The reaction of monomeric and aggregated immunoglobulins with C1. Immunochemistry 8: 1011–1020.
30. Ellerson, J. R., D. Yasmeen, R. A. Painter & K. J. Dorrington. 1972. A fragment corresponding to the C_H2 region of immunoglobulin G (IgG) with complement fixing activity. FEBS Lett. 24: 318–322.
31. Osler, A. G., & A. L. Sandberg. 1973. Alternate complement pathways. Progr. Allergy 17: 51–92.
32. Taranta, A. & E. C. Franklin. 1961. Complement fixation by antibody fragments. Science 134: 1981, 1982.
33. Scheer, P. H. & E. L. Becker. 1965. Pepsin digestion of rabbit and sheep antibodies. The effect on complement fixation. J. Exp. Med. 118: 891–904.
34. Berken, A. & B. Benacerraf. 1966. Properties of antibodies cytophilic for macrophages. J. Exp. Med. 123: 119–144.
35. Inchley, C., H. M. Grey & J. W. Uhr. 1970. The cytophilic activity of human immunoglobulins. J. Immunol. 105: 362–369.
36. Crowle, A. J. 1961. Immunodiffusion. : 1–333. Academic Press, Inc. New York, N.Y.
37. Goldwasser, R. A. & C. C. Shepard. 1959. Fluorescent antibody methods in the differentiation of murine and epidemic typhus sera; specificity changes resulting from previous immunization. J. Immunol. 82: 373–380.
38. Goldwasser, R. A. & C. C. Shepard. 1958. Staining of complement and modifications of fluorescent antibody procedures. J. Immunol. 80: 122–131.
39. Trautman, R. & K. M. Cowan. 1968. Preparative and analytical ultracentrifugation. *In* Methods in Immunology and Immunochemistry. Volume II. Physical and Chemical Methods. C. A. Williams & M. W. Chase, Eds. Chap. 7: 81–118. Academic Press, Inc. New York, N.Y.
40. Mancini, G., A. O. Carbonara & J. F. Heremans. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2: 235–254.
41. Fahey, J. L. & E. M. McKelvey. 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immunol. 94: 84–90.
42. Fiset, P., R. A. Ormsbee, R. Silberman, M. G. Peacock & S. H. Spielman. 1969. A microagglutination technique for detection and measurement of rickettsial antibodies. Acta Virol. 13: 60–66.
43. Anacker, R. L., E. G. Pickens & D. B. Lackman. 1967. Details of the ultrastructure of *Rickettsia prowazeki* grown in the chick yolk sac. J. Bacteriol. 94: 260–262.
44. Popow, P. P. & R. D. Golzowa. 1933. Zur Kenntnis der Wasserstoffionenkonzentration im Darm einiger blutsaugender Arthropoden. Arch. Schiffs-Trop. Hyg. 37: 465, 466.
45. Rockstein, M. 1964. The Physiology of Insecta. Academic Press, Inc. New York, N.Y.
46. Tyeryar, F. J., Jr., E. Weiss, D. B. Millar, F. M. Bozeman & R. A. Ormsbee. 1973. DNA base composition of rickettsiae. Science 180: 415–417.
47. Wisseman, C. L., Jr. To be published.
48. Dalton, D. D. & C. L. Wisseman, Jr. To be published.
49. Waddell, A. D. et al. In preparation.
50. Brown, D. T. et al. In preparation.
51. Wisseman, C. L., Jr. et al. To be published.

VIRULENCE OF *RICKETTSIA PROWAZEKI* FOR HEAD LICE *

E. S. Murray and S. B. Torrey

Department of Microbiology
Harvard School of Public Health
Harvard University
Boston, Massachusetts 02115

Introduction

Lice that infest man colonize in three separate areas: the clothes, the hair of the head, and the hair in the pubic area. The separateness of these colonizations is sufficiently distinct for human lice to be categorized as body (or clothes) lice, head lice, and crab (or pubic) lice.

The crab louse (*Pediculis pubis*) is markedly different anatomically and physiologically from both head and body lice; we will address ourselves to the subject of virulence of *R. prowazeki* for the crab louse in a subsequent paper. Head and body lice are considered by some entomologists to belong to two distinct species: *Pediculis humanus corporis* and *capitis*. However, the majority opinion seems to be that head and body lice are at opposite ends of a single species.[1] Regardless of taxonomic differences, it is clear that, geographically on man, colonies of head lice rarely migrate to the clothes and colonies of body (clothes) lice rarely migrate to the hair on the head; furthermore, almost without exception, eggs of head lice are laid on hair and eggs of body (clothes) lice are laid on clothes.

During this century, a further distinction between body and head lice has become clear-cut in the developed nations. Since 1900, infestation with body lice has markedly decreased in the United States, Western Europe, and in other nations with high standards of living. There remains, however, a wide prevalence of head lice, particularly on school children, in many of these countries. Sporadically, head lice reach epidemic proportions, such as in England in 1972, when more than 150,000 school children were reported infested. In Boston during the winter of 1973–74, an epidemic of head lice plagued the schools. Two school nurses who provided us with combed-out head lice informed us that they had examined the clothes and heads of hundreds of children with head lice. They insisted that they had not found any lice living in the clothes.

This greater prevalence of head lice over body lice is now also becoming noticeable in traditional endemic typhus areas of Eastern and Southeastern Europe, such as Bosnia, Yugoslavia.[2] Head lice are therefore still prevalent in the world and are becoming more available to be the sole transmitters of typhus, if they do transmit it.

Transmission of typhus from man to man by the human body louse (*P.*

* Supported mainly by contract DADA 17-70-C-0054 with the Research and Development command through the office of the Surgeon General, Department of the Army, Washington, D.C. and partly by the training program in Rickettsiology and Virology 5T1-AI0014-15 of the NIAID.

humanus corporis) was demonstrated by Nicolle *et al.* in 1909. Since 1909 Nicolle's discovery has been confirmed many times, and no other vector (except possibly, the flea in certain atypical instances) has been implicated in the typhus transmission cycle.

However, there is little accurate information on the significance of head lice in the transmission of typhus. In 1912, Goldberger and Anderson [3] conducted inconclusive studies on the infectability of the head louse. Weyer's experiments were also suggestive but not conclusive.

The present study was performed to determine whether head lice could be infected with *R. prowazeki*, and, if so, would the infection be fatal to the louse and would the rickettsiae be shed in the feces and therefore be available, as with infected body lice, to transmit and disseminate the disease.

Materials and Methods

Source of Lice

Body lice used in this experiment were obtained from a normal colony of *P. humanus corporis* that had been adapted to feeding on a rabbit. This colony was supplied by the United States Department of Agriculture in 1964 and has been maintained with daily feedings on a succession of rabbits in our laboratory for more than 10 years. Lice are maintained by placing them on a shaved rabbit's belly and allowing them to feed for approximately 30 min daily. Between feedings, the lice are kept in a desiccator in a 29°C incubator with humidity maintained at about 60%. At intervals of 1–2 years, lice from this colony are removed, sacrificed, dissected, smeared on slides, and stained by Giemsa, Giménez, or immunofluorescence for the presence of rickettsiae. No rickettsiae have ever been demonstrated in smears of lice from this colony.

All head lice used in this experiment were obtained from Boston school children with obvious head louse infestations. A special fine-toothed comb was used to comb out the adult and instar lice into plastic bags. In the laboratory, lice were retrieved from the plastic bags and placed in a special metal louse feeding capsule.†

Physiologic Characteristics of Head Lice

There were no characteristic marks of head lice. However, head lice appeared to be more active than body lice. Furthermore, they preferred to move around on hairs rather than on felt cloth, which body lice preferred. The most obvious characteristic was that head lice consumed very little food, even when starved. Frequently, it was necessary to inspect a head louse under a dissecting microscope to determine if it had imbibed blood. A body louse usually fed itself to repletion, with a large, maximally distended abdomen. In addition, head lice would not, or could not, effectively feed through the bolting silk that covered one face of the metal feeding box. In contrast, all stages of body lice, from nymph to adult, readily fed through the bolting silk.

† These special feeding boxes were designed and donated by Dr. Anka Sitar of the Institute of Health Protection of Serbia, Belgrade, Yugoslavia.

Strain of Infecting Rickettsia

The Ankara strain of *R. prowazeki* was used to infect both body and head lice in this experiment. The Ankara strain was obtained from a case of classic louse-borne typhus in Ankara, Turkey by Dr. John C. Snyder in 1943. The pool of rickettsiae used was the 16th yolk sac passage in embryonated eggs of the original Ankara strain. Ankara pool 66H1397–50% yolk sac in PGS [5] was thawed, diluted to 10%, and spun at 1000 rpm for 10 min. The middle layer was removed and refrozen at —80°C to be used later (see below).

Manner of Infecting Lice

Rabbit Inoculation

We chose to infect lice by intravenous inoculation of a rabbit in the manner described by Snyder and Wheeler.[6] The lice were subsequently fed on the shaved belly of the infected rabbit. On the Day 0 of infection, 5.25 ml of 10% Ankara strain yolk sac material (see above) was thawed. This material was inoculated into the ear vein of a recently weaned 800-g rabbit.

Head Lice

The prepared colony of 39 wild head lice was immediately (11:30 AM) put on the cleanly shaved belly of the rabbit in a metal corral and was allowed to feed for 30 min, after which time the head lice ran about; they showed no interest in feeding. All 39 lice were examined under a dissecting microscope. No red blood could be seen in the gut of seven of the lice; these lice were discarded. The remaining 32 lice were put in a 29°C incubator. Four hours later, 16 (or 50% of those that fed initially) were placed on the same infected rabbit for a second feeding in the same manner. Most of these 16 lice fed for 15–30 min before detaching and starting to run around aimlessly. They were put back into the metal box in the 29°C incubator, rejoining the remaining 16 head lice that had fed only once. Thereafter, these head lice were fed and handled as described later.

Body Lice

Sixty body lice (50 adults and 10 newly hatched nymphs) were placed on the shaved rabbit's belly in a metal corral at 3:30 PM (4 hr after the inoculation, see above). They were allowed to feed for approximately 30 min, at which time almost all had fed to repletion, with distended abdomens. The 55 fed lice were put in a metal feeding box and placed in the 29°C incubator. Thereafter, they were fed and manipulated as described in the next section.

Method of Feeding Lice After They Were Infected with R. prowazeki

Body lice were fed on the forearm of one of us (E.S.M.) once daily at approximately 9 AM. The body lice were kept continually in a metal feeding

box, one face of which was covered with bolting silk, which had holes large enough for the lice to feed through but not large enough for even the nymphs to escape. It was found that the body lice would feed to repletion once a day through the bolting silk with the metal box strapped tightly to the forearm. This was happily convenient by allowing the author (E.S.M.) to strap the body louse metal feeding box on the right forearm and take the head lice out of their metal box and put them on the skin of the left forearm. Thus, the right hand was free to manipulate the head lice. After the 9-AM feeding, the body lice were kept in the 29°C incubator until 5 PM and then were put in a pocket close to the body and kept there from 5 PM to approximately 9 AM the next day.

Head lice were fed loose on the forearm of the author (E.S.M.) three times a day, usually at 9 AM, 5 PM, and 11 PM. Head lice were allowed to feed as long as they desired. However, they rarely fed to repletion, almost always only partially. Between feedings, they were kept in a metal feeding box. Between 9:30 AM and 5 PM, they were maintained in a 29°C incubator (see *Body Lice*). Between 5 PM and 9 AM the next day, they were kept in a pocket close to the body. Head lice did not feed well through the bolting silk in the metal box feeder. They were always removed from the box and allowed to feed while free on the skin.

Immunofluorescent Testing of the Lice and Feces

Collecting Dead Lice

The metal feeding box that contained the infected body lice was opened only once every 24 hr, namely, when they were fed, at about 9 AM. Any dead lice were removed at this time and placed in a separate small vial at ±4°C until processed. The infected head lice were examined three times daily at approximately 9 AM, 5 PM, and 11 PM. Dead lice were removed and placed at ±4°C in separate vials until processed.

Smearing Lice

The technique for smearing lice was developed by Dr. Jacob A. Gaon of the Department of Epidemiology in the Sarajevo Medical School, Sarajevo, Yugoslavia. A small drop of water was placed slightly away from the center of a microscope slide. The louse to be smeared was placed in the drop and under a dissecting microscope. With a cataract knife, a cut was made at the junction of the thorax and abdomen. Then, under ordinary vision, the abdomen was pulled away from the thorax and dragged into the dry central area of the slide, where, as it was dragged, the gut tissues dried out, were pulled apart, and then were stretched into thin layers suitable for staining.

Feces of lice were emulsified in very small drops of sterile water and dried on the slide.

Immunofluorescent (IF) Staining of Louse Smears

A simple standard three-layer indirect IF test was performed. The louse (or feces) smears represented the *R. prowazeki* antigen to be tested for its

presence or absence. A high titered (1:5120) human serum from a Brill Zinsser disease patient was used at 1:80 (or 64 units) as the known positive *R. prowazeki* serum or second layer. Antihuman γ-globulin conjugated with fluorescein isothiocyanate was used as the third layer.

RESULTS

Developing a Stable Colony of Normal Wild Head Lice

One of the major impediments to performing the experiment became evident immediately after obtaining our first dozen wild head lice. The lice died quickly when we fed them once a day, which was our usual procedure. When we changed to two feedings per day, they still died. We noticed that they ate very little at each feeding. They appeared much more sensitive to variations in humidity and temperature. They were also more active and seemed to exhaust their blood meal rapidly. Moreover, they could not feed regularly through bolting silk in a feeding box, in contrast to the ability of our departmental colony of rabbit-fed body lice to do so.

We finally developed a feeding method by which we could keep head lice alive for a reasonable period of time (20–30 days) for experimentation. We did not attempt to develop a scheme for maintaining an optimal egg-laying colony for several generations. Our method consisted of feeding lice obtained in the wild outside a feeding box on the clean skin of the forearm, putting them on and picking them off after they ceased feeding. Three feedings were given at approximately 8-hr intervals at 9 AM, 5 PM, and 11 PM.

Precautions and Procedures with Lice After Infection with R. prowazeki

The lice were tightly taped inside a metal feeding box and were then sealed in a petri dish before being placed inside a pocket and kept close to the skin from 5 PM to 9 AM. Between 9 AM and 5 PM, the metal feeding box was kept in a desiccator jar inside a 29°C incubator at about 60% humidity. After the lice were infected with virulent *R. prowazeki*, they had to be handled by the author alone in special isolated areas.

The author had contracted louse-borne typhus in a laboratory in Cairo, Egypt in 1944 and had a serum antibody titer of 1:320 against *R. prowazeki* at the time of the experiment. A previous experiment with *R. prowazeki* strain E had demonstrated that blood meals that contained *R. prowazeki* antibodies did not alter the infection in the louse. Wisseman *et al.*[7] have also shown that typhus antibodies are not inhibitory in the louse gut.

Difficulties in Maintaining Wild Head Lice

TABLE 1 summarizes some of the difficulties involved in preparing a group of wild head lice for experimentation. On Day 0, two groups of lice were obtained in plastic bags. Lice had been combed into the bags from children's infested heads. On Day 1, 28 of 42 adults and 26 of 40 instars fed on the author's arm. By Day 2, all 28 adults who had fed survived, whereas only 14 instars remained of the original 40.

Day 7, the daily mortality rate had markedly decreased, but there were only 20 survivors of 42 original adults and 8 survivors of 40 instars. Instars exhibited a high mortality rate at first but after adaptation appeared sturdier and lived longer than adults.

With only 28 seasoned head lice available on the day of the experiment, we added a group of 11 new wild head lice, mixed adults and instars, which had been sent to us the night before. Therefore, we had 39 head lice for the infection experiment, although we expected a high mortality from the unseasoned 11 mixed lice.

Louse Infection Data

On 6/19/74, we selected 50 adults and 10 newly hatched instars from our normal rabbit-fed colony of body lice. On the same day, we had 39 head lice

TABLE 1

PREPARING A NORMAL HEAD LOUSE COLONY FOR EXPERIMENTAL INFECTION

Beginning Date 6/12/74	Louse Feedings			
	Adults		Instars	
Day	Fed Total	Dead *	Fed Total	Dead *
0	42	—	40	—
1	28/42	—	26/40	—
2	28/28	14	14/26	14
3	21/28	0	10/14	12
4	20/21	7	8/10	4
7	20/20	1	8/8	2
Total	20/42	22	8/40	32

* Found dead in the morning before feeding.

(see above). The 39 head and 60 body lice were infected as described in the MATERIALS AND METHODS. TABLES 2 and 3 record the results after infection of the lice. The first three lines of TABLE 2 summarize the deaths from Days 0 to 4. The body lice fed well, 55 of 60 in one feeding. Of the 39 head lice, 32 fed poorly to fairly well in one or two feedings (see MATERIALS AND METHODS). By the fourth postinfection day, only 33 of 55 body lice and 16 of 32 head lice had survived. These 49 lice, which were manipulated in detail, represent the main part of the experiment.

TABLE 3 records the deaths from Day 5 onward as they occurred each day and the results of IF tests on the dead lice smeared and of tests on batches of feces removed from the feeding boxes on various days and tested by IF. As can be seen, all head lice dead from Days 5 to 9 were positive; of the 16 lice alive on Day 5, only three survived until Day 9, and these three, when sacrificed and tested, were also found to be infected with *R. prowazeki*. Of the 28 body lice alive on Day 5, five survived until Day 15. Of the 23 that died, 18 were positive:

HEAD AND BODY LICE INFECTED BY FEEDING ON RABBIT INOCULATED INTRAVENOUSLY WITH *R. prowazeki* [TEST FOR INFECTION: IMMUNOFLUORESCENCE (IF)]

Day After Infection	Data	Body Lice	Head Lice
0	total lice put on infected rabbit	60	39
0	total successfully fed	55 *	32 †
0–4	total dead	22	16
0–4	no. lice infected / total tested	0/3	2/6 ‡
5–9	no. lice infected / total tested	—	16/16
6–15	no. lice infected / total tested	22/28	—

* One 30-min feeding only: it began 4 hr after rabbit infected intravenously.
† Two 30-min feedings: one immediately after infecting the rabbit intravenously (all 32) and another 4 hr later for ½ colony (16).
‡ One positive on Day 0, another positive on Day 2.

TABLE 3

HEAD AND BODY LICE INFECTED BY FEEDING ON RABBIT INOCULATED INTRAVENOUSLY WITH *R. prowazeki* [TEST FOR INFECTION: IMMUNOFLUORESCENCE (IF)]

Day After Infection	Body Lice: No. Lice Infected / Total Examined	Body Lice: Feces	Head Lice: No. Lice Infected / Total Examined	Head Lice: Feces
0–4	see TABLE 2		see TABLE 2	
5	0/5 *		1/1 *	+, ++ †
6	4/4	±, ± †	5/5	++++, +++
7	3/4	±, ++	—	+++, +++
8	1/1	+, ++	3/3	+++, +++, ++++
9	3/3	++	7/7	
10	0/1	+++	3 L=live	
11	1/1	+++	3 M=moribund	
12	4/5		1 D=dead	
13	0/1	++, +++		
14	—			
15	6/8 5 L, 3 D			
Control lice	0/4	(−)	0/4	(−)

* Numerator, number of lice positive for *R. prowazeki;* denominator, total number tested by IF.
† ±, Suspicious; +, few definitely with rickettsiae; ++, +++, ++++. moderate to massive rickettsial infection.

four of the five living and sacrificed on Day 15 were positive for *R. prowazeki*. Feces from head lice were moderately positive for *R. prowazeki* on Day 5 and were heavily positive by Day 8. Feces from body lice were only suspicious on Day 6, definitely positive on Day 7, and quite strongly positive on Day 10 and later.

Smears of four normal head lice and four normal body lice and the feces they passed were negative for *R. prowazeki*.

Referring again to TABLE 2, of the many lice of both kinds that died through the fourth experimental day, three body lice and six head lice were tested. None of the body lice were positive by IF, but two of the six head lice had a few distinct rickettsiae by IF.

From Days 5 to 9, all 16 dead and sacrificed head lice were positive for *R. prowazeki*. From Day 6, when the first body louse was demonstrated to be positive, through the 15th day of experimental infection, 22 of the 28 body lice were positive by IF.

In a previous pilot study that employed various staining methods, IF was superior to either Giemsa or Giménez. In this pilot experiment, seven of eight head lice infected with the Ankara strain of *R. prowazeki* were positive for rickettsiae by the IF test. Because of the superior results with IF in testing smears of lice and feces in the pilot test, we used this test exclusively in the main experiment.

DISCUSSION

This experimental work clearly demonstrates that the head louse (*P. humanus capitis*) is highly susceptible to virulent *R. prowazeki*. From the fifth day of infection onward, all 16 head lice tested, the 13 that died and three that were sacrificed alive on the ninth postinfection day, displayed massive infection of gut tissues.

As mentioned previously, in a pilot experiment prior to the main experiment, seven of eight head lice infected with the Ankara strain of *R. prowazeki* were demonstrated by IF to be infected with typhus rickettsiae. Furthermore, in both the pilot and the main experiment, all pooled feces of infected head lice from the sixth day onward were demonstrated by IF to be heavily contaminated with *R. prowazeki* rickettsiae. Head lice, therefore, appear to be potential transmitters of *R. prowazeki* under optimal epidemiologic circumstances.

It was surprising that the body lice that fed on the same infected rabbit in the main experiment exhibited a slightly lower infection rate (78%) and a longer time lag for the infection to develop (maximum 15 days). There are several possible explanations.

The head lice fed for 30 min on the rabbit *immediately* after it had been infected intravenously with *R. prowazeki*. Because of an accident, we were not able to feed the body lice on the same rabbit until 4 hr after it had been intravenously infected. Snyder and Wheeler fed groups of body lice on a rabbit at various intervals after intravenous infection of *R. prowazeki*. Their results make an interesting comparison with ours.

Of 49 body lice fed on an infected rabbit immediately after it had been infected with *R. prowazeki* intravenously, 48 (98%) were positive for rickettsiae on smear testing. Of a second group of 19 body lice that were placed on the same rabbit for the first time 8½ hr after the intravenous infection, only 13 (68%) were ultimately demonstrated to be infected. Thus, the body lice fed later had both a lower infection rate and a longer survival time as a group than those fed immediately. This result may be a partial or complete explanation for our differences between infections in the body and head lice.

However, there were other differences in experimental details that might be significant. For example, the body lice were only examined once daily, at which time dead lice were removed to the refrigerator. Head lice, conversely, were examined three times daily; therefore, head lice were removed more promptly after death, so that head louse smears and test samples were, in general, in better condition than those from body lice. Furthermore, the three small feedings per day of the head lice might produce quite different infection conditions in the gut when compared to the single daily gut-distending blood meal that the body louse had to digest. Finally, a domesticated colony of body lice fed over several decades on rabbit blood may possibly have developed some natural immunity to typhus.

One may question why we fed half (16) of the head louse colony a second time 4 hr after the first meal. In fact, we were distressed by the small amount of blood ingested by most head lice at the first meal. For almost half of the lice, we had to use a dissecting scope to determine the presence of red blood in the gut. We were not certain that the head lice had imbibed sufficient blood to become infected. However, when we fed the head lice a second time on the rabbit, we were more concerned that the rabbit blood might be toxic to the lice.[8] Therefore, we "scatter-basketted our eggs" and only gave half of the head louse colony infected rabbit blood on the second occasion; we hoped for at least some infectious meals and some survivors. Unfortunately, our data are not sufficiently precise to state the exact toxicity of the rabbit blood or to make a firm conclusion as to whether mortality of lice that fed twice on the rabbit was higher than that for those that fed only once.

SUMMARY

Wild head lice were obtained by combing out adult and instar lice from the uncut hair of school children. Normal body lice were selected from a colony of rabbit-adapted body lice obtained from the United States Department of Agriculture and maintained in the Department of Microbiology for more than 10 yr. Thirty-nine head lice and 60 body lice were fed on a rabbit that had been injected intravenously with a 10% suspension of a yolk sac pool from eggs heavily infected with the Ankara strain of virulent *R. prowazeki*. Five days after infection, 33 body lice and 16 head lice had survived and were feeding on a volunteer. Between Days 5 and 9, 13 head lice were dead or moribund and all of them were positive by IF for *R. prowazeki*. The three surviving head lice were also positive. Tests on the 33 body lice showed that 22 were positive for *R. prowazeki*, including four of the five body lice that survived until Day 15. In summary, head lice can be readily infected with *R. prowazeki* and disseminate virulent *R. prowazeki* organisms in their feces. Thus, theoretically, head lice appear to be highly potential as transmitters of *R. prowazeki* under optimal epidemiologic circumstances.

ACKNOWLEDGMENTS

We thank Drs. John C. Snyder, Andrew Spielman, and J. William Vinson for their invaluable advice and help in designing the experiment and in working out technical details of handling the lice. We also thank Mrs. Barbara Walsh and Mrs. Rita Cunningham for their imaginative and expert technical assistance in providing live entomologic specimens. Mrs. Judith Spielman provided indispensable assistance in growing and processing infectious inocula and in infecting the rabbit.

REFERENCES

1. BUXTON, P. A. 1939. The Louse. Edward Arnold & Co. London, England.
2. GAON, J. A. 1973. Louse eradication programs in Yugoslavia. *In* Proceedings of the International Symposium on the Control of Lice and Louse-Borne Diseases. Pan American Health Organization Scientific Publication No. 263. Washington, D.C.
3. GOLDBERGER, J. & J. F. ANDERSON. 1912. The transmission of typhus fever with especial reference to transmission by the head louse (*Pediculus capitis*). Public Health Rep. (US) **27:** 297–307.
4. WEYER, F. 19xx. Personal communication.
5. BOVARNICK, M. R., J. C. MILLER & J. C. SNYDER. 1950. The influence of certain salts, amino acids, sugars and proteins on the stability of rickettsiae. J. Bacteriol. **59:** 509–522.
6. SNYDER, J. C. & C. M. WHEELER. 1945. The experimental infection of the human body louse *Pediculus humanus corporis* with murine and epidemic louse-borne typhus strains. J. Exp. Med. **32:** 1–20.
7. WISSEMAN, C. L., JR., J. L. BOESE, A. D. WADDELL & D. J. SILVERMAN. This monograph.
8. VINSON, J. W. 1962. Personal experience with lice and rabbit blood in Mexico.

MURINE TYPHUS RICKETTSIAE IN THE ORIENTAL RAT FLEA *

S. Ito, J. W. Vinson, and T. J. McGuire, Jr.

Department of Anatomy
Harvard Medical School
and Department of Microbiology
Harvard University School of Public Health
Boston, Massachusetts 02115

INTRODUCTION

Rickettsia mooseri, the etiologic agent of murine typhus, is transmitted fro its natural rodent host to man by the oriental rat flea, *Xenopsylla cheopi* When these rickettsiae are imbibed in an infectious blood meal, they propag in the midgut epithelium of the flea and are excreted in the feces, which th becomes the actual vehicle of infection.[2-4] A similar sequence of events occu in the transmission of epidemic typhus rickettsiae by the infected human bo louse. Adult rat fleas may under normal circumstances live for many mont Infection of the flea with *R. mooseri* can occur only during the parasitic ad stage, and the life-span of the flea is not shortened by the infection.[5]

Although electron microscopic features of the infection of the body lou by *Rickettsiae prowazeki* have been examined in several studies,[5-12] no cor parable investigations have been made of rickettsial infection in oriental r fleas. Indeed, ultrastructural studies on the flea midgut epithelium have so f been limited to a short report by Reinhardt *et al.*[13] on the midgut of the orient rat flea and two other species and to an earlier description by Richards a Richards [11] of a novel beaded layer that forms the basement lamina that unde lies the midgut epithelium of the flea, *Ctenophthalmus*.

Herein, we intend to provide further information on normal midgut ep thelium of *X. cheopis* and to elucidate some of the ultrastructural features the infection of this species by murine typhus rickettsiae.

MATERIALS AND METHODS

The Wilmington strain [15] of *R. mooseri* used in the present study represen the eighth passage in the yolk sacs of developing chick embryos since it w received in this laboratory in 1946. The stock pool of this strain was a 50 suspension of infected yolk sac in sucrose-PG,[13] aliquots of which were store at −70° C.

Our colony of *Xenopsylla cheopis*, originally obtained from the United Stat Department of Agriculture, Entomology Research Division, Gainesvill Florida, has been maintained in our laboratory. Rearing procedures followe those recommended by Cole.[16] Fasted adult fleas of unknown age were use

* Supported in part by grant AI 11508 from the National Institute of Allergy an Infectious Diseases.

PAHO 1973

This brief and, of necessity, spotty summary provides some evidence to support the thesis that typhus infections in various forms and the typhus potential now constitute a grossly underestimated, worldwide problem deserving close attention.

SOURCE NOTES

1. Information herein on reported cases of louse-borne typhus was taken largely from the epidemiologic reports of the World and Pan American Health Organizations.
2. Much, but not all, of the pertinent literature, as well as concepts of typhus epidemiology and control, was reviewed for my paper, "The Use of the Living Attenuated E Strain Typhus Vaccine in Epidemic and Endemic Situations," presented at the 17th Oholo Biological Conference at Zichron Yaakov, Israel, in March 1972. Figures 3 and 4 herein were prepared to accompany that paper.
3. Some of the information herein about typhus in Burundi and Bolivia is contained in the following unpublished reports by my coworkers and me:
   a. Report on investigations of the etiology of the alleged typhus epidemic in Burundi. Submitted to WHO on September 15, 1967.
   b. Preliminary report on a controlled field trial of the attenuated living E strain typhus vaccine during an epidemic of louse-borne typhus in Burundi, May-September 1969. Submitted to WHO on February 10, 1970; addendum No. 1 submitted November 5, 1970.
   c. Report on a field trip to Bolivia, July 8-20, 1968. Submitted to PAHO.
   d. Interim progress report on a pilot typhus vaccination project in Bolivia. Submitted to PAHO on February 15, 1972.
4. Some of the information herein is derived from unpublished data in the Department of Microbiology, University of Maryland School of Medicine, about specimens collected by collaborating investigators in Ethiopia, Mexico, and former West Pakistan, by local health officials in those countries, or by departmental field teams.
5. Finally, some of the information in this paper has come from personal communications with physicians and hospital personnel in Afghanistan, Burundi, Rwanda, and Uganda.

Edward S. Murray[1] and Jakob A. Gaon.[2]

Incidence of Rickettsia prowazeki *infections in an endemic focus of louse-borne typhus: factors influencing the occurrence of epidemics.* Louse-borne typhus, as Hans Zinsser has aptly described it, has not only been one of the major scourges of mankind but has also considerably changed the course of history through many of the strategic and catastrophic epidemics it has caused. The disease has, at one time or another, occurred almost everywhere in the world. With the improvement in living standards and the advances in technology of the past 100 years or so, however, particularly modern plumbing, the bathtub, central heating, washing machines, and the like, the louse has been reduced to impotence in most of the world's countries.

Wherever there has been marked reduction in the human body louse population, the incidence of typhus has shrunk to a few sporadic, recrudescent cases. The United States is a good example. The last epidemic of louse-borne typhus is reported to have occurred in Philadelphia in 1877, but during the 95 years since then many hundreds—and quite probably thousands—of recognized and unrecognized cases of Brill-Zinsser disease have occurred in this country.

Of particular interest is the fact that lice have not been eradicated in the United States. Lice can still be collected from alcoholics in the outpatient departments of the large hospitals in Boston and New York, the very areas were the largest numbers of Brill-Zinsser infections have occurred.

In the United States, then, the necessary elements are present for typhus epidemics to start, namely, the presence of lice, cases of recrudescent typhus, and susceptible persons. The fact that there have been no epidemics and in fact no suspected cases of genuine

[1] Department of Microbiology, Harvard School of Public Health, Boston, Massachusetts, USA.
[2] Institute of Epidemiology, Sarajevo University, Sarajevo, Yugoslavia.

primary louse-borne ty
an obvious explanatio
hold level for transmi
United States is far
potential of the num
numbers of recrudesce

But there are areas
Bolivia, Burundi, and
threshold is being exc
to discuss one such ar
—where the critical
exceeded for many
potential for transm
typhus appears recen
that level.

Widespread epid
typhus occurred in
Wars I and II. D
World War II, sporad
continued to occur
in that period show
of Bosnia 50 to 95
tion had suffered
were therefore pot
ettsia prowazeki, as
dates for contracting

Active measures
lice and curtail typh
tween 1954 and th
than 57 documen
typhus epidemics
demics have slowly
have now ceased.
cases of louse-born
1967. The inciden
continued unabate
and only since 19
abating. The com
Brill-Zinsser disea
of louse-borne typ
threshold level in
below that level f

We believe the
involved in the p
typhus. They are
cases, the prevale
availability of s

CONTROL MEASURES

nd Jakob A. Gaon.[2]
ı prowazeki *Infec-*
ıcus of louse-borne
ng the occurrence of
ɛ typhus, as Hans
bed it, has not only
scourges of mankind
y changed the course
of the strategic and
it has caused. The
or another, occurred
he world. With the
tandards and the ad-
the past 100 years or
y modern plumbing,
heating, washing
the louse has been
ı most of the world's

ɛen marked reduction
ıuse population, the
as shrunk to a few
cases. The United
e. The last epidemic
is reported to have
a in 1877, but during
many hundreds—and
ls—of recognized and
Brill-Zinsser disease
ıuntry.
t is the fact that lice
d in the United States.
ed from alcoholics in
ents of the large hos-
New York, the very
t numbers of Brill-
occurred.
, then; the necessary
r typhus epidemics to
ence of lice, cases of
d susceptible persons.
ve been no epidemics
ited cases of genuine

logy, Harvard School of
Massachusetts, USA.
gy, Sarajevo University,

primary louse-borne typhus for 95 years has an obvious explanation: the critical threshold level for transmission of typhus in the United States is far above the combined potential of the numbers of lice and the numbers of recrudescent cases that exist.

But there are areas of the world such as Bolivia, Burundi, and Peru where the critical threshold is being exceeded. We would like to discuss one such area—Bosnia, Yugoslavia—where the critical threshold has been far exceeded for many years but where the potential for transmission of louse-borne typhus appears recently to have fallen below that level.

Widespread epidemics of louse-borne typhus occurred in Bosnia during World Wars I and II. During the decade after World War II, sporadic, often large epidemics continued to occur there. Serologic surveys in that period showed that over large areas of Bosnia 50 to 95 per cent of the population had suffered louse-borne typhus and were therefore potential carriers of *Rickettsia prowazeki*, as well as possible candidates for contracting Brill-Zinsser disease.

Active measures to reduce the numbers of lice and curtail typhus were carried out. Between 1954 and the present, however, more than 57 documented small and medium typhus epidemics have occurred. Such epidemics have slowly decreased in number and have now ceased. The last documented cases of louse-borne typhus occurred in April 1967. The incidence of Brill-Zinsser disease continued unabated through 1967 and 1968, and only since 1969 has it shown signs of abating. The combined potential of lice and Brill-Zinsser disease cases for transmission of louse-borne typhus fell below the critical threshold level in 1967 and has remained below that level for the past five years.

We believe there are four basic factors involved in the potential for transmission of typhus. They are the number of Brill-Zinsser cases, the prevalence and density of lice, the availability of susceptible persons, and a very important factor which we will describe presently.

As to the number of Brill-Zinsser disease or recrudescent typhus cases, Dr. Gaon has been studying typhus in Bosnia since 1946 and all cases in the region are reported to him at his institute. He sends out investigators who take blood samples from patients, their families, and close neighbors.

Over the past 18 years we have devised tests to differentiate the sera collected by the institute: those from louse-borne typhus cases, those from Brill-Zinsser disease cases, and those from healthy individuals who possess residual antibodies from a typhus attack suffered years before. By correlating the groups of individuals showing evidence of past or present typhus with others who show no evidence of previous typhus infection, we hope to draw a picture of the flow of *R. prowazeki* through the Bosnian community over the years.

Table 1 shows the total number of cases of primary louse-borne typhus and Brill-Zinsser disease that have been reported to and investigated by Dr. Gaon's institute since 1954. There was a total of 1,847 cases, of which 313 were primary louse-borne typhus and 1,534 were Brill-Zinsser disease cases.

These data are better shown in Figure 1, from which it is evident that the drop to zero of primary louse-borne typhus cases in the 1967-69 period was not preceded by a drop

Table 1. Incidence of louse-borne typhus and Brill-Zinsser disease in Bosnia, Yugoslavia, 1954-71.

Period	Brill-Zinsser cases	Louse-borne typhus cases	Totals
1954-56	293	114	407
1957-59	251	84	335
1960-62	256	86	342
1963-65	239	20	259
1966-68	303	9	312
1969-71	192	—	192
	1,534	313	1,847

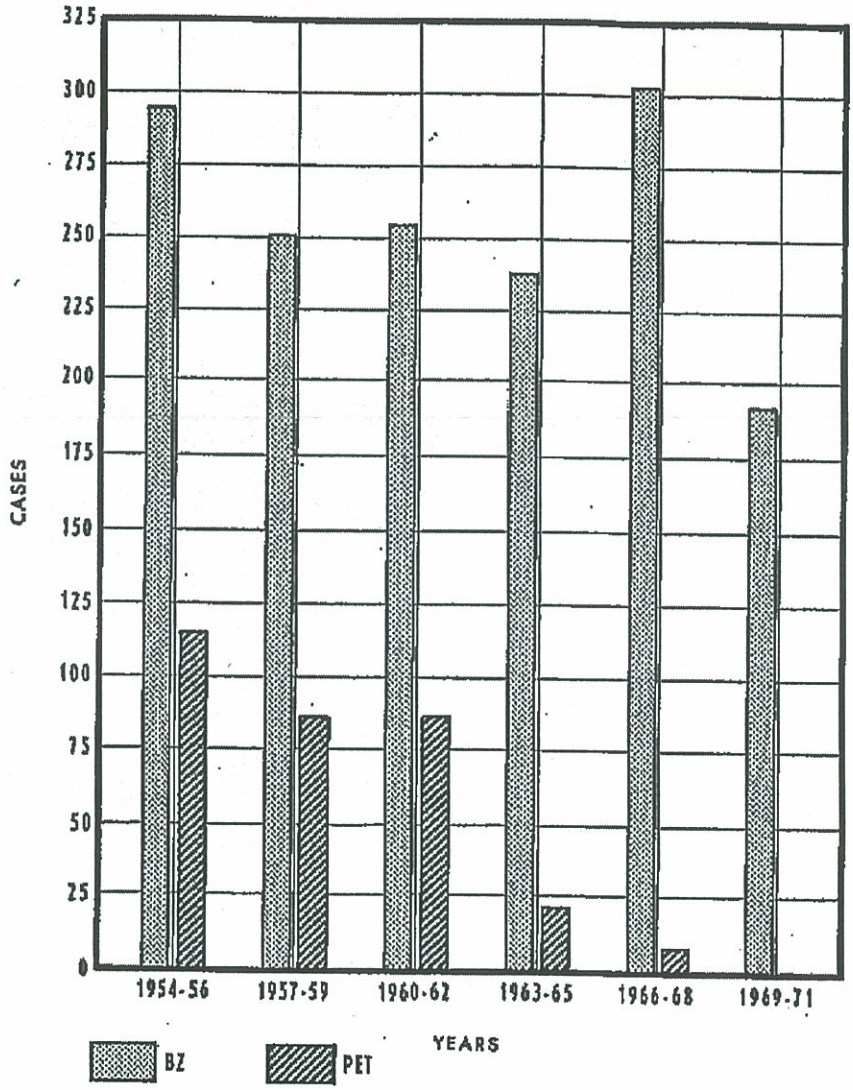


Figure 1. Primary epidemic typhus (PET) and Brill-Zinsser disease (BZ) in Bosnia, Yugoslavia, by three-year periods, 1954-71.

in the incidence of Brill-Zinsser disease cases. In fact, in 1966, 1967, and 1968, just when transmission of *R. prowazeki* from Brill-Zinsser disease cases to susceptible persons was disappearing, the effective reservoir of typhus, i.e., the number of persons with Brill-Zinsser disease, was at its highest level in 15 years.

The second factor, louse infestation, is obviously exceedingly important in transmis-

sion. Dr. Gaon ha
for the reduction
density in Bosnia.

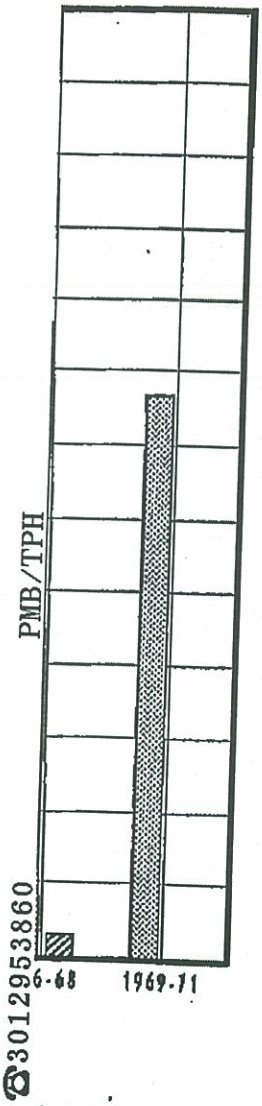
The third factor
tible persons. Tab
residual immunity
typhus endemic foc
is in the table's he
centages recorded
about 2,500 cases
see, about 81 per
born before 1941
through the typhu
II still have eviden
About 57 per cen
war show such ev
of those born afte
infected in the
occurred sometim
. One might ask
stopped in Bosnia
Brill-Zinsser dise
ber of susceptible
Dr. Gaon found
lence. Why did
drop below the c
. There is one
must be include
transmission pot

Table 2. Residual im
places in Bosnia,

| Sur
Place a |
|---|
| Skahovica (1951) |
| Skahovica (1966) |
| Nova Kasaba (19 |
| Vlasic (1967) |
| All Bosnia (1966- |
| All Bosnia (1969- |
| All Bosnia (1972 |
| Travnik (1972) |

Total Percenta

a 44/45 = 44 ind



slavia, by three-year periods.

er of persons with Brill-
t its highest level in 15

, louse infestation, is
important in transmis-

sion. Dr. Gaon has described the program for the reduction of louse prevalence and density in Bosnia.

The third factor is the number of susceptible persons. Table 2 shows the status of residual immunity of individuals living in typhus endemic foci in Bosnia. Our interest is in the table's headings and the total percentages recorded at its bottom. We studied about 2,500 cases for residuals. As you can see, about 81 per cent of people who were born before 1941 and who therefore lived through the typhus epidemics of World War II still have evidence of immunity to typhus. About 57 per cent of those born during the war show such evidence, and 4 to 8 per cent of those born after it show evidence of being infected in the sporadic epidemics that occurred sometime after the war.

One might ask why typhus transmission stopped in Bosnia in 1967. The incidence of Brill-Zinsser disease was still high, the number of susceptible persons was increasing, and Dr. Gaon found a 3 per cent louse prevalence. Why did the transmission potential drop below the critical threshold level?

There is one more factor, a fourth, that must be included in calculating the typhus transmission potential. That is the number of lice that a single Brill-Zinsser disease patient can infect. The expectable percentages of lice that such a person can infect are shown in Table 3.

Two decades ago Dr. John C. Snyder and I fed lice on Brill-Zinsser patients in New York, Boston, and Philadelphia. Table 3 shows the results on seven persons on whom we were successful in infecting lice. We fed between 77 and 300 lice on each of these patients, sometimes for three or four days and at other times for only a half-day.

As can be seen, the percentage of lice infected averaged between one and four lice per patient in five of the cases. Two cases did have higher louse infection rates, but, interestingly enough, they were the two in our study that resulted in death. In studying 700 cases in Bosnia over the past 15 years, Dr. Gaon has found only two deaths. It thus appears that patients who are to die are capable of infecting more lice than those who are not.

The rare infection of lice on Brill-Zinsser patients contrasts sharply with S. Burt Wolbach's results in feeding lice on patients with primary louse-borne typhus in Poland after World War I. His primary louse-borne typhus cases infected more than 50 per cent of the lice exposed to them.

Table 2. Residual immunity as evidence of prior louse-borne typhus infection by serum surveys at various dates and places in Bosnia.

Survey Place and Date	Dates of Birth				
	Before 1941	1941-45	1946-50	1951-60	1961-70
Skahovica (1951)	44/45*	3/5	0/6	—	—
Skahovica (1966)	47/50	8/9	2/16	1/51	—
Nova Kasaba (1958)	117/129	11/23	1/20	—	—
Vlasic (1967)	34/41	1/1	0/20	0/4	—
All Bosnia (1966-68)	374/546	26/70	4/94	3/116	0/21
All Bosnia (1969-71)	400/529	40/78	4/43	3/86	1/23
All Bosnia (1972 [6 months])	87/123	12/18	3/20	2/26	0/9
Travnik (1972)	71/94	70/94	4/7	1/7	1/4
	1264/1557	171/298	18/226	10/290	2/57
Total Percentages	81	57	8	3	4

* 44/45=44 individuals with positive residual antibodies among 45 tested.

Table 3. Percentage of lice infected by feeding on Brill-Zinsser disease patients.

Patient number	No. of lice fed	Time lice fed on patient (in days)	Percent of lice infected (approx.)		Data[a] on Patients	
					Weil-Felix[b]	Age
1	300	4	1/100		20	48
4	400	3	1/100		10	59
13	180	1/2	2/100		20	41
14	157	1/2	2/100		10	55
8	154	4	4/100		20	20
15	108	2-1/2	9/100	Died	40[c]	72
7	77	3	16/100	Died	280[c]	38

[a] Probable interval between first and second attacks (in listed order): 28, 30, 34, 37, 3, 44, 37.
[b] Denominator of titer.
[c] Died on 10th day.

In summary, the reservoir of rickettsiae in Brill-Zinsser disease cases will remain with us as long as there are people alive who have suffered an attack of louse-borne typhus, and that means for at least another hundred years. To completely eradicate lice appears to be an impossible dream.

But the experience in the typhus endemic focus of Bosnia, where the transmission of louse-borne typhus has fallen below the critical threshold level, indicates that even in the presence of large numbers of Brill-Zinsser disease cases, a high proportion of susceptible persons in the population, and considerable louse infestation, typhus fever will not occur when the combined potential of the four factors involved in louse-borne typhus transmission can be reduced below the critical transmission threshold level.

Luis Gamarra Gutiérrez.[1] *Pilot program for the control of typhus in Bolivia.* Typhus has long been a serious problem in that part of Bolivia over 2,500 m in altitude known as the *altiplano*, and has affected all settlements in it except cities, i.e., departmental capitals (Figure 1). The area's extent is 216,463 km², or 16 per cent of the national territory. The affected departments—Bolivia's principal political divisions—are La Paz, Cochabamba, Oruro, and Potosí, and to a lesser extent Chuquisaca and Tarija; only three departments, Pando, Beni, and Santa Cruz, have not been affected at one time or another.

The *altiplano's* population of 2,130,000 (about 43 per cent of the national population) consists largely of Indians living in considerable poverty. Because of the cold and their lack of custom they bathe infrequently, and many never. Lousiness is common and in some areas universal, and on its heels comes typhus. In the past we in the Ministry of Social Welfare and Health's National Institute of Communicable Diseases could do nothing but fight the brush fires of typhus by applying DDT and treating with chloramphenicol, and when outbreaks ended we withdrew.

Our inability to deal with the typhus problem in the long run led us to consider the possibility of mass vaccination, and to that end we concluded an agreement with the Pan American Health Organization under which Dr. Charles L. Wisseman, Jr., and his Department of Microbiology at the University of Maryland would produce a E strain vaccine that we would field-test. This pilot program has been carried out in three phases, preparation, immunization, and surveillance.

[1] National Institute of Communicable Diseases, La Paz, Bolivia.

Figure 1. Typhus in Bo

During the prep
localities in three
—Chacarilla and
huanaco and Des
ment and Yampa
ment—for pilot
censused the pop