VIRULENCE OF *RICKETTSIA PROWAZEKI* FOR HEAD LICE *

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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.*

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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 [4] 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 atfer 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.

By 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.

TABLE 2

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		Head Lice	
	No. Lice Infected		No. Lice Infected	
	Total Examined	Feces	Total Examined	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.

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