeve permits daily inspection of the ticks without removing the entire application (Branagan 1969).

Tail bags: They were used on calves and were simply very long narrow variations of the rabbit's body bags. They were firmly fixed to the tail root and switch with zinc oxide plaster.

Feeding Techniques

Application of ticks to host: The tubes containing the ticks were placed inside the bag which was then fastened. The ticks were released by removing the caps, working through the cloth. To prevent larvae escaping, a two centimeter wide strip of zinc oxide plaster was placed over the zip fastener.

Plate 1. Daily observation and collection of ticks.

Plate 2. Restraint of rabbit for tick collection.

Observation and collection: Daily observations were made. Larvae not attached after 48 hours, were removed and counted. Dropped, engorged instars were collected, weighed and counted. For collections from the body bag the rabbit was restrained by placing it on a stout wire platform in a standard household basin. (See Plates 1 and 2). The platform was about two centimeters off the bottom of the basin and this held the engorged instars which dropped or were brushed off the rabbit, until put into tubes.

Collections from ear bags were made by shaking the engorged instars out into a plastic bucket.

An electric diaphragm pump (Dymax 2, Charles Ansten Ltd., Weybridge.) was used with the modified collection tube described earlier and the active unfed instars were easily collected. (See Fig. 1). This is a modification of the method described by Matthewson and Hughes (1978).

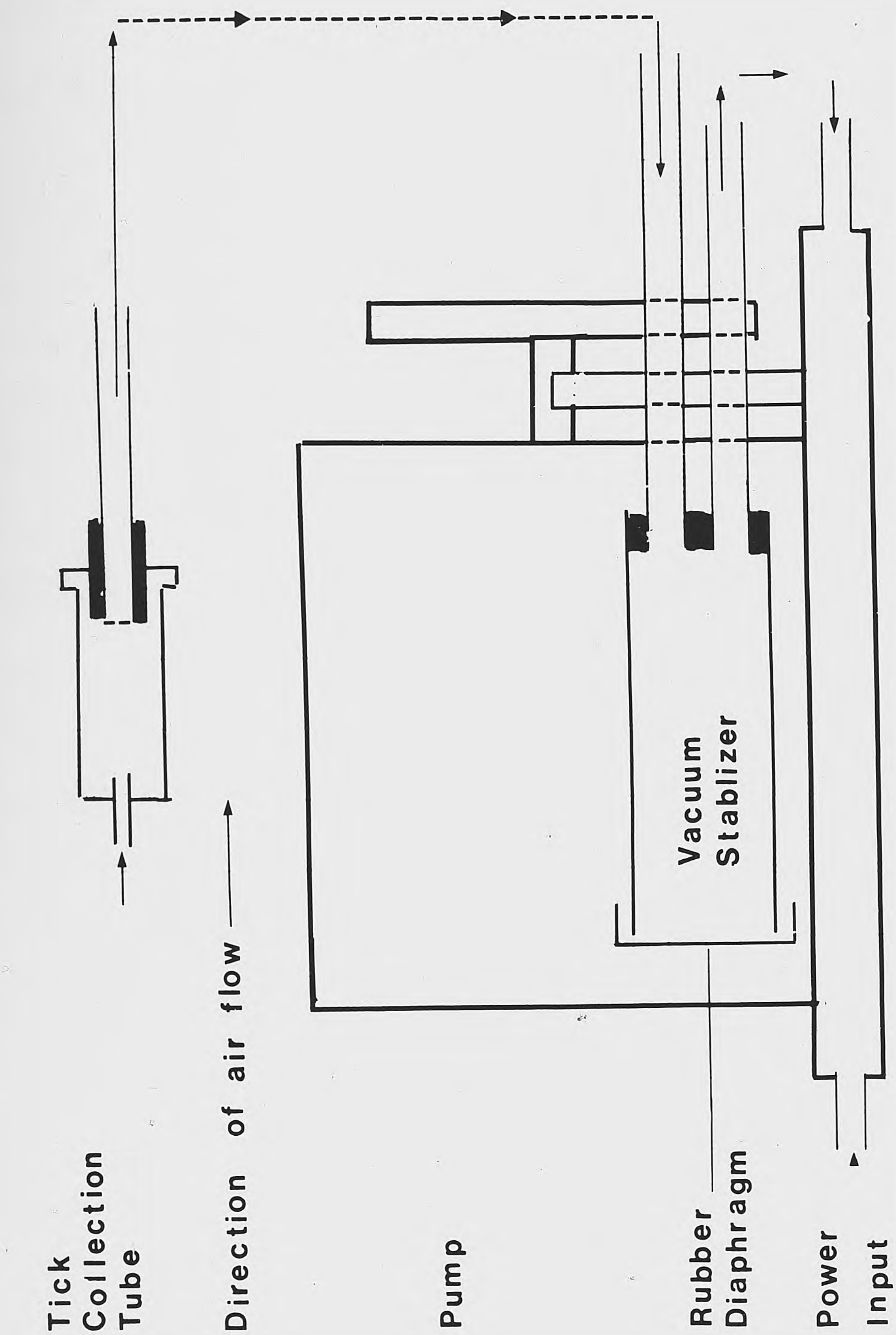
Miscellaneous

Tick handling in the laboratory: A number six, sable hair, watercolour brush was used for handling the ticks and the suction pump was used to assist in counting the highly active unfed larvae and nymphs. A tally counter was used when counting large numbers. All tick work in the laboratory is carried out in a tray (35 x 20 cms.) surrounded by a moat of antiseptic/detergent solution (Savlon, I.C.I. Ltd.).

Weighing: Ticks were weighed on a substitution balance (Oertling R. 20, Oertling Ltd., Orpington). It has a sensitivity of 0.1 mg

FIGURE 1.

Pump for unfed nymph collection.



and is accurate to $\pm$ 0.05 mg.

The rabbits were weighed on a spring pan balance (Sherring 5 kg., by Sherring Ltd., Edinburgh-Glasgow). It has a sensitivity of 10 grms.

Blood collection and examination: In Experiment 2 , blood was collected from the rabbits, twice a week. The blood was taken from an ear vein and 0.5 ml. put into a collection tube containing E.D.T.A. as anticoagulent. Total red cell and total white cell counts, packed cell volumes and mean cell volumes estimations were made using an electronic counter and analyser (Coulter Counter Fn, Coulter MCV/HCT accessory and Haemoglobinometer, Coulter Electronics Ltd., Dunstable). Blood smears were made and stained by Giemsa for differential white cell counts.

Rabbit housing: The rabbits were kept in individual rabbit cages, in an isolation room in the small animal house at the C.T.V.M. The cages measured 19x24x16 ins. high and had a slatted floor over a droppings tray. The temperature in the isolation room was between 10 ^{o}C and 21 ^{o}C during the first experiment and between 16 ^{o}C and 22 ^{o}C in the second. The relative humidity was between 40 and 65 percent.

Calf housing: The calves were kept in individual pens at the C.T.V.M. urban isolation unit. The temperature varied between 18 ^{o}C and 22 ^{o}C and the relative humidity between 60 and 70 percent.

Rabbit feeding: A standard pelleted diet (SGl, Oxoid Ltd.) was fed ad-lib with a handful of hay daily and occasionally carrots.

Calf feeding: The calves were offered hay and propriety calf weaner nuts.

Body surface temperature and relative humidity: The temperature next to the skin of the rabbits was measured with an electrical resistance temperature probe (Sanwa Multitester, Sanwa Ltd., Tokyo). To ensure consistancy of results the probe was calibrated with a standard mercury thermometer at room temperature daily.

The relative humidity inside the body bag was measured daily with a simple dial-type hygrometer (Edney, England). It was calibrated by preparing a standard curve using an Assman aspirated hygrometer (Casella, London).

Statistical methods: Standard statistical methods were applied. Most calculations were made with a pocket calculator (Commodore, England).

EXPERIMENTAL DESIGN AND RESULTS

Experiment 1

Aims

Observe the normal feeding behaviour of *H. a. excavatum* (Razi strain) on a rabbit and try a method for collecting unfed nymphs.

Design

Four thousand larvae of *H. a. excavatum* (Razi strain) were removed from storage at 15°C and 85 percent relative humidity. I assumed a normal egg batch would supply three thousand larvae. They were applied to the body of a male rabbit and this was designated day 0.

All larvae unattached on day 2 were removed and counted. Detached, engorged instars were collected daily. Unfed nymphs which were wandering over the host were picked off with fine disecting forceps and the suction pump. All were counted and each day's collection weighed. They were stored at 25°C and 75 percent relative humidity.

The experiment was terminated on day 28 by removing the remaining nymphs with forceps.

The developing instars were examined approximately every day and the minimum development periods recorded. Later those which had successfully moulted, were counted.

Results

General: Table 6 shows the feeding pattern obtained on this rabbit. The number of ticks collected each day as the feed progressed is shown diagramatically in figure 2. The minimum development periods of the larvae and nymphs and the weight of the ticks collected were used as indicators of the normality of the feed and are compared with the results of other feeds in a separate section. (Minimum development times and the collection weights of the ticks).

Eighty two percent of the larvae moulted successfully and all of the nymphs excepting five which were detached at the end of the experiment.

Observations: Within 24 hours very approximately seventy percent of the larvae had attached along the back with approximately twenty percent on the flanks and five percent on the abdomen. The first three days' collections consisted of mobile, engorged larvae but subsequent collections had a rising proportion of immobile larvae with hard outer coats. I believe they were dislodged by the body bag rubbing the rabbit's clipped hair.

Many recently moulted nymphs were seen lying inside their larval coats still on the host. They could be carefully levered or picked out of them with fine disecting forceps to join others wandering over the host but only those nymphs already out of their coats were collected. The collection tube attached to the suction pump was most satisfactory when collecting nymphs from the clipped area of the rabbit. Those nymphs holding onto long unclipped hair were best removed with forceps. The yellow to dark brown wandering nymphs were easily seen against the host's white coat but distinguishing those in their larval coats was more difficult because only the end of the coat was open. With experience more were recognised.

Mobile and immobile engorged nymphs were collected and on day 28 four adults were found. Three had started feeding while the other was waiting.

The rabbit accepted tick collection well when restrained in the basin and became very docile with repeated handling. It was irritated by the ticks and where several were engorging closely together there was an erythema. At the end of the experiment I thought it had lost a lot of weight.

FIGURE 2

Experiment 1

Hyalomma a. excavatum (Razi stain)

Number of ticks collected each day as the feed progresses.

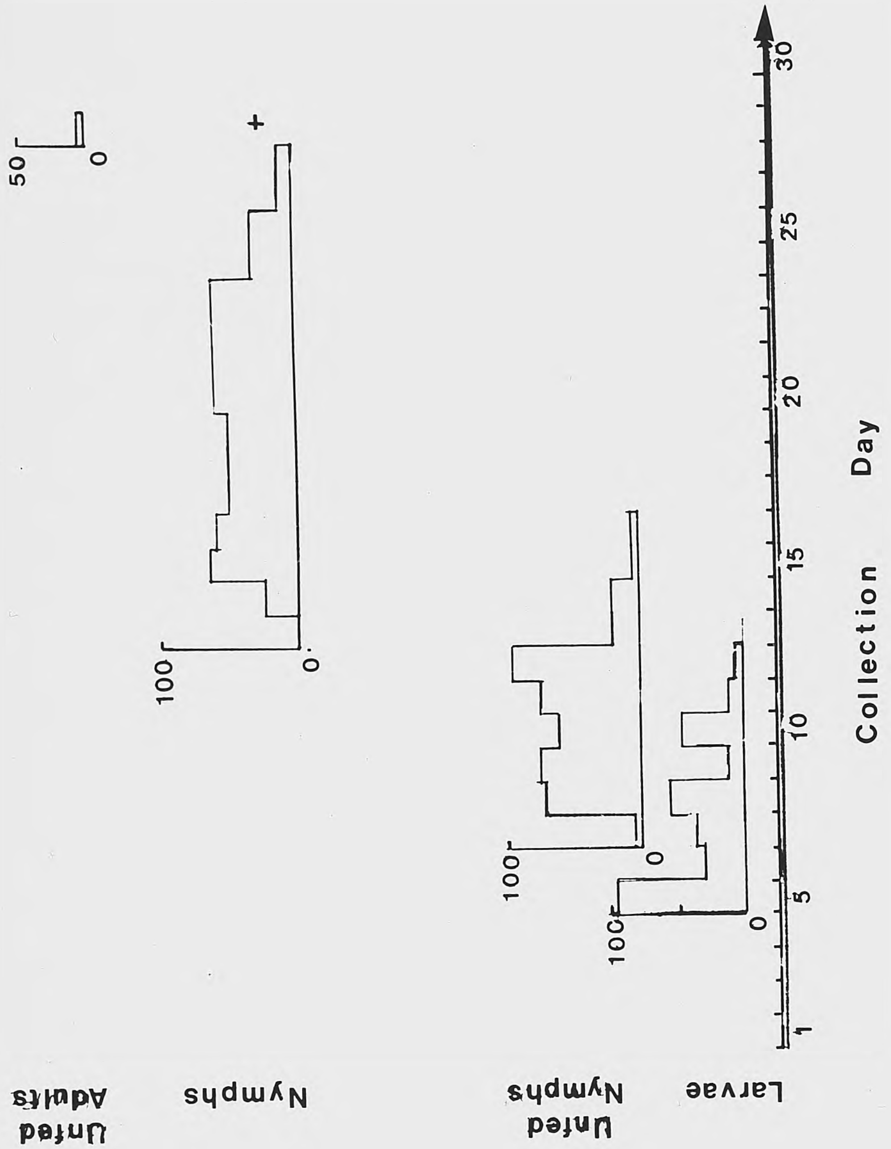


TABLE 6

Feeding performance of H. a. excavatum (Razi strain) on a rabbit. (Experiment 1).

No. larvae on:	4,000 approx.
No. larvae removed on day 2:	700 approx.
Engorged larvae	
Number:	278
Collection range (days):	5-13
mean (days):	7.1
Unfed nymphs	
Number:	429
Collection range (days):	7-16
mean (days):	10.6
Engorged nymphs	
Number:	597*
Collection range (days):	13-28+
mean (days):	19.6*
Adults	
Number:	4**
Collection day	28

* Not including 23 removed on day 28.

** Removed on day 28.

Experiment 2

Aims

i) Can immobile larvae with hard outer coats be removed from the host and moulted in an incubator?

ii) Is it possible to collect more unfed nymphs directly from the host by collecting those still hardening in their larval coats?

iii) How quickly do unfed nymphs which have moulted *in situ* re-attach?

iv) What are the effects on the host of tick feeding?

Design

This work was done initially with two rabbits feeding *H. a. excavatum* (Razi strain) and then in a reduced version, with one rabbit for each of *H. a. excavatum* (Berlin strain) and *H. a. anatolicum* (Ludhiana strain). Three thousand larvae were applied to the body of each rabbit. The larvae of *H. a. excavatum* (Iran) were counted first, while those of the other two strains were estimated by weighing egg batches and roughly dividing them to give very approximately 1,000 moulted larvae per tube.

A series of further observations were made in addition to the basic ones recorded in Experiment 1.

Unfed nymph collection: In order to anticipate the moulting of the nymphs on the host and recognise those lying in their larval coats, two-host larval development was simulated by incubation in the laboratory. Only detached larvae of H. a. excavatum (Razi strain) were used, as sufficient experience had been gained to recognise nymphs of the other two strains.

The incubation temperature was determined by measuring the rabbits' body surface temperature daily for a week with an electrical temperature probe. The ear surface temperature was also measured, as was the relative humidity inside the body bag. The incubation temperature used was 34°C, with a relative humidity of 85 percent. A maximum of about 20 larvae from each day's collection from each rabbit were incubated in this environment and examined daily for moulting. The rest were controls and incubated at 25°C and 80 percent relative humidity.

Thus nymphs were recognised from the start of moulting on the host. Those lying in their larval coats were picked or levered out with fine forceps to be collected with those wandering over the host by the suction pump. The Razi strain nymphs were stored initially at 25°C and 80 percent relative humidity but later were kept at 15°C and 85 percent relative humidity. The Berlin and Ludhiana strains were stored at the lower temperature.

Removal of two-host larvae for moulting in the laboratory: The number of mobile and immobile larvae of the Razi strain in each day's collection was recorded and when the majority were immobile, approximately 50 were removed daily from each rabbit. Approximately 90 percent were hard enough to be collected with fine forceps whithout being crushed. They were incubated at 34°C and 85 percent relative humidity and the resulting nymphs were collected after 15 days and the remainder incubated a further 12 days.

Because of heavy losses of moulted nymphs of the Razi strain, the larvae of the other two strains were incubated at 25°C and 80 percent relative humidity and the resulting nymphs were collected weekly for storage at 15°C and 85 percent relative humidity. Only immobile larvae of the Berlin strain were collected and they were included with those removed from the rabbit. Approximately 400 larvae of the Ludhiana strain escaped and attached to the ears because the zip fastener was not covered by plaster. Most were removed and destroyed but on days four and six, some were removed intact and were included with those larvae which were removed later or collected as immobile larvae.

Duration of two-host nymphs' waiting period: Fifty newly collected nymphs of the Razi strain were applied to one of each rabbits' ears. Those which were unattached the next day were collected with the suction pump, counted and re-applied. Those still unattached after two days were counted and discarded.

Longevity of unfed nymphs: When taking nymphs from storage for further work the survivors were counted to ascertain the suitability of the storage environment.

Rabbit health when feeding ticks: The rabbits were weighed weekly throughout the experiments. Blood samples for routine haematology were taken twice a week from the two rabbits used as hosts for the Razi strain.

Results

General: Again more than 90 percent of larvae attached within 24 hours and larvae and nymphs restricted by the body bag preferred feeding on the back.

The feeding patterns on the four rabbits are shown in Table 7. The numbers of ticks collected as the feeds progressed are shown diagramatically in figures 3 to 6. There is a difference of approximately one day between the mean collection times from the two different hosts of H. a. excavatum (Razi strain), and both of these feeds progressed faster than that of H. a. excavatum (Berlin strain). However, some unfed nymphs of the Berlin strain were left on the host to obtain more engorged nymphs for maintainance of the colony. They were reluctant to attach and 110 were removed on day 15 and 12 on day 19. Had they been removed earlier, either as larvae or nymphs the collection pattern would have more closely resembled a 'normal' distribution. The nymphs of Berlin strain were slow to engorge.

In contrast, all instars of H. a. anatolicum (Ludhiana strain) were collected earlier and over a shorter period than those of either strain of H. a. excavatum. The difference in mean collection times of unfed nymphs of H. a. anatolicum (Ludhiana strain) and H. a. excavatum (Berlin and Razi strains) is highly significant ($P < 0.01$).

The minimum development periods of larvae and nymphs and the weight of the ticks were used as a check on the normality of the feed and are compared with other feeds in another section (Minimum development times and the collection weights of the ticks). Nine of the ten *H. a. excavatum* (Berlin strain) engorged nymphs moulted as did all Razi and Ludhiana strain nymphs.

Unfed nymph collection: The simulation of two-host larval development successfully paralleled the first moulting of nymphs *in situ*. Table 8 shows the results of larval development at 34°C and 85 percent relative humidity. The body surface temperature of each rabbit was 34 $\pm$ 1°C and 35 $\pm$ 1°C and the ear surface temperatures were 32 $\pm$ 1°C and 31 $\pm$ 2°C. There was no difference between rabbits, but the difference between body and ear surface temperatures was significant ($P < 0.05$). The relative humidity inside each body bag was 32 $\pm$ 5 percent and 27 $\pm$ 7 percent and the difference is not significant. A more humid environment was chosen for incubation to aid the survival of the nymphs but some died before they were collected. Both live and dead were included in the percentage moult (see Table 8). The overall moult was 87.25 percent and is comparable with that of 82.8 percent of the 58 larvae incubated at 25°C as controls.

The later collections of larvae contained an increasing proportion of immobile larvae and although the mean moulting times tended to fall as that proportion rose, the range widened.

Of all larvae applied to the four rabbits 23.5 percent were removed as unfed nymphs. The collection of unfed nymphs from each rabbit often took more than an hour to complete. Good lighting was essential, as was the basin, described earlier, to restrain the rabbit.

Removal of two-host larvae for moulting in the laboratory:

The results of incubating larvae removed from the host are shown in Table 9. Some nymphs died before they were collected for storage at 15°C, but they were included when calculating the percentage moult. The Berlin and Ludhiana strains were incubated at a lower temperature than the Razi strain and had a lower mortality. The proportion of larvae moulting varied considerably from batch to batch but the collections made when nymphs were first appearing on the host were the most viable. Of all larvae collected 54.4 percent moulted and this was significantly less than the Razi strain larvae which dropped naturally from the host (See Table 8) ($P < 0.01$).

Duration of two-host nymphs' waiting period: Of the 100 nymphs applied to the rabbits' ears, eighteen were unattached after one day and four, two live and two dead, were unattached after two days. A total of 92 engorged nymphs were subsequently collected between days 5 and 10 with a mean engorgement time of 7.25 days.

Longevity of unfed nymphs: Table 10 gives the number of nymphs surviving different storage conditions. The mean number of days is the storage time of the mean collection. One day's collection of the Ludhiana strain may have suffered severe losses due to overcrowding, giving this strain a false, abnormally low survivability.

Rabbit health when feeding ticks: The average daily weight losses of the four rabbits over the experiment are shown in Table 11. Each lost approximately 0.5 percent of its initial body weight per day. The results of routine haematological examination on two rabbits is shown in Table 12 and are evidence of haemoconcentration as the feed progresses.

All of the rabbits were irritated by the ticks and frequently tried to scratch. Their skin was thickened where the ticks had fed. Both rabbits with ear bags showed excessive lachrymation and mild conjunctivitis.

Figure 3

(Experiment 2)

Hyalomma a. excavatum (Razi strain), Feed 1.

Number of ticks collected each day as the feed progresses.

(i) Engorged nymphs applied to ear as unfed nymphs.

(ii) Engorged two-host nymphs.

Figure 4

(Experiment 2)

Hyalomma a. excavatum (Razi strain), Feed 2.

Number of ticks collected each day as the feed progresses.

(i) Engorged nymphs applied to ear as unfed nymphs.

(ii) Engorged two-host nymphs.

FIGURE 3

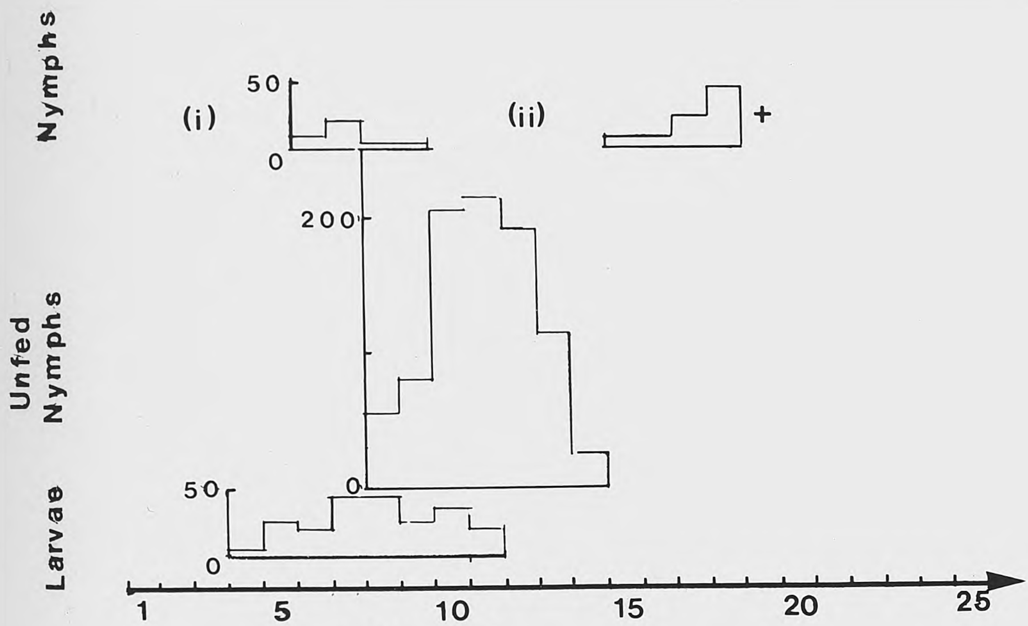


FIGURE 4

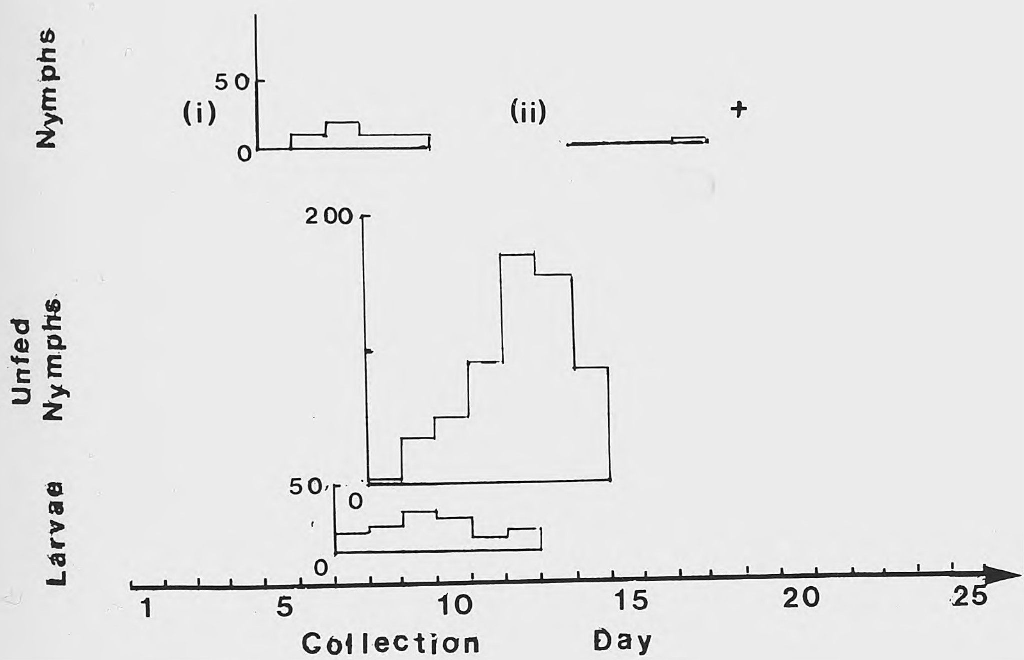


Figure 5

(Experiment 2)

Hyalomma a. excavatum (Berlin strain), Feed 1.

Number of ticks collected each day as the feed progresses.

Figure 6

(Experiment 2)

Hyalomma a. anatolicum (Ludhiana strain), Feed 1.

Number of ticks collected each day as the feed progresses.

FIGURE 5

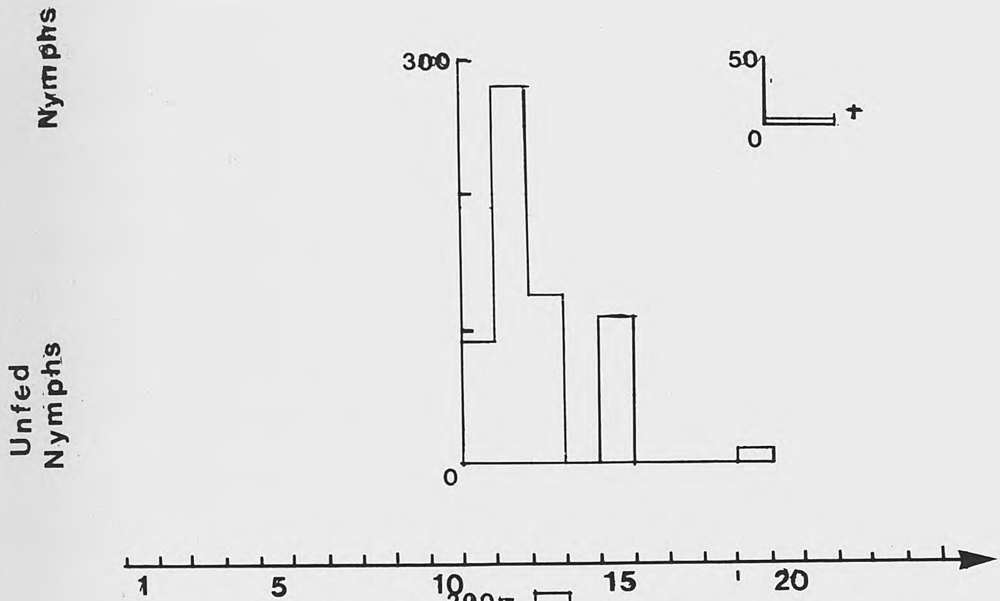


FIGURE 6

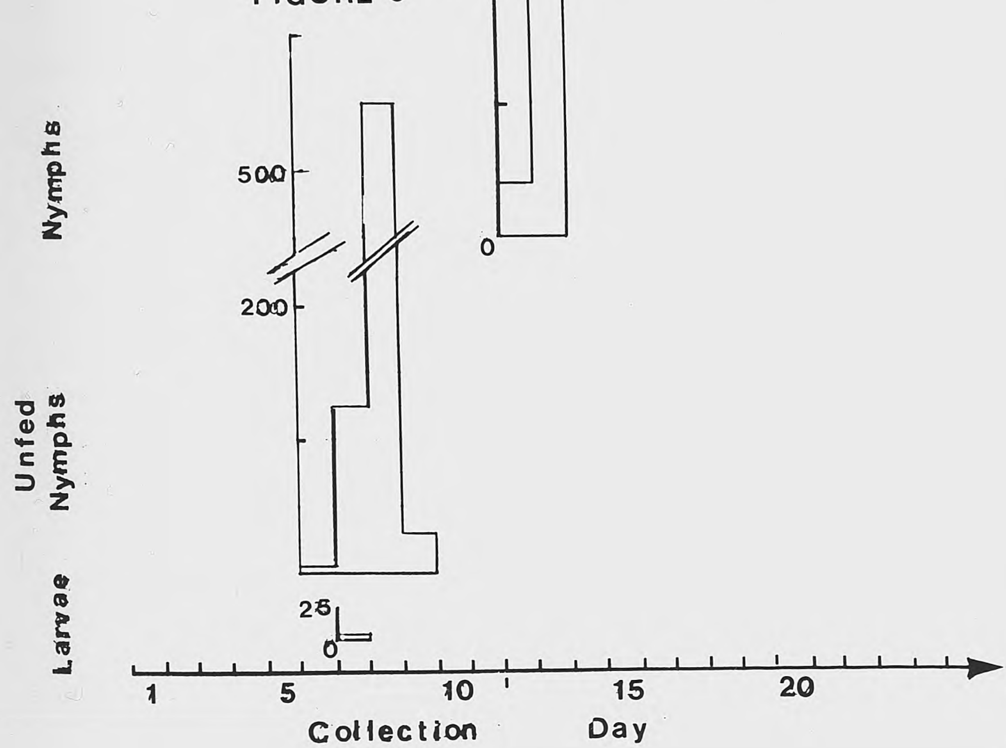


TABLE 7

Feeding performance of Hyalomma anatolicum subspecies on four rabbits. (Experiment 2).

	H. a. excavatum			H. a. anatolicum
	Razi	Razi	Berlin	Ludhiana
No. larvae on:	3000	3000	3000	3000
	755	835	579	-
Mobile larvae				
Number:	152	66	0	4
Collection range (days):	4-11	7-11	-	7
Collection mean (days):	7.1	8.3	-	7.0
Immobile larvae				
Number:	61	44		
Collection range (days):	6-11	7-12		
Collection mean (days):	9.8	10.7		
Removed larvae			Removed and Immobile larvae	
Number:	179	231	188	137
Collection range (days):	10-14	10-14	11-13	4-9
Unfed nymphs				
Number:	892	603	616	708
Collection range (days):	8-14	8-14	11-19	6-9
Collection mean (days):	11.0	12.0	12.6	7.8
Engorged nymphs (Body)				
Number:	89*	11**	10***	248
Collection range (days):	15-18+	14-18+	20-22+	12-13
Collection mean (days):	-	-	-	12.8

* not including 188 removed on day 18.

** not including 80 removed on day 18.

*** not including 25 removed on day 22.

TABLE 8

Larval development at 34°C, 85 percent R.H. (Days) (Experiment 2)

H. a. excavatum (Razi strain)	Collection day							
	4	5	6	7	8	9	10	11
Feed 1								
No. collected (mobile/immobile)	5/0	20/0	16/2	19/1	20/0	18/8	5/22	2/17
Moult range (days)	4-6	4-7	4-6	3-6	4-6	1-9	1-7	1-17
mean	4.7	4.7	5.2	5.2	5.2	4.3	3.1	4.5
No. moult (live/dead)	3/2	20/0	15/1	19/0	18/1	23/0	19/0	10/1
% moult	100	100	88.9	95.0	95.0	88.5	65.5	57.9
Feed 2								
No. collected (mobile/immobile)				13/1	20/0	26/3	3/19	2/7
Moult range (days)				2-7	5-6	3-8	1-19	1-8
mean				4.6	5.3	5.0	6.8	4.8
No. moult (live/dead)				9/1	19/0	28/0	13/8	8/1
% moult				71.4	95.0	96.6	95.5	100

TABLE 9

Results of incubating larvae removed from host. (Experiment 2).

Collection day	No. larvae incubated	No. unfed nymphs moulted (live/dead)	Total	Percentage moult
H. a. excavatum (Razi strain) - incubated at 34°C, 85 percent R.H.				
10	54	29/1	30	55.5
	65	46/2	48	73.8
11	63	43/8	51	81.0
	47	26/4	30	63.8
12	33	16/3	19	57.6
	46	21/2	23	50.0
13	25	6/4	10	40.0
	51	9/8	17	33.3
14	4		0	0
	22	4/2	6	27.3
	410		234	57.1
H. a. excavatum (Berlin strain) - incubated at 25°C, 80 percent R.H.				
11	53	42/0	42	79.2
13	122	50/4	54	44.3
13	13	5/1	6	46.2
	188		102	54.3
H. a. anatolicum (Ludhiana strain) - incubated at 25°C, 80 percent R.H.				
4	9	4/0	4	44.4
6	56	38/2	40	71.4
8	70	12/7	19	27.1
9	2	1/0	1	50.0
	137		64	46.7

TABLE 10

Survival of *Hyalomma anatolicum* subspecies unfed nymphs.

(Experiment 2).

Environment	Strain	Number Stored	Number Surviving	Percentage Survival
Mean 72 days at 25°C, 80% R.H. + 37 days at 15°C, 85% R.H.	Razi	429	7	1.6
Mean 66 days at 25°C, 80% R.H. + 37 days at 15°C, 85% R.H.	Razi	278	36	12.9
After 12 days at 34°C, 4 days at 25°C + 37 days at 15°C, 85% R.H.	Razi	200	139	69.5
Mean 21 days at 25°C, 80% R.H. + 37 days at 15°C, 85% R.H.	Razi	1577	1258	79.8
Mean 13 days at 15°C, 85% R.H.	Berlin	613	560	91.4
Mean 18 days at 15°C, 85% R.H.	Ludhiana	708	455	64.3

TABLE 11

Rabbit weight loss when feeding ticks. (Experiment 2).

Strain of H. anatolicum subspp.

Feed	Razi 1	Razi 2	Berlin 1	Ludhiana 1
Initial weight (Kgs):	2.735	3.280	3.910	3.850
Total loss (grms):	230	410	350	290
Duration of feed (days):	18	18	21	13
Daily loss (grms):	12.8	22.8	16.7	22.3
$\frac{\text{Daily loss}}{\text{Initial wt.}} \times 100$:	0.47	0.69	0.43	0.58

TABLE 12

Results of Routine Haematology. (Experiment 2).

Day	Total R.B.C. X 10 ^{12}/L	Haematocrit %	Haemoglobin g%	M.C.V.	Total W.B.C. X 10 ^{9}/L	Differential % Neut.	% Lymph.
H. a. excavatum (Razi strain), Feed 1.							
0	5.16	34.1	10.2	7.0	12.9	28	67
4	5.4	38.6	12.4	7.0	9.4	32	60
7	5.93	39.0	12.9	6.4	11.8	24	72
11	6.25	46.3	14.9	7.2	9.7	23	68
15	6.21	43.8	14.0	6.0	6.7	18	76
18	6.15	39.8	14.0	6.3	10.6	33	68
H. a. excavatum (Razi strain), Feed 2.							
0	5.59	39.4	12.7	6.9	8.3	30	65
4	5.41	39.3	11.9	7.1	4.7	29	64
7	5.76	41.3	13.3	7.0	11.5	38	57
11	5.88	40.3	14.0	6.7	9.2	36	60
15	7.68	45.8	15.4	5.7	11.6	36	57
18	7.38	43.4	14.7	5.7	14.0	29	65

Experiment with calves.

I am grateful to Mr. C.G.D. Brown and Dr. A.R. Walker for the opportunity to observe the unfed nymphs produced in the earlier experiments and larvae feeding on two calves. The calves were infected with *T. annulata* (Hissar strain) and the ticks were for use in further studies of the parasite. The collection of engorged instars was shared while I was responsible for the subsequent weighing, counting and observation.

My aims

Observe the feeding of larvae and nymphs of the three strains of *H. anatolicum* subspecies on calves.

Design

The strains of tick were applied to the two calves thus:-

Berlin strain larvae and nymphs on the right ear.

Ludhiana strain larvae and nymphs on the left ear.

Razi strain larvae and nymphs on the tail.

Both larvae and nymphs had been stored at 15°C and 85 percent relative humidity. The nymphs had been counted while the numbers of larvae were estimated as before. The larvae were applied two days after the nymphs.

Routine observations were made daily and the engorged instars were stored at 25°C, 80 percent relative humidity. The bags were removed and the feeding sites examined when it was anticipated the feed had ended.

Results

The engorgement times of the larvae and nymphs of the three strains on two calves are shown in Table 13. *Hyalomma a. anatolicum* (Ludhiana strain) larvae and nymphs complete engorgement significantly earlier than those of *H. a. excavatum* (Berlin and Razi strains) when the latter results are combined. ($P < 0.01$ for larvae and nymphs).

Table 14 shows the number and percentage of successful engorgements on calves. One day's collection of two strains was mixed and I assumed 70 percent of the larvae, and half of the nymphs, were of the Ludhiana strain, and the rest were the Razi strain. Few *H. a. excavatum* (Berlin and Razi strains) larvae engorged but more than 30 percent of the nymphs of all three strains and larvae of *H. a. anatolicum* (Ludhiana strain) engorged and were collected.

The proportion of successful moults is shown in Table 15. Of all larvae 95.9 percent moulted and 40.4 percent of all nymphs moulted. The minimum moulting periods and the weight of the engorged ticks was used as a check on the normality of the feed and are compared with the results of the other experiments in the following section (Minimum development times and the collection weight of ticks).

TABLE 13

Feeding performance of *Hyalomma anatolicum* subspp. on two calves.

	H. a. excavatum				*H. a. anatolicum*	
Strain:	Razi		Berlin		Ludhiana	
Feed:	1	2	1	2	1	2
Engorged larvae						
Collection range (days):	4-10	5-11	6-9	7-11	3-5	5-8
mean (days):	6.0	9.2	7.8	8.0	4.8	6.2
Engorged nymphs						
Collection range (days):	6-12	6-13	8-11	9-13	5-7	6-10
mean (days):	9.7	8.5	10.1	10.6	5.2	7.9

TABLE 14

Number and percentage of successful engorgements of Hyalomma anatolicum subspecies on two calves.

Strain	Feed	No. put on	Engorgements No.	Engorgements Percentage	
Larvae					
Berlin	1	500	46	9.2	6.9
	2	500	23*	4.6	
Razi	1	500	40**	8.0	9.3
	2	500	53	10.6	
Ludhiana	1	500	101	20.2	44.3
	2	500	342**	68.4	
Nymphs					
Berlin	1	300	183	61.0	41.7
	2	300	67	22.3	
Razi	1	500	161**	32.2	31.8
	2	500	157	31.4	
Ludhiana	1	250	114	45.6	31.2
	2	250	42**	16.8	

* Includes one two-host nymph.

** One collection was mixed. Assumed 22 larvae were Razi and 51 were Ludhiana. Thirteen nymphs each.

TABLE 15

Percentage of larvae and nymphs of *Hyalomma anatolicum* subspecies from calves, moulting.

Strain	Feed	Percentage moult
Larvae		
Berlin	1	91.3
	2	86.4
Razi	1	88.9
	2	86.8
Ludhiana	1	97.0
	2	99.0
Nymphs		
Berlin	1	24.0
	2	56.7
Razi	1	29.1
	2	49.7
Ludhiana	1	46.5
	2	89.7

Minimum development times and the collection weights of the ticks. (All Experiments).

Both factors were a check on the normality of each feed. The minimum development times of larvae and nymphs from all experiments are presented in Table 16. The mean weights of the daily collections of larvae, unfed nymphs and engorged nymphs from rabbits are shown in Figures 7, 8 and 9 respectively. Collections of three or less larvae were not included and the weight of larvae of H. a. excavatum (Berlin strain) and H. a. anatolicum (Ludhiana strain) were not recorded.

Trend lines were calculated for each series of mean weights of the ticks from rabbits and are described opposite the respective figures. All the lines have negative gradients except those of the unfed nymphs of H. a. excavatum (Berlin strain) and H. a. anatolicum (Ludhiana strain).

The mean collection weights of the larvae and nymphs from the two calves are shown in Figures 10 and 11. Using the peak mean weight of engorged nymphs of H. a. excavatum (Razi strain) the five feeds on rabbits were compared with the two on calves. Nymphs fed on calves were significantly lighter than those from rabbits.

$t = 47.42$ 5 degrees of freedom

($P < 0.001$).

TABLE 16

Minimum development times of *Hyalomma anatolicum* subspecies larvae and nymphs from rabbits and calves at 25°C, 80 percent relative humidity.

Rabbits

Strain	Razi			Berlin	Ludhiana
Experiment	1	2	2	2	2
Feed	1	1	2	1	1
Minimum development period (days)					
mobile larva-nymph	6	7	7	7	5
nymph-adult	N.R.	22	23	25	21

N.R. - not recorded.

Calves

Strain	Razi		Berlin		Ludhiana	
Experiment Feed	1	2	1	2	1	2
Minimum development period (days)						
mobile larva-nymph	11	11	11	10	12	11
nymph-adult	21	20	19	21	17	20

Figure 7

Mean daily collection weights of engorged larvae from rabbits.

●——● Experiment 1, *Hyalomma* *a.* *excavatum* (Razi strain).

Trend line:- Y = 5.87 - 0.026 X

▲——▲ Experiment 2, *Hyalomma* *a.* *excavatum* (Razi strain), Feed 1.

Trend line:- Y = 12.07 - 0.625 X

■——■ Experiment 2, *Hyalomma* *a.* *excavatum* (Razi strain), Feed 2.

Trend line:- Y = 10.1 - 0.38 X

FIGURE 7

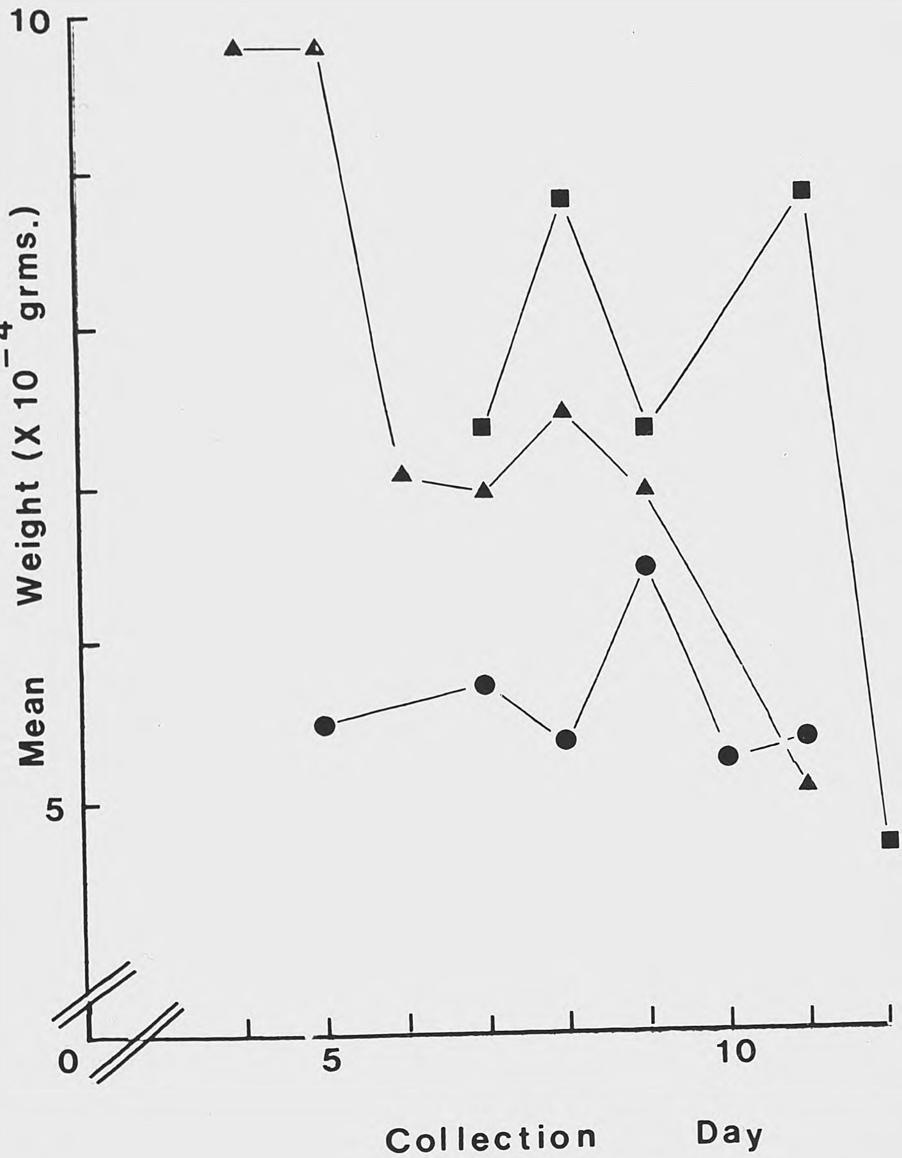


Figure 8

Mean daily collection weights of unfed nymphs from rabbits.

Experiment 1, *H. a. excavatum* (Razi strain).

Trend line:- $Y = 2.48 - 0.003\ X$

Feed 1. Experiment 2, *H. a. excavatum* (Razi strain),

Trend line:- $Y = 3.99 - 0.11\ X$

Feed 2. Experiment 2, *H. a. excavatum* (Razi strain),

Trend line:- $Y = 3.19 - 0.034\ X$

Feed 1. Experiment 2, *H. a. excavatum* (Berlin strain),

Trend line:- $Y = 2.9 + 0.01\ X$

Feed 1. Experiment 2, *H. a. anatolicum* (Ludhiana strain),

Trend line:- $Y = 1.20 + 0.15\ X$

FIGURE 8

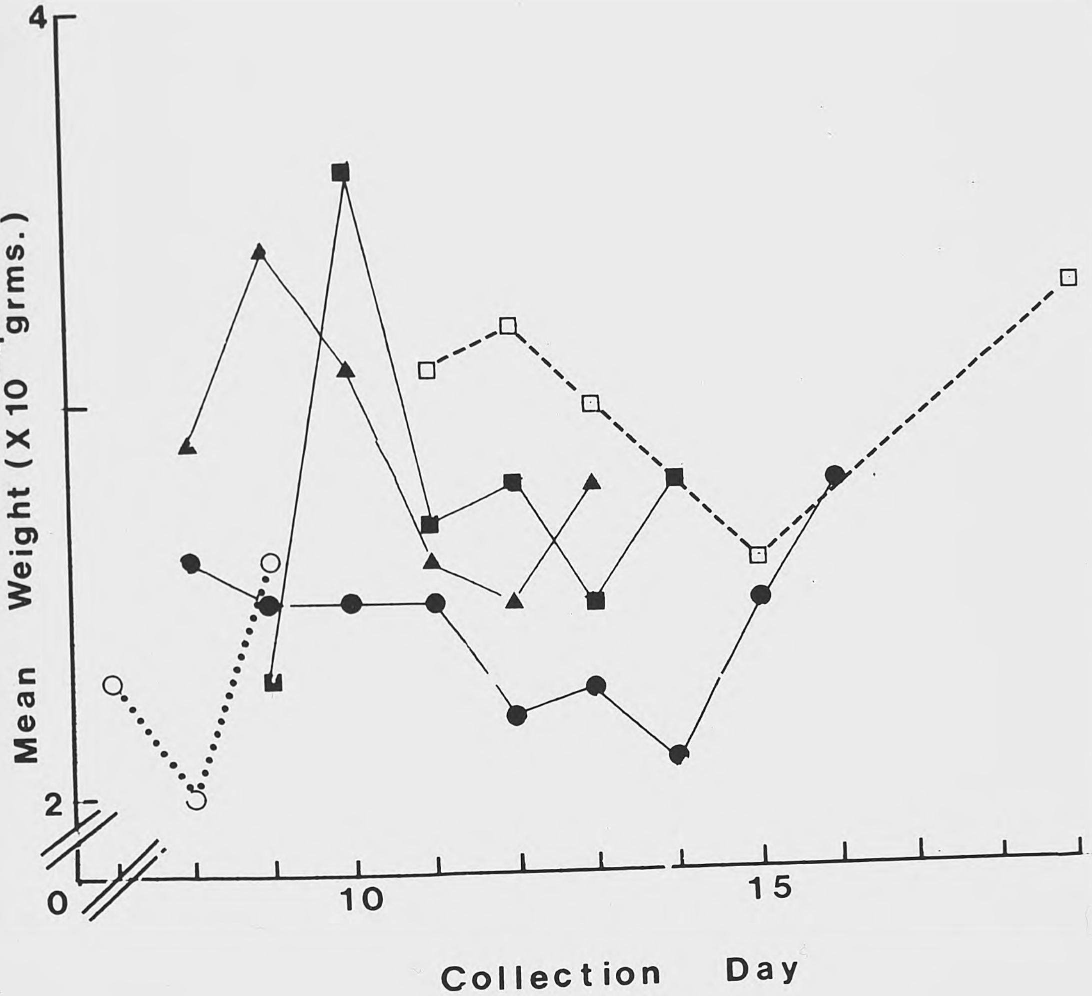


Figure 9

Mean daily collection weights of engorged nymphs from rabbits.

●———●	Experiment 1, *H. a. excavatum* (Razi strain). Trend line:- Y = 5.17 - 0.15 X
△••••••••△ Feed 1 (Ear).	Experiment 2, *H. a. excavatum* (Razi strain), Trend line:- Y = 5.85 - 0.21 X
▲••••••••▲ Feed 1 (Body).	Experiment 2, *H. a. excavatum* (Razi strain), Trend line:- Y = 5.66 - 0.19 X
□------□ Feed 2 (Ear).	Experiment 2, *H. a. excavatum* (Razi strain), Trend line:- Y = 4.33 - 0.11 X
■------■ Feed 2 (Body).	Experiment 2, *H. a. excavatum* (Razi strain), Trend line:- Y = 6.25 - 0.23 X
□ Feed 1.	Experiment 2, *H. a. excavatum* (Berlin strain),
○ Feed 1.	Experiment 2, *H. a. anatolicum* (Ludhiana strain),

FIGURE 9

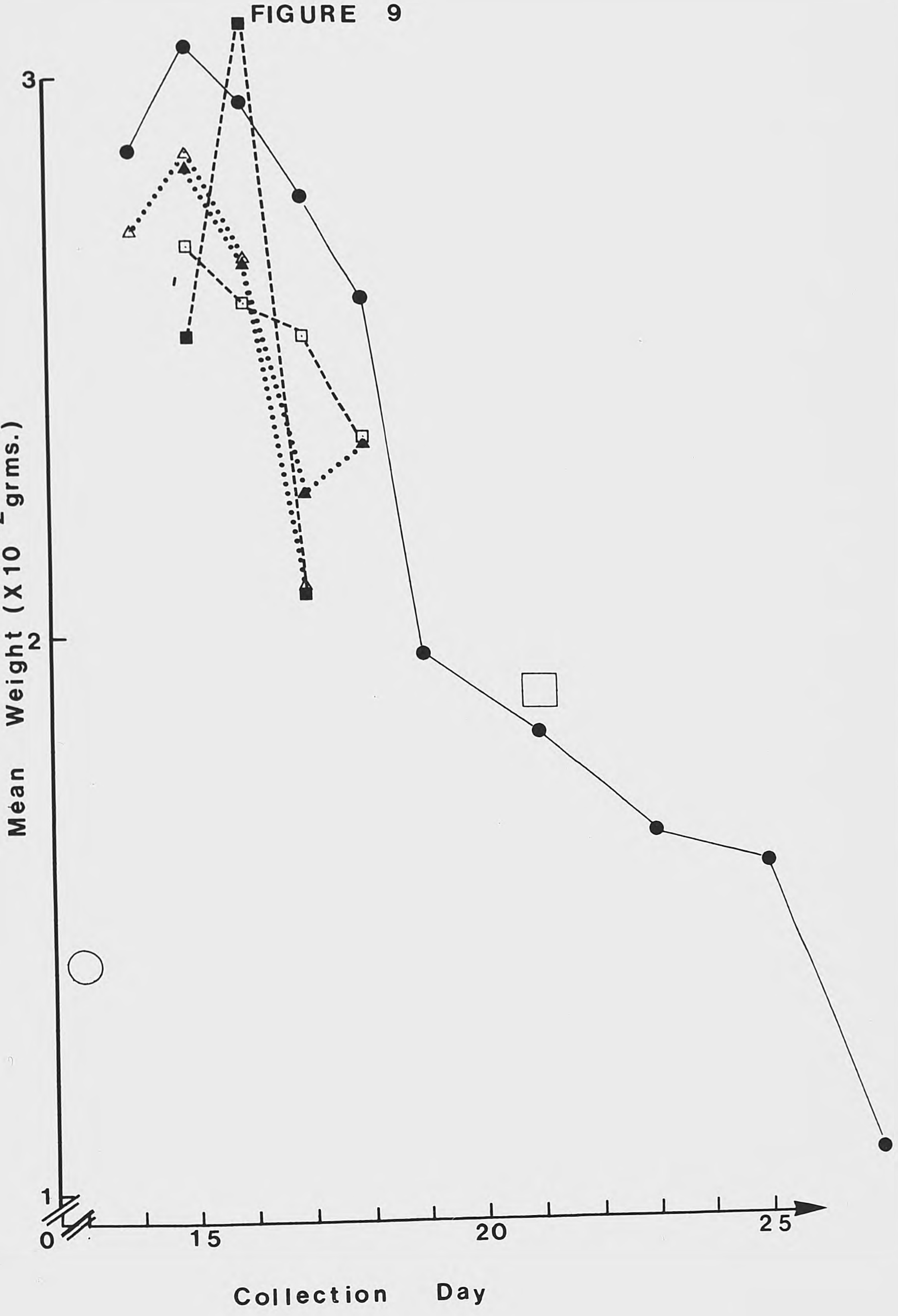
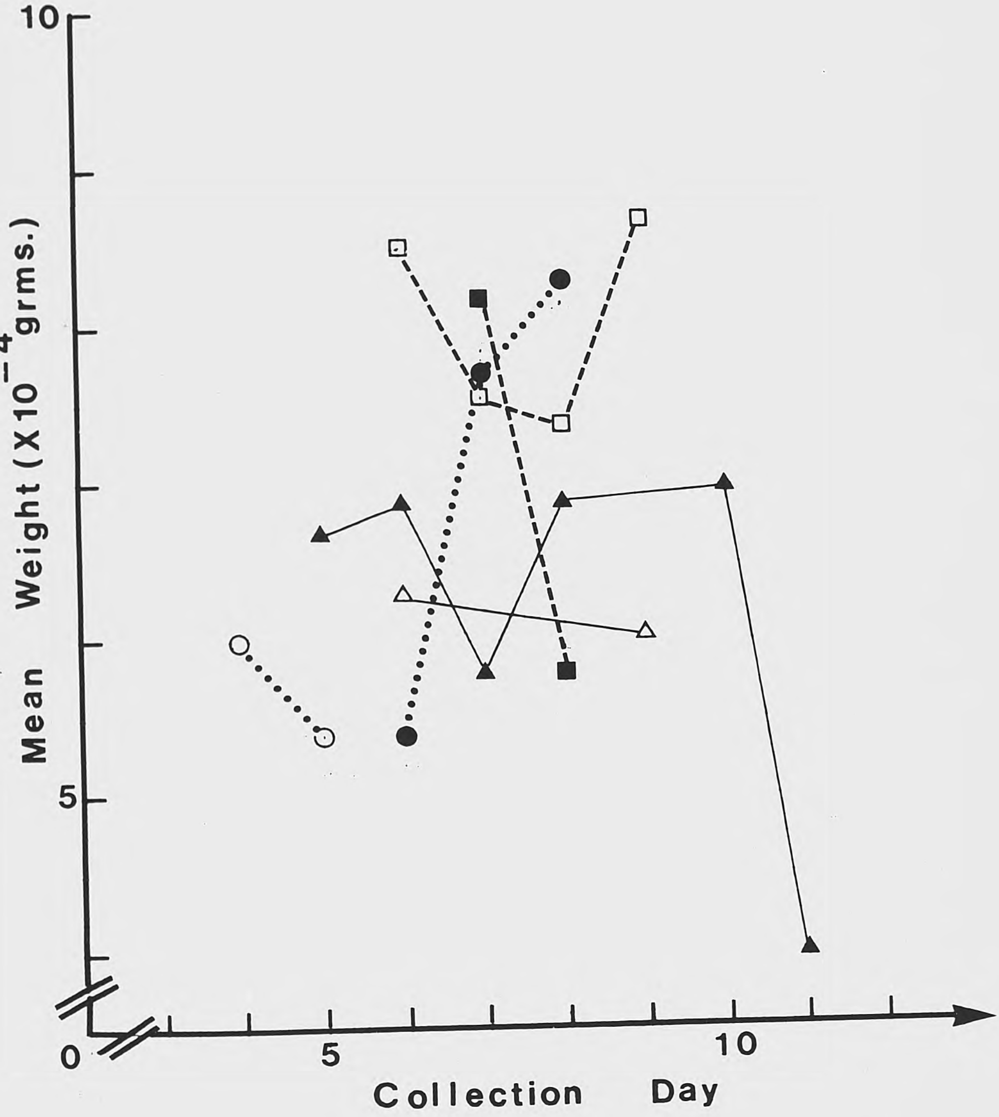


Figure 10

Mean daily collection weights of engorged larvae from calves.

△——△	*H. a. excavatum* (Razi strain), Feed 1.
▲——▲	*H. a. excavatum* (Razi strain), Feed 2.
□------□	*H. a. excavatum* (Berlin strain), Feed 1.
■------■	*H. a. excavatum* (Berlin strain), Feed 2.
○•••••••○	*H. a. anatolicum* (Ludhiana strain), Feed 1.
●•••••••●	*H. a. anatolicum* (Ludhiana strain), Feed 2.

FIGURE 10

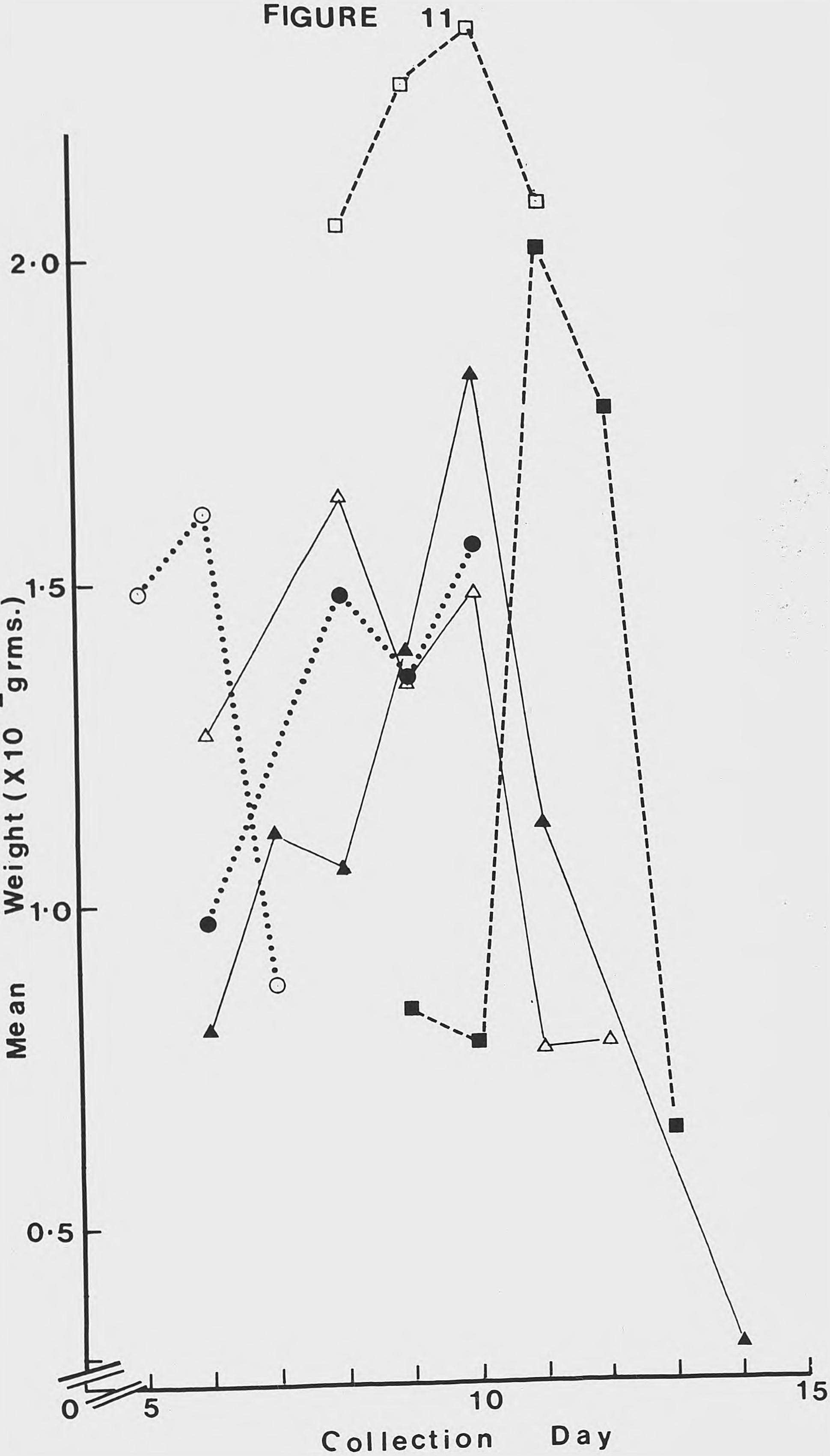


<u>Figure 11</u>

Mean daily collection weights of engorged nymphs from calves.

△———△	*H. a. excavatum* (Razi strain), Feed 1.
▲———▲	*H. a. excavatum* (Razi strain), Feed 2.
□------□	*H. a. excavatum* (Berlin strain), Feed 1.
■------■	*H. a. excavatum* (Berlin strain), Feed 2.
○·······○	*H. a. anatolicum* (Ludhiana strain), Feed 1.
●·······●	*H. a. anatolicum* (Ludhiana strain), Feed 2.

FIGURE 11



DISCUSSION

Feeding *Hyalomma anatolicum* subspecies on rabbits.

Normality of the feeds

The minimum development times of the larvae and nymphs and the mean weight of the daily tick collections were used to check that the ticks had developed normally and engorged successfully. The minimum development times found in these experiments were all about seven days for the larval-nymphal moult and 23 days for the nymphal-adult moult. They are similar to those found by other writers when consideration is given to the temperature related nature of moulting times (Snow, 1969). (See Tables 1 and 2 in Literature Review).

The mean weights of the daily collections of larvae from all three *H. a. excavatum* (Razi strain) feeds decreased as each feed progressed but were greater than 4.9 X 10^{-4} grams, found as the mean for the same strain by Al-Janabi (1976). The number collected each day showed a normal distribution or were biased towards the earlier collections. Later collections contained a rising proportion of immobile larvae. Thus the fall in larval weight as the feeds progressed may have been because the first collections contained many heavier but still 'normal' larvae and the later collections contained larvae as partially developed nymphs. These later collections showed a much greater range of moulting times at 34°C than the earlier collections, and is evidence of larval-nymphal development on the host.

The mean weights of the daily collections of unfed nymphs from all five feeds showed a consistent fall from the first or second collection to the second last, and then a rise at the last collection. Hyalomma a. excavatum (Berlin strain) nymphs' weights were similar to those of H. a. excavatum (Razi strain) in Experiment 2 but both the H. a. excavatum (Razi strain) nymphs in Experiment 1 and H. a. anatolicum (Ludhiana strain) nymphs were lighter. Both the decline in weight and the lighter nymphs in Experiment 1 could have resulted from a rising proportion of nymphs, missed in earlier collections and which having failed to re-attach, lost weight before being collected later. The H. a. excavatum (Razi strain) nymphs in Experiment 1 were only collected if they had left their larval coats and so had stayed on the host longer than those in Experiment 2. All stages of H. a. anatolicum are smaller than H. a. excavatum according to Hoogstraal and Kaiser (1959) as was found here.

The rise in weight in the last collections probably resulted from a few recently attached nymphs collected then.

The weights of engorged nymphs from all three H. a. excavatum (Razi strain) feeds on the body and two on ears were very similar and showed a consistent decline as long as the feeds were allowed to continue. In the first experiment this was until adults emerged on the host. The early results were about the mean of 2.32×10^{-2} grams found by Al-Janabi (1976) for two-host nymphs of this strain dropped between days 13 and 18. The initial part of the decline may have been because the earliest and later collections contained individuals at the extreme range of normality and perhaps an increase in host resistance.

The continual decline in Experiment 1 was caused by the increasing number of partially developed, one-host ticks collected. All of this strain's nymphs collected moulted successfully. The nymphal feed of H. a. excavatum (Berlin strain) was atypical, as the nymphs attached reluctantly and the few which engorged were a third lighter than the first collections of H. a. excavatum (Razi strain) nymphs. Also, one of the ten collected failed to moult. However, the mean weight of H. a. anatolicum (Ludhiana strain) was in the middle of the range of weights found by Snow (1970) for this subspecies with guinea-pig hosts. All moulted. Thus this feed was typical and again showed H. a. anatolicum to be lighter than H. a. excavatum.

Feeding pattern

Comparisons of the feeding patterns obtained here with those of other workers are complicated by the removal of ticks at different stages. The first experiment was the most prolonged and only wandering, unfed nymphs were removed, excepting four adults, three of which had started feeding, and some immobile nymphs removed when the feed was terminated. These findings, plus the observation that immobile nymphs were contained in later collections (presumably rubbed off the host), are evidence of a one-host cycle for H. a. excavatum (Razi strain). This was additional to two- and three-host cycles also found by Hoogstraal and Kaiser (1959) and Berdyev (1974) for this subspecies, and Al-Janabi (1976) for this strain. The two later feeds of this strain were terminated before this incidental finding could be confirmed. However in all three feeds of this strain a much smaller proportion of ticks showed a three-host cycle than the 50 percent found by both Hoogstraal and Kaiser (1959) and Al-Janabi (1976). The duration of the larval feeds was consistently between four and eleven days and agreed with the findings of these two authors. The collections of H. a. excavatum (Razi strain) unfed nymphs in Experiment 2 were completed in fewer days than in Experiment 1 because two-host larvae were removed, as were nymphs still in their larval coats. The nymphal feeds of this strain were not comparable with other work.

The H. a. excavatum (Berlin strain) feed may have been atypical, particularly the nymphal feed but here it was a two-host tick, unlike the Razi strain. Unfed nymph collections were made over a longer period than those of the other strains for reasons discussed earlier but they also started later. Both host cycle and feeding time may be evidence of strain variation.

The H. a. anatolicum feed progressed much faster than those of either strain of H. a. excavatum. The unfed nymph collection was completed significantly earlier and the nymphal feed completed before any nymphs of H. a. excavatum had dropped. It was mainly a two-host tick on rabbits, as found by Sardey and Ghafoor (1971). Others claim it shows both two- and three-host cycles on rabbits

(Serdyukova, 1946 a; Avastthi and Hiregauder, 1969; Hooshmand-Rad and Hawa, 1973 b). This difference may have resulted from a strain variation as suggested for H. a. excavatum. The nymphs of this strain dropped after 12 to 13 days, much earlier than the 16 to 24 days found by Sardey and Ghafoor (1971). Probably this strain was a particularly rapid feeder.

Larval collection and moulting

There was no difference in the proportion of all larvae which dropped, moulting at 34°C or 25°C. Still mobile larvae when incubated at 34°C took a minimum of four days to moult while similar larvae took seven days at 25°C. The post moult mortality was higher at 34°C but earlier collection for storage at 15°C may have alleviated this. Similar findings were made when two-host larvae were removed for incubation at 34°C and 25°C. However significantly less larvae removed from the host moulted. This resulted from damage during removal and the increasing number of non-viable larvae collected as the feed progressed and most viable larvae completed their development on the host. Losses from injury could be minimised by only removing those with hard outer coats which entails waiting until the in situ moult to nymphs has begun. If one decides to collect most unfed nymphs after they have moulted on the rabbit, the number of such collections required can be reduced, safely, by stripping the remaining larvae from the rabbit once the peak nymph collection has passed. However, collection of unfed nymphs from the rabbit is time consuming and it is possible, by sacrificing only a few, to advance the day when all remaining ticks on the rabbit are removed, to one or two days following the first appearance of newly moulted nymphs. The incubation temperature for the larvae can be chosen to suit the time unfed nymphs are required. As the nymphs appear they should be stored at 15°C to minimise post-moulting mortality.

Collection of unfed nymphs

Simulation of two-host larval development by incubating three-host larvae at 34°C greatly assisted the initial, early recognition of newly moulted, unfed nymphs in their old larval coats. Collection of those nymphs from their larval coats in addition to those wandering over the host increased the collection from 10.7 percent of H. a. excavatum (Razi strain) larvae applied in Experiment 1, to 29.7 and 20.1 percent of larvae of the same strain and 20.5 and 23.6 of larvae of H. a. excavatum (Berlin strain) and H. a. anatolicum (Ludhiana strain) respectively in Experiment 2. This increase was obtained while concurrently removing two-host larvae and resulted in a dramatic reduction in the number of nymphal engorgements. However, as noted earlier this procedure is time consuming, taking about an hour per rabbit per collection day. Also these collections must be done daily, as when newly-collected nymphs were applied to rabbits' ears, 92 percent attached within 24 hours.

Over all feeds approximately a third of larvae applied were later collected as engorged larvae or as nymphs. The loss was much greater than that found by Hoogstraal and Kaiser (1959) who recovered all ticks applied and Al-Janabi (1976) who recovered about half. Both applied less than 400 larvae and used the ears of rabbits. It was to the ears that larvae of H. a. anatolicum (Ludhiana strain) escaped and as noted earlier 92 percent of newly collected nymphs engorged when applied to the ears. Al-Janabi (1976) only recovered half of 56 nymphs he applied to a rabbit's ears. Therefore while it is probable that immature instars of H. anatolicum subspecies attach better when applied in smaller numbers to the ears, there is some evidence that recently moulted, two-host nymphs attach better than three-host nymphs which were probably held more than a month at 25°C, 80 percent relative humidity. But because of the number of unfed nymphs required the body must be used.

Storage of unfed nymphs

Although not the result of systematic observation, the findings about the longevity of unfed nymphs can be related to practical colony management. The rate of mortality was greater than expected from the work of Snow (1969). He found half should be dead by day 125 if stored at 18°C, day 40 if stored at 25°C and day 20 if stored at 38°C; all at 85 percent relative humidity. His results were for three-host unfed nymphs of H. a. anatolicum from guinea-pig hosts. Most findings here relate to H. a. excavatum but probably more important is they were two-host ticks and so exposed to a temperature of about 34°C and relative humidity of about 30 percent while hardening. Thus newly-moulted nymphs should be collected as soon as possible for storage at 15°C and 85 percent relative humidity.

Rabbit health

That the use of the same host for successive infestations of ticks results in a decline in the number of engorgements and degree of repletion, and a marked response in the host, has been shown for Rh. appendiculatus using rabbit hosts by Branagan (1974) and for Dermacentor andersonii using guinuea-pig hosts by Allen and Wikel (1978). A tendancy for adult H. a. excavatum (Razi strain) to produce local oedema and malaise in rabbits was described by Al-Janabi (1976). As only immatures were fed and each rabbit infested only once, only skin thickening and irritation was noticed in these experiments. A steady loss of half a percent of the body weight each day was probably due to the rabbits' discomfort interrupting their feeding. These losses were not considered unusual by Graham-Marr (personal communication) and he suggested supplementing the ration with two teaspoonfuls of Minadex (Glaxo) per rabbit per day.

Although haematology was limited to only two rabbits, there was no evidence of a leucocyte response, the counts being within the ranges quoted by Schalm (1965). The haemoconcentration may be caused by irritation from the ticks interrupting the rabbits drinking or it may be a more fundemental response to the secretions of the ticks.

Thus these rabbits can carry an infestation of about 3,000 H. anatolicum subspecies larvae and although these ticks are three-host on gerbils (Meriones tnstrami) (Hadani et al, 1969; as H. excavatum), rabbits are easier to handle and using the methods described here should provide many more unfed nymphs than gerbils. Guinea-pigs may prove to be the ideal hosts. Snow (1969) found that by applying the larval product of 500 H. a. anatolicum eggs to guinea-pigs, 80 percent of the resultant stages dropped as engorged three-host larvae, saving time collecting two-host larvae and unfed nymphs from rabbits. Other species of Hyalomma ticks have two-host cycles on rabbits and it may be possible to adapt these methods described here to produce unfed nymphs of them.

Feeding *Hyalomma anatolicum* subspecies on calves.

Normality of the feeds

The larval minimum development times were considerably longer than those from rabbits and the modal value of 11 days was at the top of the range found by Serdyukova (1946 b) and Chaudhuri *et al* (1969), and later than that found by Daubney and Said (1951). However they used a higher environmental temperature for moulting ticks. The same situation was found with the nymphal development times and the times for larval and nymphal development were consistant for all feeds, the resulting instars had probably developed typically.

The mean weights of the daily collections of *H. a. excavatum* larvae were comparable with those from rabbits, but no larval feed showed any general trend in mean daily engorged weights. The mean weights of *H. a. anatolicum* larvae were generally heavy when compared with the range of 2.0 to 6.0 X 10^{-4} grams found by Snow (1969) using guinea-pig hosts. A 90 percent moult is final proof that the larvae engorged well.

A trend was apparent in the mean weights of the daily collections of nymphs. There was a sharp rise over the first few days to a peak and then a fall. Of the four *H. a. excavatum* feeds only one, that of the Berlin strain, gave daily mean weights which were all equal to those found on the rabbits. The other Berlin strain feed gave similar values on only two days and the peak weights of *H. a. excavatum* (Razi strain) nymphs were significantly lighter than those from rabbits. Most *H. a. anatolicum* (Ludhiana strain) nymph collections had mean weights which equalled the mean weight of nymphs from rabbits. Those which did not, had few members but all means came within the range of 0.6 to 1.8 X 10^{-2} grams found by Snow (1969) using guinea-pig hosts.

Thus I considered all the larval engorgements were typical but only the two H. a. anatolicum (Ludhiana strain) and one H. a. excavatum (Berlin strain) nymphal feeds were typical. All those ticks which moulted, did so with normal minimum development times and so they were also considered normal.

Feeding patterns

Hyalomma a. excavatum was principally a three-host tick on cattle. Only a single two-host nymph of H. a. excavatum (Berlin strain) was found along with a few immobile larvae in the last collections which may have arisen from mobile larvae trapped in the bags. Hyalomma a. anatolicum was confirmed to be a three-host tick on cattle as found by others (Serdyukova, 1946 a; Daubney and Said, 1951; Delpy, 1952; Avatthi and Hiregauder, 1969; Chaudhuri et al, 1969; Bhattacharyulu et al, 1975).

The two larval feeds of each strain were comparable in duration, except perhaps those of H. a. excavatum (Razi strain)as, although they were collected on the same days their mean feeding times were at opposite ends of the range. Both larval feeds of H. a. anatolicum proceeded faster than the larval feeds of H. a. excavatum (Berlin and Razi strains)on the same calf. This was also the case for the nymphal feed but only one H. a. excavatum feed was considered normal. These results agreed with the finding on rabbits that *H. a. anatolicum* (Ludhiana strain) feeds rapidly. But when compared with the results of other authors only the larval feeds and one of the nymphal feeds came entirely within the range found for *H. a. anatolicum* by Daubney and Said (1951). Also they were longer than the feeding times found by Serdyukova (1946 b) and Chaudhuri *et al* (1969). These discrepancies are because Serdyukova was working in the field and Chaudhuri *et al* used adult hill bulls repeatedly as hosts while the calves used here had not been previously exposed to tick challenge.

Success of feeding *H. anatolicum* subspecies on calves

The low number of successful engorgements and poor moulting performance of the nymphs were disappointing. The preference of *H. a. excavatum* immatures for small animal hosts was stated by Hoogstraal and Kaiser (1959) and explained how only 10 percent of larvae of Berlin and Razi strains engorged compared with the much greater success with *H. a. anatolicum* larvae. But there was no such difference between subspecies in the number of nymphal engorgements. About a third of the nymphs of each strain applied, engorged. This compares poorly with the 92 percent engorgement of *H. a. excavatum* (Razi strain) two-host nymphs applied to rabbits' ears immediately after collection and over 75 percent engorgements when nymphs of the same strain and from the same batch as was applied to the calves, were applied to a rabbit's ears (Walker, personal communication). It is impossible to conclude the reason for this with only two calves which were suffering, concurrently, acute tropical piroplasmosis.

Theileria annulata infection may have reduced the viability of the ticks and certainly over 80 percent of the adults of all three strains had infected salivary glands but *H. a. anatolicum* (Ludhiana strain) adults had a much higher individual infection rate (Walker, personal communication) and all strains had a poor moulting rate. *Theileria annulata* infection in the calves may also have caused the poor engorgement and moulting rates. Alternatively, the change in host from a rabbit to a calf could have been at fault but *H. a. anatolicum* immatures prefer large mammals as hosts (Hoogstraal and Kaiser, 1959). These possibilities and others, such as incorrect handling, chemical contamination from an unknown source or some microbial infection in the ticks, await further work, ideally with clean calves.

CONCLUSIONS

1. One-, two- and three-host cycles were observed when H. a. excavatum (Razi strain) was applied to a male rabbit. Because later feeds were terminated earlier no other one-host cycles were observed but two-host cycles were shown for H. a. excavatum (Berlin strain) and H. a. anatolicum (Ludhiana strain) and two- and three-host cycles confirmed for H. a. excavatum (Razi strain) on rabbits.

2. On calves, three-host cycles were observed for all three strains of H. anatolicum subspecies. Also recovered was a two-host nymph of H. a. excavatum (Berlin strain).

3. Unfed nymphs of all three strains of H. anatolicum subspecies were obtained by modifying the feeding pattern on rabbits. This was done in two ways. Nymphs which had moulted on the host were collected, both as they wandered over the host and as they lay in the old larval coats. Others were obtained by removing from the host, larvae, which would otherwise shortly moult in situ, for moulting in the laboratory. Approximately half of these larvae moulted.

4. Losses of unfed nymphs are high, particularly if stored for longer than a few weeks at temperatures greater than 15°C, relative humidity 85 percent.

5. Rabbits showed only minor losses of weight when 3,000 larvae were applied to their body. The accepted handling well with a simple method of restraint.

ACKNOWLEDGEMENTS

This dissertation was carried out at the Centre for Tropical Veterinary Medicine, University of Edinburgh, as part of the degree of Master of Science, under a Ministry of Overseas Development natural resources studentship award.

I should like to thank the Director and staff of the C.T.V.M. and in particular my supervisor Dr. A.R. Walker, Mr. C.G.D. Brown for allowing me to follow the feeding performance of the unfed nymphs on calves, Mr. W. MacLeod for technical assistance, Miss S. McKellar for help at all times and my typist.

REFERENCES

AL-JANABI, B.M. (1976). Transmission of *Nocardia farcinica* by two ixodid species. Ph.D. Thesis, University of Edinburgh.

ALLEN, J.R. and WIKEL, S.K. (1978). Acquisition of tick resistance in guinea-pigs - in "Tick-borne diseases and their vectors". Edited by J.K.H. Wilde. Centre for Tropical Veterinary Medicine, University of Edinburgh; 75-76.

AVASTTHI, B.L. and HIREGAUDER, L.S. (1969). Habits of *Hyalomma anatolicum anatolicum* Koch, 1844, a most common tick of cattle in Gujaret state. Gujaret Vet., 3, 13-15.

BAILEY, K.P. (1960). Notes on the rearing of *Rhipicephalus appendiculatus* and their infection with *Theileria parva* for experimental transmission. Bulletin of Epizootic Diseases of Africa, 8, 33-43.

BARNETT, S.F. (1977). Theileriosis - "Parasitic protozoa." Vol. IV. Edited by J.P. Krier, Academic Press, New York and London; 77-133.

BERDYEV, A. (1972). The duration of the life cycle of *Hyalomma asiaticum* Schulze and Schlottke in natural biotopes. (In Russian; English summary). Parazitologiya, Leningrad, 6, 30-34. Translation seen.

BERDYEV, A. (1973). A contribution to the ecology of *Hyalomma anatolicum excavatum* (Parasitiformes, Ixodidae) in Turkmenia. (In Russian; English summary). Zoologicheskiy Zhurnal, 52 (2), 191-196. Abstract in Biological Abstracts, 56 (10), 56223.

BERDYEV, A. (1974). On the cycle of development of Hyalomma anatolicum excavatum Koch (Parasitiformes, Ixodidae) in Turkmenia. (In Russian). Meditsinskaya Parazitologiya, Moskva, 43 (1), 38-43. Translation seen.

BHATTACHARYULU, Y., CHAUDHURI, R.P. and GILL, B.S.(1975). Transstadial transmission of Theileria annulata through common ixodid ticks infesting Indian cattle. Parasitology, 71, 1-7.

BRANAGAN, D. (1969). The maintainance of Theileria parva infections by means of the ixodid tick Rhipicephalus appendiculatus. Tropical Animal Health and Production, 1, 119-130.

BRANAGAN, D. (1974). The feeding performance of the ixodid Rhipicephalus appendiculatus Neum. on rabbits, cattle and other hosts. Bulletin of Entomological Research, 64, 387-400.

BUXTON, P.A. and MELLANBY, K. (1934). The measurement and control of humidity. Bulletin of Entomological Research, 25, 171-175.

CASALS, J., HENDERSON, B.E., HOOGSTRAAL, H., JOHNSON, K.M. and SHELOKOV, A. (1970). A review of Soviet viral hemorrhagic fevers, 1969. Journal of Infectious diseases, 122 (5), 437-453.

CHAUDHURI, R.P., GILL, B.S. and KHAN, M.H. (1975). Studies on transmission of Babesia bigemina. Annales de la Société Belge de Médecine Tropicale, 55 (4), 327-332. Summary in Veterinary Bulletin (1976), 46 (10), 5747.

CHAUDHURI, R.P., SHRIVASTAVA, S.C. and NAITHANI, R.C. (1969). On the biology of the ixodid tick Hyalomma (Hyalomma) anatolicum anatolicum Koch, 1844 (Acarina: Ixodidae). Indian Journal of Animal Science, 39 (3), 257-268.

DAS, H.L. and SUBRAMANIAN, G. (1972 a). The biology of Hyalomma (Hyalomma) marginatum isaaci (Sharif, 1928) Kaiser and Hoogstraal, 1963 (Acarina: Ixodidae). Acarologia, 13 (3), 497-501.

DAS, H.L. and SUBRAMANIAN, G. (1972 b). Biology of Hyalomma (H.) dromedarii Koch, 1844 (Acarina: Ixodidae). Indian Journal of Animal Science, 42, 285-289.

DAUBNEY, R. and MAHLAN, E.R. (1967). Viral encephalomyelitis of equines and domestic ruminants in the Near East. Part 1, Research in Veterinary Science, 8 (3), 373-397.

DAUBNEY, R. and SAID, M.S. (1951). Egyptian fever of cattle. The transmission of Theileria annulata (Dschunkowsky and Luhs 1904) by Hyalomma excavatum, Koch, 1844. Parasitology, 41 (3-4), 249-260.

DELPY, L.P. (1949). Essai critique de synonymie du genre Hyalomma C.L. Koch 1844 depuis Linné, 1758. Annales de Parasitologie Humaine et Comparée, 24 (5-6), 464-494.

DELPY, L.P. (1952). Rôle des Hyalomma dans la transmission de la theileriose bovine. Biology et taxonomie des espèces en cause. Report before the 14th International Veterinary Congress (London, August 8-13th, 1949), 2, 89-94.

FELDMAN-MUHSAM, B. (1948). On larvae and nymphs of some species of Palestinian Hyalomma. Parasitology, 39 (1-2), 138-147.

FELDMAN-MUHSAM, B. (1954). Revision of the genus Hyalomma. I. Description of Koch's types. Bulletin of the Research Council of Israel, 4, 150-170.

FELDMAN-MUHSAM, B. (1962). Revision of the genus Hyalomma. III. H. lusitanicum Koch and H. anatolicum Koch. Parasitology, 52, 211-219.

FELDMAN-MUHSAM, B. and MUHSAM, H.V. (1966). On the duration of larval and nymphal quiescence in male and female ixodid ticks. Bulletin of Entomological Research, 57, 101-106.

FELDMAN-MUHSAM, B. and SATUREN, I.M. (1961). Notes on the ecology of ixodid ticks of domestic stock in Israel. Bulletin of the Research Council of Israel, 10 (1-3), 53-61. Summary in Review of Applied Entomology (1963), 51, 195-196.

HADANI, A., CWILICH, R., RECHAV, Y. and DINAR, Y. (1969). Some methods for the breeding of ticks in the laboratory. Refuah Vet, 26, 87-100 .

HADANI, A., TSUR-TCHERNOMORETZ, I., PIPANO, E. and SENFT, Z. (1963). Studies on the transmission of Theileria annulata by ticks (Ixodidea, Ixodidea) I. Hyalomma excavatum. Report before the Israeli section of the Society of Protozoologists. First annual meeting, 4 April 1963, Jerusalem, Israel. Journal of Protozoology, 10 supp., 35.

HOFFMANN, G., HÖRCHNER, F., SCHEIN, E. and GERBER, H-C. (1971). Saisonales Auftreten von Zecken und Piroplasmen bei Hausturen in den asiatischen Provinzen der Türkei. (German; English summary). Berliner und Münchener Tierarztleche Wochenschrift, 84, 152-156.

HOOGSTRAAL, H. (1956). African Ixodoidea, Vol. 1, Ticks of the Sudan. U.S. Naval Medical Research Unit No. 3, Cairo, Egypt. Research Report Naval Medicine 005.050 - 29.07.

HOOGSTRAAL, H. (1971). Biological patterns in Afghanistan tick fauna. Abstracts. Third International Congress of Acarology, Prague, August 31st to September 6th, 1971; 87.

HOOGSTRAAL, H. (1978). Biology of ticks - in "Tick-borne diseases and their vectors". Edited by J.K.H. Wilde. Centre for Tropical Veterinary Medicine, University of Edinburgh; 3-14.

HOOGSTRAAL, H. and KAISER, M.N. (1959). Observations on Egyptian Hyalomma ticks (Ixiodoidea, Ixodidae). Annales of the Entomological Society of America, 52, 243-261.

HOOSHMAND-RAD, P. (1977). Theileriosis in ruminants of Iran - in "Theileriosis: Report of a Workshop held in Nairobi, Kenya, 7-9 December 1976". Edited by J.B. Henson and M. Campbell. Ottowa, International Development Research Centre, lDRC-086e; 12-14.

HOOSHMAND-RAD, P. and HAWA, N.J. (1973 a). Malignant Theileriosis of sheep and goats. Tropical Animal Health and Production, 5, 97-102.

HOOSHMAND-RAD, P. and HAWA, N.J. (1973 b). Transmission of Theileria hirci in sheep by Hyalomma anatolicum anatolicum. Tropical Animal Health and Production, 5, 103-109.

JAGANNATH, M.S., ALWAR, V.S. and LALITHA, C.M. (1972). Life history of Hyalomma marginatum issaaci Sharif, 1928 (Acarina: Ixodidae). Indian Journal of Animal Science, 42 (7), 512-516.

KAISER, M.N. and HOOGSTRAAL, H. (1963). The Hyalomma ticks (Ixodoidea, Ixodidae) of Afghanistan. Journal of Parasitology, 49 (1), 130-139.

KAISER, M.N. and HOOGSTRAAL, H. (1964). The Hyalomma ticks (Ixodoidea, Ixodidae) of Pakistan, India and Ceylon, with keys to subgenera and species. Acarologia, 6 (2), 257-286.

KEMP, G.E. (1978). Tick-borne viruses - in "Tick-borne diseases and their vectors". Edited by J.K.H. Wilde. Centre for Tropical Veterinary Medicine, University of Edinburgh; 409-432.

KUIMA, A.U. and PAK, T.P. (1975). Biocoentric relationships between Crimean haemorrhagic fever virus, ixodid ticks and their hosts. Communication 2. (In Russian; English summary). Ekologiya Vinisov, No. 3, 107-112. Summary in Review of Applied Entomology (1977), 65 (3), 786.

LE RICHE, P.G., ALTAN, Y., CAMPBELL, J.B. and EFSTATHIOU, G.C. (1974). Ticks (Ixodoidea) of domestic animals in Cyprus. Bulletin of Entomological Research, 64, 53-63.

LEWIS, E.A. and FOTHERINGHAM, W. (1941). The transmission of *Theileria parva* by ticks. Parasitology, 33, 251-277.

LIEBISCH, A. and ZUKARI, M. (1978). Biological and ecological studies on ticks of the genera *Boophilus*, *Rhipicephalus* and *Hyalomma* in Syria - in "Tick-borne diseases and their vectors". Edited by J.K.H. Wilde. Centre for Tropical Veterinary Medicine, University of Edinburgh; 150-162.

LOTOTSKY, B.V. and POKROVSKY, S.A. (1946). An experiment in the organisation of a minimum complex of measures against haemosporidosis in northern Tadzhikistan. (In Russian). Izvestiya Tadzhikskogo filiada Akademii nauk SSSR, 6, 66-74. Summary in Review of Applied Entomology (1947), 35, 74-75.

MARKOV, A.A., STEPANOVA, N.I. and LAPTEV, V.I. (1966). Application of xenodiagnostic and complement fixation tests for cattle *Theileria* differentiation. Proceedings 1st International Congress of Parasitology (Rome, September 21-26, 1964). Edited by A. Corradetti. Permagon Press, Oxford, 1, 275-276.

MATHEWSON, M.D. and HUGHES, G. (1978). The establishment of cultures of two and three-host ticks in the laboratory and their use in screening of candidate ixodicides - in "Tick-borne diseases and their vectors". Edited by J.K.H. Wilde. Centre for Tropical Veterinary Medicine, University of Edinburgh; 231-240.

MAZLUM, Z. (1968). *Hyalomma asiaticum asiaticum* Schulze and Schulottke, 1929. Its distribution, hosts, seasonal activity, life cycle and role in transmission of bovine theileriosis in Iran. Acarologia, 10 (3), 437-442.

MAZLUM, Z. (1970). Vectors of *Theileria hirci* in south and south east of Iran. Annales de Parasitologie Humaine et Comparée, 45, 523-526.

MAZLUM, Z. (1971). Ticks of domestic animals in Iran: geographical distribution, host relations and seasonal activity. (In Persian; English summary). Revue de la Faculté Vétérinaire Université de Tehran, 27 (1), 1-32.

MEHLHORN, H. and SCHEIN, E. (1976). Elektronenmiknoskopische untersuchugen an entwicklungsstadien von *Theileria parva* (Theiler, 1904) im darm der ubertragerzecke Hyalomma anatolicum excavatum (Koch, 1844). (In German; English summary). Tropenmedizin und Parasitologie, 27, 182-191.

MEHLHORN, H. and SCHEIN, E. (1977). Electron microscope studies of the development of kinetes of *Theileria annulata* Dschunkowsky and Luhs, 1904 (Sporozoa, Piroplasmea). Journal of Protozoology, 24 (2), 249-257.

NEITZ, W.O. (1956). A consolidation of our knowledge of the transmission of tick-borne diseases. Onder-stepoort Journal of Veterinary Research, 27, 115-163.

PEGRAM, R.G. (1976). Ticks (Acanria: Ixodoidea) of the northern regions of the Somali Democratic Republic. Bulletin of Entomological Research, 66, 345-363.

PERVOMAISKY, G.S. (1954). Variation in pasture ticks (Acanria, Ixodidae) and its significance for systematics. (In Russian). Trudy Vsesoyuznogo Entomologicheskogo Obshchestva. Akademiya nauk SSSR, 44, 62-201. In English: NAMRU 3-75.

PIPANO, E. (1977). Basic principale of Theileria annulata control - in "Theileriosis: Report of a Workshop held in Nairobi, Kenya, 7-9 December 1976". Edited by J.B. Henson and M. Campbell. Ottowa, International Development Research Centre, lDRC-086e; 55-65.

POMERANTZEV, B.I. (1946). Les tiques (Ixodidae) de la faune de l'URSS et de pays limitrophes. (In Russian; French title). Opredeliteli po Faune SSR. Izdavaemye Zoologicheskim Institutom Akademii nauk SSR, (26), 28pp.

POMERANTZEV, B.I. (1950). Ixodid ticks (Idodidae). (In Russian). Faune SSR. Paukoobraznye, n.s., (41), 4 (2), 224pp. English translation by A. Elbl (1959), edited by G. Anastos. The American Institute of Biological Sciences; Washington, D.C. 199pp.

RAU, K.U.R. (1963). Observations on the maintainance of a laboratory strain of Hyalomma marginatum isaaci, Armed Forces Medical Journal, 19 (1), 40-48.

RAY, H.N. (1950). Hereditary transmission of Theileria annulata infection in the tick, Hyalomma aegypticum Neum. Transactions of the Royal Society of Tropical Medicine and Hygiene, 44, 93-104.

RIEK, R.F. (1968). Babesiosis - in "Infectious blood diseases of man and animals". Vol. II. Edited by D. Weinman and M. Ristic. Academic Press, New York and London; 220-265.

ROBSON, J. and ROBB, J.M. (1967). Ticks (Ixodoidea)of domestic animals in Iraq . Journal of Medical Entomology, 4 (3), 289-293.

ROBSON, J., ROBB, J.M. and AL-WAHAYYIB, T. (1968). Ticks (Ixodidae) of domestic animals in Iraq. Part 2. Journal of Medical Entomology, 5 (1), 27-31.

ROBSON, J., ROBB, J.M. and HAWA, N.J. (1968 a). Ticks (Ixodidae) of domestic animals in Iraq. Part 3. Journal of Medical Entomology, 5 (2), 257-261.

(1968 b). Ticks (Ixodidae) of domestic animals in Iraq. Part 4. Journal of Medical Entomology, 5 (2), 261-264.

(1969). Ticks (Ixodidae) of domestic animals in Iraq. Part 5. Journal of Medical Entomology, 6 (2), 120-124.

ROBSON, J., ROBB, J.M., HAWA, N.J. and AL-WAHAYYIB, T. (1969 a). Ticks (Ixodidae) of domestic animals in Iraq. Part 6. Journal of Medical Entomology, 6 (2), 125-127.

(1969 b). Ticks (Ixodidae) of domestic animals in Iraq. Part 7. Journal of Medical Entomology, 6 (2), 127-130.

SAMISH, M. and PIPANO, E. (1978). Transmission of Theileria annulata by two and three host ticks of the genus Hyalomma (Ixodidae) - in "Tick-borne diseases and their vectors". Edited by J.K.H. Wilde. Centre for Tropical Veterinary Medicine, University of Edinburgh; 371-372.

SARDEY, M.R. and GHAFOOR, M.A. (1971). Life cycle deviation in Hyalomma anatolicum anatolicum Koch, 1844, fed on rabbit. Indian Journal of Animal Science, 41 (10), 960-962.

SCHALM, O.W. (1965). Veterinary Hematology, Second edition. Lea and Febiger, Philadelphia; 664pp.

SCHEIN, E. (1975). On the life cycle of Theileria annulata (Dschunkowsky and Luhs, 1904) in the midgut and hemolymph of Hyalomma anatolicum excavatum (Koch, 1844). Zeitschrift fur Parasitenkunde, 47 (2), 165-167.

SERDYUKOVA, G.V. (1946 a). On the cycle of development of the tick Hyalomma anatolicum anatolicum Koch. (In Russian; English summary). Bulletin de l'Academie des sciences de l'URSS, Séries Biologique, 2-3, 199-202. Summary in Review of Applied Entomology (1947), 35, 140.

SERDYUKOVA, G.V. (1946 b). Instances of the local mass increase of the tick Hyalomma anatolicum anatolicum Koch in Tadzhikistan and their causes. (In Russian). Izvestiya Tadzhikskogo filiada Akademii nauk SSR, 6, 60-63. Summary in Review of Applied Entomology (1947), 35, 73-74.

SNOW, K.R. (1969). The life-history of Hyalomma anatolicum anatolicum Koch, 1844 (Ixodoidea, Ixodidae) under laboratory conditions. Parasitology, 59, 105-122.

SNOW, K.R. (1970). The quantity of blood imbibed by Hyalomma anatolicum anatolicum Koch, 1844 (Ixodoidea, Ixodidae). Parasitology, 60, 53-60.

SONENSHINE, D.E. and ZIV, M. (1971). Ecological studies on ticks infesting sheep and small mammals in an unimproved semi-desert pasture in Israel. Journal of Medical Entomology, 8 (6), 683-686.

WARNECKE, M. (1978). A survey of the infestation rate of ixodid ticks with piroplasms in Turkey - in "Tick-borne diseases and their vectors". Edited by J.K.H. Wilde. Centre for Tropical Veterinary Medicine, University of Edinburgh; 166-167.

WILDE, J.K.H. (1977). The control of theileriosis - in "Theileriosis: Report of a workshop held in Nairobi, Kenya, 7 - 9 December 1976". Edited by J.B. Henson and M. Campbell. Ottowa, International Development Research Centre, lDRC-086e; 76-85.