

mosa; *O. quadrasi* from Leyte and Mindanao, P.I.; and *Pomatiopsis lapidaria* from the Potomac River near Washington, D.C. were exposed to strains of *Schistosoma japonicum* from Shanghai, China; Kofu, Japan; and Chang Hua, Formosa. *O. hupensis* was readily infected with the Chinese and Japanese strains but in these tests could not be infected with the Formosan strain. *O. nosophora* was susceptible to both the Japanese and Formosan strains but was refractory to the Chinese strain. *O. formosana* became infected with the Formosan strain but could not be infected with the Chinese and Japanese strains. *O. quadrasi* was susceptible only to the Formosan strain while *P. lapidaria* was infected with the Chinese and Formosan strains but not the Japanese strain.

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EXPERIMENTAL INFECTION OF THE AFRICAN RELAPSING
FEVER TICK, *ORNITHODOROS MOÛBATA* (MURRAY),
WITH *BORRELIA LATYCHEVI* (SOFIEV)

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The Asiatic relapsing fever spirochete, *Borrelia latychevi*, was isolated by Sofiev (1941) in Middle Asia from rodents, *Rhombomys opimus* and *Gerbillus eversmanni*, and from its natural vector, the argasid tick, *Ornithodoros tartakovskyi*. Recently, Baltazard and collaborators (1952) reported the unsuccessful attempts to transmit this spirochete by *O. erraticus* (small and large form) and *O. normandi*

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and stated that *O. tholozani* and *O. moubata*, as well as the North American argasid ticks, could not serve as vectors of this organism.

In studies of the life cycle of spirochetes in various tick vectors, the development and persistence of *B. latychevi* in *O. moubata* were observed. It is the purpose of this paper to report these observations which are partially in disagreement with those of Baltazard *et al.*

MATERIALS AND METHODS

The *O. moubata* used for the following experiments were from clean stock maintained at the Rocky Mountain Laboratory. This stock originated from ticks collected in the Belgian Congo by Dr. R. Geigy, Director of the Swiss Tropical Institute. Dr. M. Baltazard, Director of the Pasteur Institute, Teheran, Iran, kindly sent us infected *O. tartakovskyi* from which a strain of *B. latychevi* was isolated. This species of relapsing fever spirochete produces only a mild infection in adult rabbits, white mice, and man and fails to infect white rats, guinea pigs, or dogs (Sofiev, 1941; Pospelowa-Shtrom, 1940). New-born rabbits, however, are highly susceptible, since from 70 to 100 per cent of these animals, infected either by tick bite or by inoculation of infectious blood, die in 15 to 20 days. During this period, a steady increase in the number of spirochetes in the peripheral blood can be observed (Baltazard *et al.*, 1952).

In order to carry out the experiments to be described, large numbers of ticks and infected hosts were needed. The following procedure was employed. Infected *O. tartakovskyi* were allowed to feed on a normal white mouse. Tail blood of this animal was examined daily. As soon as spirochetes were observed, the blood was used to inoculate groups of normal mice. When these animals were known to have spirochetemia, second and fourth nymphal stages and adults of *O. moubata* were allowed to feed on them. After the ticks had engorged, they were stored at room temperature in desiccator jars in which a relative humidity of 79 per cent was maintained by a saturated solution of ammonium chloride. In order to follow the behavior of *B. latychevi* in *O. moubata*, several ticks were dissected each day and their organs were examined by dark-field technique according to methods described by Burgdorfer (1951).

In addition to this procedure, haemolymph and coxal fluid from the ticks were examined for spirochetes. The haemolymph was obtained by amputating the distal portion of the legs, the coxal fluid by applying a warm needle or forceps to the ventral side of the ticks, which respond to such irritation with the expulsion of this fluid.

Smears prepared from the blood of infected mice were examined under the microscope, using a magnification of 450 and ordinary direct illumination.

RESULTS

1. *Infection and persistence of B. latychevi in second nymphal stage O. moubata.* Two hundred *O. moubata* in the second nymphal stage were fed on a mouse which had in the peripheral blood an average of two to three spirochetes per microscopic field. Fifty-three of 90 ticks examined during the first 75 days after the infective feeding were shown to be carriers of active spirochetes. The organisms were regularly found in the haemolymph, central ganglion, coxal organs, and salivary glands after the fifth day following the infective feeding. An increasingly heavy infiltra-

tion could be observed in the central ganglion and in the coxal organs, whereas in the salivary glands fewer spirochetes were found as the period of infection increased.

2. *Infection and persistence of B. latychevi in fourth nymphal stage O. moubata.* One hundred and ten *O. moubata* in the fourth nymphal stage were fed upon mice which had in the peripheral blood an average of four spirochetes per microscopic field. Twenty days after the ticks had engorged, the survival of *B. latychevi* was checked by examination of haemolymph. Spirochetes in varying numbers were found in 70 of 110 ticks studied. All of the ticks which did not show spirochetes in the haemolymph were dissected and examined. Heavy infections were noted in central ganglions, coxal organs, and salivary glands of 15 of these 40 ticks. Spirochetes could also be found in the walls but never in the lumen of the Malpighian tubules. Direct examinations of ticks previously shown to be infected revealed that *B. latychevi* persists in the tissues for more than 150 days.

3. *Infection and persistence of B. latychevi in adult O. moubata.* Fifty adult *O. moubata*, both males and females, were fed on infected mice whose peripheral blood contained an average of three spirochetes per microscopic field. Thirteen of the ticks were dissected and tested at intervals from 10 to 340 days after the infective

TABLE 1.—*Transovarial transmission. Presence of B. latychevi in eggs and progeny of infected O. moubata as revealed by dark-field examination*

Tick No.	Date of Infection	Date of Oviposition	No. of Eggs			No. of Nymphs		
			Tested	Positive	Negative	Tested	Positive	Negative
1	7-7-52	8-13-52	33	16	17	None		
2	"	9-15-52	50	0	50	25	0	25
3	12-6-52	4-5-53	30	30	0	20	12	8
4	"	"	15	12	3	20	12	8
5	"	4-6-53	15	15	0	25	17	8
6	"	"	15	14	1	15	1	14
7	"	6-14-53	21	15	6	None		
8	"	"	26	18	8	20	15	5

feeding. It was found that *B. latychevi* developed and persisted also in adult *O. moubata*. The spirochetes penetrate the gut wall, enter the body fluid, and then attack the various organs. Central ganglion, coxal organs, salivary glands, and the walls of the Malpighian tubules are regularly parasitized. In addition, the spirochetes were found in the tissues of the genital system of adult female ticks, especially in the ovarian tissue and egg follicles. The genital system of male ticks was not examined. Examination of eggs from 8 and of nymphs from 6 infected female ticks indicated that transovarial transmission can occur. Spirochetes could be found in eggs of 7 and in nymphs of 5 infected *O. moubata* (see Table 1).

4. *Transmission experiments.* Theoretically, *O. moubata* could transmit relapsing fever spirochetes by (a) excretions of the Malpighian tubules, (b) coxal fluid, which is ejected shortly before the tick finishes feeding, and (c) saliva, *i.e.*, by bite. Although the spirochetes were found in each of the organs which secrete these fluids, transmission through the excretions of the Malpighian tubules would not be expected since no spirochetes were found in the lumen of this organ or in the so-called "tick feces."

The coxal fluids of several hundred *O. moubata* were examined for the presence of spirochetes. With a few exceptions all fluids contained spirochetes in varying numbers. Seventy-three transmission experiments were then performed to determine whether or not infected *O. moubata* could transmit *B. latychevi* to adult white

TABLE 2.—Number of spirochetes in peripheral blood of adult mice, suckling mice, and new-born rabbits inoculated with *B. latychevi*

	Number of spirochetes* found																	
	Days after inoculation																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Adult mice																		
No. 1	0	0	1	45	3	4	1	4	14	8	1	—	1	1	0	0	1	0
No. 2	0	0	1	59	4	1	1	3	4	0	0	—	0	0	0	0	0	0
Suckling mice																		
No. 1	0	1	1	15	92	7	43	52	47	4	48	—	15	11	5	14	4	8
No. 2	0	1	2	27	79	32	39	39	140	4	12	—	14	12	0	1	1	8
New-born rabbits																		
No. 1	0	0	20	202	300	300	104	130	21	108	164	+						
No. 2	0	1	16	180	140	300	300	80	120	+								

— = Not examined.

+ = Died.

* The number of spirochetes was estimated by counting the organisms observed over a period of 5 minutes in a blood smear stained by Giemsa's method.

mice or new-born rabbits. Ticks in lots of 3 to 10 were employed in each experiment. None of the animals became infected.

To explain the negative results, it was postulated that either the quantity of spirochetes transmitted by saliva and coxal fluid was not sufficient to produce a frank infection in the animals or the virulence of *B. latychevi* had been decreased by passage through *O. moubata*. In order to determine which of the above explanations was valid, a comparative study was made of the course of infection in experimental animals inoculated either with suspensions of infected *O. moubata* or with blood from a mouse upon which infected *O. tartakovskyi* had fed.

Six suckling mice, 6 adult mice, and 6 new-born rabbits were inoculated with 0.25 ml. of citrated blood (12 drops of tail blood containing one spirochete per microscopic field, diluted in 2.0 ml. citrate solution) from a mouse which had been infected by *O. tartakovskyi*. All animals became infected. The course of infection in the peripheral blood of two animals of each group is shown in Table 2. It was found that the number of spirochetes in the blood of adult mice was less than the number in the blood of suckling mice and that spirochetemia persisted for a greater length of time in the latter animals. In new-born rabbits, however, an extremely heavy infection occurred, which caused death of all the animals.

In a parallel experiment, the animals were inoculated with suspensions of infected *O. moubata*. The ticks had been fed on infected mice and were then held for periods of 90 to 355 days before they were used. Varying numbers of ticks were triturated in 1.0 to 3.0 ml. of saline, and 0.25 ml. of the suspension, which was proved to contain spirochetes, was inoculated intramuscularly into suckling mice,

TABLE 3.—Results of attempts to infect mice and rabbits by injecting suspensions of *O. moubata* infected with *B. latychevi*

No. of triturated ticks	Days after infective feeding	Suckling mice		Adult mice		New-born rabbits	
		Positive	Negative	Positive	Negative	Positive	Negative
10	92	0	6	—	—	—	—
10	92	—	—	1	5	—	—
2	104	—	—	0	6	0	2
1	138	—	—	0	4	—	—
2	184	—	—	—	—	1	1
1	210	—	—	3	4	—	—
4	212	—	—	—	—	0	4
3	212	—	—	—	—	1	3
1	355	—	—	—	—	1	3

— = None injected.

adult mice, and new-born rabbits. As will be noted in Table 3, only a few animals became infected and in most instances the inocula did not produce infection.

DISCUSSION

Since *B. latychevi* persisted and multiplied in the tissues of *O. moubata*, including the coxal organs and salivary glands, there was a possibility that this species of tick would transmit these spirochetes as did *O. tartakovskyi*. However, none of the mice or new-born rabbits used in transmission experiments became infected, and a reason for the apparent discrepancy was sought. *B. latychevi* is relatively less virulent than other species of relapsing fever spirochetes, but it does cause a frank infection in mice and particularly in new-born rabbits when transmitted by its natural vector, *O. tartakovskyi*, or when infected blood is employed as an inoculum (see Table 2). The fact that suspensions of heavily infected *O. moubata* inoculated into these animals failed, with a few exceptions, to produce an infection suggests that *B. latychevi*, by passage through *O. moubata*, partially loses its original virulence.

SUMMARY

B. latychevi persists through all stages of *O. moubata* studied. It is found consistently in the central ganglion, the coxal organs, the salivary glands, the walls of the Malpighian tubules, and in the tissues of the genital system, as well as in the progeny of infected ticks.

Attempts to infect mice and new-born rabbits by feeding infected *O. moubata* were unsuccessful, and inoculation of suspensions of heavily infected ticks into these animals failed, except in a few instances, to produce infection. The results of these experiments indicate, therefore, that there is a change in the virulence of *B. latychevi* induced by passage through *O. moubata*.

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