INFECTION OF SMALL RODENTS WITH *COXIELLA BURNETI* BY THE PERORAL ROUTE

Species	Antibodies				*C. burneti* in spleen (No. positive/ total no. tested)
	3 weeks after infection		13 weeks after infection		
	No. positive/ total no. tested	Titre	No. positive/ total no. tested	Titre	
Experiment 1. Infection by drinking contaminated water					
A. flavicollis	6/8	—	—	—	8/8
C. glareolus	4/6	—	—	—	5/6
White mice	1/5	—	—	—	2/5
Experiment 2. Infection by eating contaminated roll					
A. flavicollis	2/3	1 : 160 1 : 640	3/3	1 : 80 1 : 80 1 : 80	1/3
White mice	2/5	1 : 40 1 : 160	0/5	—	0/5

inoculated in 0.5-ml amounts intraperitoneally into 3–5-g white mice. After 3 weeks the mice were examined for the presence of antibody in their sera by complement fixation.

In both experiments on peroral infection positive results were obtained, as shown in the accompanying table. Wild rodents were found to be almost 100% infected, but white mice showed a significantly decreased susceptibility to infection by the peroral route. The titre of antibodies in wild mice 3 weeks after the infection was 1: 640; after 13 weeks the level of antibodies decreased to 1: 80. In white mice, antibody titres of 1 : 160 were found in only a few cases and the titres disappeared entirely after 13 weeks. Mice which died from other causes during the experiments were not included in the results.

Supplementary results showing the possibility of peroral infection in small rodents were obtained with 2 subcutaneously infected milking mothers of *C. glareolus* and their sucklings, on the first and fourth days after birth.

These baby mice were infected either by drinking the mother's milk or by being in contact with infectious mother's faeces, and their sera showed positive results in complement-fixation reactions with *C. burneti* antigens.

Detection of *Coxiella burneti* in Saliva of Experimentally Infected Ticks, *Hyalomma dromedarii* Koch

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It is well known that ticks participate in the transmission of different pathogenic micro-organisms to man and to animals. These organisms are transmitted mostly in tick saliva during the sucking action, when the ticks probably introduce into the host anticoagulins, haemolysins, cytolysins and toxins. The latter can cause serious diseases in man and in animals, such as tick paralysis or sweating sickness.[a] The secretion of saliva by ticks is important in the regulation of the electrolytic and osmotic imbalance which occurs during the con-

[a] Arthur, D. R. (1962) *Ticks and disease*, Oxford, Pergamon Press.

centration of engorged host blood in their digestive organs.[b]

The transmission of rickettsiae by tick bites was reviewed by Řeháček[c] and Hoogstraal.[d] Even though this means of transmission is generally recognized, it is not always clear, especially with *Coxiella burneti*, whether it occurs with the tick saliva, by excretions of its coxal glands or by contamination with the faeces of the arthropod.

In the present study the question of *C. burneti*-transmission in tick saliva was examined and this paper gives some results of experiments in which we investigated the presence of the infective agent in the salivary glands of adult ticks, the occurrence and duration of saliva secretion and the concentration of the infective agent in the saliva.

Material and methods

Rickettsiae. Coxiella burneti, Florian strain, phase I, was isolated in 1956 by Nižňanský and Gmitter[e] from human blood and is cultivated in our laboratory by passaging in the yolk-sacs of chick embryos.

Ticks. The species of tick employed in these experiments was *Hyalomma dromedarii* Koch.[f]

Establishing the infection in ticks. Nymphs were rinsed in 70% ethanol and repeatedly washed in distilled water, immediately after engorging the blood of healthy guinea-pigs. After the nymphs were dried, 0.001 ml of yolk-sac suspension, containing 10 000 ID_{50} for chick embryos, was injected into the body cavity using a 0.25-ml tuberculin syringe with a no. 1 needle. The inoculated nymphs were placed in 50-ml Erlenmeyer flasks and were maintained at 28°C until they metamorphosed into adults.

Ascertaining the infectivity of the ticks. Ticks were tested for the presence of the infective agent before they were prepared for the saliva-secretion experiments. These examinations were done by means of tick-haemolymph smears. The haemolymph was obtained by amputating a limb, and the smears were stained by the method of Gimenez.[g]

Obtaining the tick saliva. Starved adult ticks of both sexes, fully-engorged males and females, which had engorged for different periods were all tested for saliva production. All the ticks were fed only on healthy guinea-pigs. Those examined for salivation were glued on pasting-tape, fixed to a glass slide, and inoculated in the hind part of the alloscutum with up to 0.3 ml of 1% pilocarpine, the amount depending on the period of engorgement. The secreted saliva was collected from the top of the hypostome and chelicerae of each tick by means of a very fine capillary and was either transferred to a glass slide for the preparation of a smear or was collected in a calibrated 0.1-ml pipette for measurement of the exact volume secreted. The coxiellae were again stained on the smears by the method of Gimenez.

Titration of the agent in the ticks, their salivary glands or their saliva. All the titrations were performed by intraperitoneal injections, into 8-g mice, of suspensions prepared and diluted from the ticks, the salivary glands or the saliva. Sera from these mice were examined after 4 weeks for the presence of antibodies by complement-fixation tests with *C. burneti* antigen.

Results

Only those ticks in which haemocytes showed the presence of coxiellae were included in experiments on salivation. All the ticks under investigation were found to be infected, as shown in Table 1.

We did not succeed in obtaining saliva from male ticks, whether starved or engorged, or from starved females even after repeated administration of pilocarpine: placing these arthropods in temperatures ranging from 20°C to 37°C and in more than 90% relative humidity was no more successful. The secretion of coxiellae in saliva of these ticks was indirectly confirmed by titrating the antibodies in guinea-pigs and mice on which the ticks had fed. The appearance of antibodies did not prove the transmission of coxiellae by saliva, however, since the host was also contaminated with faeces eliminated by the infected ticks during feeding.

The secretion of saliva was noted from partially or fully engorged females almost immediately after the administration of pilocarpine and continued for about 3 hours. The females tested immediately after interruption of feeding always secreted saliva. Later, in some females, secretion of saliva stopped or decreased considerably, probably because their salivary glands degenerate when egg production

[b] Tatchell, R. J. (1967) *Nature (Lond.)*, **213**, 940.

[c] Řeháček, J. (1965) *Ann. Rev. Entomol.*, **10**, 1.

[d] Hoogstraal, H. (1967) *Ann. Rev. Ent.*, **12**, 377.

[e] At the State Veterinary Institute, Bratislava, Czechoslovakia.

[f] Supplied by the Department of Diseases Occurring in Natural Foci, Gamaleja Institute of Epidemiology and Microbiology, Moscow.

[g] Gimenez, D. F. (1964) *Stain Technol.*, **39**, 135.

TABLE 1

DETAILS OF TWO EXPERIMENTS ON *COXIELLA BURNETI* SECRETION IN THE SALIVA OF INFECTED TICKS

Experiment no.	Parenteral infection of nymphs	Hatching of adults	Adults tested for infectivity (haemocytes)	Adults fed on guinea-pigs	Engorged ticks tested for infectivity		
					Date	No. with positive haemocytes/total no. of ticks	No. of ticks secreting saliva (all positive)/total no. of ticks
1	25 January	8 February (approx.)	8, 10, 12, 15, 22, 25 February and 4, 6 March	6–18 March	20 March	5/5	5/5
					22 March	5/5	5/5
					1 April	5/5	5/5
2	15 February	4 March (approx.)	22 March	25 March–4 April	4 April	5/5	5/5
					11 April	10/10	6/10
					16 April	13/13	10/13
					2 May	6/6	1/6

and laying begin. Immediately after the interruption of feeding the saliva production of partly or fully engorged ticks was practically the same: the amount of rickettsiae in the saliva of both groups of ticks was also similar.

In a few cases repeated administration of pilocarpine, or the transfer of injected females to an environment at 37°C with a relative humidity higher than 90%, stimulated saliva secretion. These environmental conditions were arranged by placing the females in closed Petri dishes, containing a piece of wet filter-paper, and incubating the dishes.

With optimal conditions, we succeeded in obtaining 0.003 ml of saliva from 1 female, but in many cases the yield was between 0.001 ml and 0.002 ml.

In 3 females, which secreted approximately the same amount of saliva during a 3-hour period, the quantity of coxiellae present in the saliva was estimated using smears. Smears prepared from the saliva every 10 minutes for 3 hours showed intense and uniform secretion of rickettsiae during the whole period—up a total of 10^9 ID_{50} for mice from the saliva of one female during the 3-hour period.

In 7 females which secreted sufficient saliva, the quantity of *Coxiella* in the saliva was determined and we were surprised at the very high values, up to 10^9 ID_{50} for mice. The quantity of rickettsiae in the bodies of female ticks was higher than in the salivary glands or the saliva; the titres of rickettsiae in the salivary glands and saliva were the same (see Table 2).

The smears of saliva from infected females showed that coxiellae were present either as separate organisms or in short chains or small bundles.

TABLE 2

TITRES OF *COXIELLA BURNETI* IN INFECTED FEMALE TICKS, *HYALOMMA DROMEDARII*

Dissection of infectious engorged females	No. of ticks	Titres of *C. burneti*		
		Whole body of female (less the salivary glands)	Salivary glands	Saliva
22 March	1	10^{10}	10^7	10^7
16 April	1	10^{10}	10^8	10^8
	2	10^{10}	10^8	10^8
	3	10^{12}	10^8	10^8
	4	10^{10}	10^6	10^6
	5	10^{10}	10^8	10^8
2 May	1	10^{11}	10^8	—[a]
	2	10^5	10^6	—[a]
	3	10^9	10^7	—[a]
	4	10^7	10^4	—[a]
	5	10^9	10^7	10^7
	6	10^7	10^7	—[a]

[a] Ticks did not secrete sufficient saliva for titration.

Discussion

The use of pilocarpine to obtain tick saliva, as described by Howell[h] and Tatchell,[i] was very successful and we agree with all Howell's observa-

[h] Howell, C. J. (1966) *J. S. Afr. vet. med. Ass.*, **37**, 236.
[i] Tatchell, R. J. (1967) *J. Parasit.*, **53**, 1106.

tions on the subject. Gregson [j] noticed, in addition, that it was possible to obtain a small volume of tick saliva by pushing a thin glass capillary on the hypostome and chelicerae of *Dermacentor andersoni* ticks, shortly after removing them from the host. We also used this method in our experiments, but with little success.

It is possible to employ other methods for the detection of infective agents in the saliva of vectors, such as the titration of the agent in the blood of the host animal, either at the site of the bite immediately after biting, or in the salivary glands of the arthropod: antibody production by the host, or the morbidity or lethal effect of an arthropod bite, or the subcutaneous injection of a known dose of the agent, may also be measured. All these methods have several disadvantages when compared with the pilocarpine method and are variable because of variations in the response of the host animal.

We conclude from our experiments that the use of pilocarpine in obtaining arthropod saliva is a suitable method not only for studying the relationship between the pathogens in the saliva and the vector, but also for obtaining good-quality samples of different infective agents from arthropods in sufficient quantity.

[j] Gregson, J. D. (1960) *Acta trop. (Basel)*, 17, 48.

Effects of Insecticides on the Feeding Activity of the Guppy, a Mosquito-eating Fish, in Thailand

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Guppies (*Lebistes reticulatus*) are well known as important predators of mosquito larvae in Thailand. In urban districts of Bangkok there are many bodies of water such as canals, ditches and ponds around and under dwellings. The water contains various kinds of organic matter, including domestic refuse, and provides suitable conditions for the development of *Culex pipiens fatigans* larvae. Large numbers of guppies are often found in such bodies of water containing high densities of mosquito larvae. However, although these fish are important in reducing the mosquito populations, applications of insecticides are still needed to obtain complete control of the larvae in these areas. Consequently, the use of selective insecticides, toxic to mosquito larvae but not to guppies, is desirable.

Several investigations on the susceptibility of *C. p. fatigans* larvae and guppies to insecticides have been undertaken in Thailand; Sasa et al.[a] concluded that fenitrothion and fenthion were suitable larvicides for controlling mosquito larvae without harmful effects on guppies. Yasuno & Kerdpipule [b] reported on the susceptibility to various insecticides of *C. p. fatigans* collected in Thailand, and an evaluation of insecticides against mosquitos was made by Lofgren et al.[c] in Bangkok.

The investigations reported in this paper were undertaken to study the effects of insecticides on the feeding activity of the guppy exposed to sublethal concentrations of toxicants. The selective toxicities of insecticides to mosquito larvae and guppies were also investigated.

Materials

Insecticides investigated included Abate, OMS-1210, OMS-1211, fenthion, fenitrothion, Dursban, ronnel, dichlorvos, diazinon, malathion, lindane, allethrin, *p,p'*-DDT and dieldrin. The compounds OMS-1210 and OMS-1211 are *o*-(2,5-dichloro-4-iodophenyl) *o,o*-diethyl phosphorothioate and *o*-(2,5-dichloro-4-iodophenyl) *o,o*-dimethyl phosphorothioate, respectively. Original stock insecticide solutions were prepared by dissolving the chemicals in ethanol at various concentrations and the stocks were kept in a refrigerator until they were tested. Quantities of each concentration of insecticide

[a] Sasa, M. et al. (1965) *Jap. J. exp. Med.*, 35, 51-62.

[b] Yasuno, M. & Kerdpipule, V., unpublished data.

[c] Lofgren, C. S., Scanlon, J. E. & Israngura, V. (1967) *Mosquito News*, 27, 16-21.