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The Use of Rabbits Used to Propagate Human Lice for Research

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The globally important human diseases of trench fever, epidemic typhus, and relapsing fever are vectored by the human louse *Pediculus humanus humanus*. Although these conditions are epidemically quiescent at present, they persist in socially dysfunctional situations of war, deprivation, and crowding. The taxonomically closely related head louse, *Pediculus humanus capitis*, does not respect economic or social status and is quite common in most countries. The 2 types of lice are now recognized as conspecific ecotypes of a single species. While the body louse has been adapted for propagation in the laboratory by feeding in vivo on live rabbits, a similar animal model has not been developed for the host-specific head louse. Accordingly, research for treatment and control of the head louse has largely been performed by using laboratory-reared body lice. This review describes methods for the propagation of body lice in the laboratory and outlines at least 4 areas of research that require sufficient numbers of aged body louse cohorts produced in rabbits for use in controlled studies: 1) pediculicide development and resistance, 2) immunity and vaccine potential, 3) endosymbiotic bacteria needed by lice for nutrition, and 4) lice as vectors of human disease. The review concludes with a discussion of several ethical issues involved with the standard method of using unsedated rabbits and recommends consideration of providing sedating anesthetics for rabbits used in louse feeding procedures.

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Introduction

Sucking lice of the Anopluran genus *Pediculus* continue to be a globally important public health concern. Human body lice, *Pediculus humanus humanus*, are important vectors of trench fever caused by *Bartonella quintana*, epidemic typhus caused by *Rickettsia prowazekii*, relapsing fever caused by *Borrelia recurrentis*, and several other bacterial pathogens including *Acinetobacter baumannii*, *Salmonella typhi*, *Serratia marcescens*, and the plague bacillus *Yersinia pestis*.[44] Since the advent of antibiotics, outbreaks of louse-vectored infections are sporadic and more quickly contained but continue to occur in unstable conditions.[14] Body louse infestation is typically associated with dysfunctional societal conditions of poverty, crowding, lack of clean clothing, unsanitary hygiene, war, and more recently, homelessness, and refugee camp conditions.[7,15,29,76,77] Untreated infestations are accompanied by allergic lesions of intense pruritus, excoriation, abrasion, and secondary infection of the skin due to scratching, reddening, and swelling of skin punctured by feeding lice.[19,63] Body lice were commonly referred to as "cooties" in the World War I era.

While human head lice, *Pediculus humanus capitis*, can be infected carriers of the same pathogens carried by body lice[5,6,10,11,77,79] and can be experimentally infected with the pathogens carried by body lice,[45] they have not been established as effective vectors of disease.[61] However, they nonetheless cause the clinical symptoms of pruritus and inflammatory lesions of the infested scalp, particularly in epidemics of school-aged children.[10,87] Head lice are also opportunistic parasites of those with an impaired immune system (for example, HIV infection).[76] Head lice are not exclusive to poor economic status and occur quite commonly, even in developed countries.[27] In the United States, to give some sense of head louse economic importance, pediculicide sales are over 240 million U.S. dollars per year, and infestation rates range from 6 to 12 million cases a year with 2 to 6 million households affected.[40] Annually, in the United States, about 1.2% of all school children are infested.[27] The cost of infestation may be estimated at more than 1 billion U.S. dollars, but the impact of lost days of learning due to the "no-nit" policy (before return to school) is more difficult to quantify.[21,35,36]

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Biology of Human Lice

Body and head lice are similar in appearance, although body lice may be slightly larger.[29,87] Illustrations of various stages of head and body lice may be seen in reference.[11] The lice are closely related, although the taxonomic relatedness of the 2 types has been debated for more than 2 centuries[57] and has been extensively investigated.[3,4,11,87] At present, they are regarded as conspecific ecotypes of a single species,[37] despite recognized genomic differences.[58,59] Phylogenetic analyses have grouped *Pediculus* lice into 6 distinct mitochondrial clades, A to F. Head lice appear in all 6 clades, but body lice belong only to clades A and D, suggesting that body lice evolved from head lice with the advent of clothing, which is required for egg deposition.[58,59] On the other hand, evolution from head lice to body lice seems not to have been a singular event because this type of evolution in clade A lice appears to take place frequently, especially in mixed infestation.[77] A molecular clock analysis indicates that body lice originated not more than 72,000 ± 42,000 y ago supporting the concept that the first human use of clothing originated at about the same time.[51] The divergent mitochondrial clades of head lice are geographically distributed and allow the study of coevolution of both the lice and their human hosts.[58]

The life cycles of these 2 ecotypes, on the other hand, are quite different. Body lice do not live on their human hosts, but periodically, 1 to 5 times daily, emerge from the seamed clothing or bedding for blood meals by puncture of capillaries on adjacent

skin. The eggs of body lice are laid in the clothing, bedding, and body hair. Head lice, by contrast, spend their entire life cycle on the scalp or attached to the hairs of the scalp. Louse eggs (called "nits") are attached to the base of the hairs and may be visible individually or as aggregate nits. In the case of both ecotypes, the eggs hatch in about 7 to 9 d, somewhat dependent on the ambient temperature. Lice undergo an incomplete metamorphosis. Hatched larval forms become first instar nymphs. As they grow, 2 more nymphal molts occur to become second and third instars, becoming adults after an additional molt. This metamorphic process takes about 20 to 21 d. The adults live for about 30 d.

Propagation of Lice on Rabbits

The initial attempts to propagate body lice for research used human volunteers,[23,83] a method still in use in certain circumstances.[81,84,85] Human volunteers used as hosts for temporary colonies of head lice find the practice irritating and arduous because of the frequency of feeding activity, at least 4 to 5× per day, and many individuals develop allergic immune responses of pruritus, inflammation, and secondary infection.[84,90] Because of the logistic limitations of this approach, efforts were made to develop an animal host for sustainable louse colonies under laboratory conditions, first successfully made by Culpepper in the 1940s at his lab in Orlando, FL.[23-25] The success of this model enabled investigators around the world to similarly establish colonies of body lice by using starter lice from the Orlando lab, or de novo, by using the same techniques.[83] In vitro techniques have been developed for the propagation of both body and head lice and are further discussed below. At present, the use of laboratory-reared body lice in rabbit hosts remains the predominant mode for the production of same-age, timed cohorts of the various life cycle stages for research and testing. Body lice are accepted as an appropriate species for the investigation of pediculicide efficacy against head lice.[31] Laboratory-reared human body lice are important for the development and efficacy testing of pediculicides for control of head lice; this is an ongoing need because head lice cultures suitable for research have not been successfully established in an animal model.[31,84]

Propagation of body lice for research begins with receipt of a starter shipment of adult lice from an established lab or by de novo collection of adult lice from a human infestation.[22,33,83] Several authors recommend that start-up, de novo colonies from lice collected in the field be first fed on human volunteers before being transferred to rabbit feeding.[22,83] Timing is important because adult lice can only live 2 to 3 d without a blood meal from the host. The adults are received or placed on patches of black cloth, and the patches are placed in covered culture dishes or covered glass or steel bowls. The lice are maintained under incubator conditions of about 30 to 32 °C and relative humidity of about 50%, conditions that mimic the ambiance under clothing. Variations of temperature, humidity, frequency of feeding, and type of blood have been studied to determine their effect on breeding and to establish optimal conditions for production.[25,31,34,55,56,62,69,80] Adult female lice will lay about 3 to 10 eggs per day and a total of about 60 to 100 over their lifespan.

Feeding takes place by removing host rabbits from their cages and bringing them to a procedure area with tabletop devices that allow the rabbits to be restrained in dorsal recumbency by strapping the legs. The abdominal skin is prepared by shaving with an electric clipper. Dishes of lice with separate cohorts of the sequential metamorphic stages are brought from the incubator. The cloth patches with adherent lice are then removed and placed directly on the shaved abdominal skin of the rabbit. The lice are allowed to feed for 15 to 30 min, and then the patches and any fed lice on the skin are brushed back into the dishes and returned to the incubator. For optimal production in the laboratory, all stages of lice must be fed at least every other day (4× per wk). The fed adults live and procreate for 20 to 21 d or more after hatching. Under conditions of natural infestation, lice from human clothing or bedding will die within a week if unable to acquire a human host.

In recent years, the inability to find an effective animal host for the rearing and production of human head lice, *P. humanus capitis*, has led to efforts to develop an in vitro technique to produce quantities of lice at various stages of the life cycle for research use. These efforts began early,[30,37,38,53,61,74] but more effective devices and membrane materials have since been developed for this purpose (see references below). Different devices have been developed but all use a reservoir that holds warmed rabbit or human blood. A suitable membrane material (for example, American Can Parafilm M or similar) is stretched over the reservoir, and the substrate patch material with attached lice is placed directly on the membrane for a timed feeding period. Devices have been developed for the production of both head and body lice.[12,28,48,52,62,69,88,90]

Research Applications

The following section presents brief introductions to 4 areas of research that use lice. The literature based on the subject areas is extensive and may be gleaned by cross-referencing. The intent here is not to exhaustively review and cite the subject area but rather to illustrate the abundant body of research related to the human louse that uses rabbits and derived methodology to provide life-stage forms necessary for particular areas of research. These subject areas are as described below.

Pediculicide development and resistance. Given the public health and economic importance of louse control and eradication, pediculicide development and the concurrent problem of pediculicide resistance are active areas of research. The history of pediculicide development shows that over time lice will develop resistance to current and subsequent chemical pediculicides as they are put into use.[2,26,89,90,91] This research area is directed primarily at head lice because pediculicides are not useful in the treatment of body lice. Body lice are treated by correction of personal hygiene and hot water laundering of clothes and bedding because the lice and nits do not reside on the body.

Insecticide resistance is particularly problematic in the control of human head lice for several reasons: 1) the parasites are obligate blood feeders and are exposed to pediculicides at all life stages; 2) lice are highly fecund with short generational time and, 3) many of the numerous pediculicidal products actually share a common chemistry that promotes cross-resistance.[21] The historic development of common pediculicides since the use of dichlorodiphenyltrichloroethane (DDT) in the 1940s and documentation of sequential resistance have been cited.[2,21,22,60,78]

The measurement of both efficacy and resistance to pediculicides can be evaluated objectively only by exposing test lots of lice to insecticides applied via an inert carrier.[16,17] This approach is the basis of the WHO test and subsequent variants of this standardized procedure.[41,54,75,78,88] These tests have used cohorts of test lice propagated in rabbits,[2,42] head lice collected from infested humans,[16,43,72] and head or body lice propagated by the in vitro membrane feeding method.[90]

Resistance is usually encountered as poor clinical response to treatment with a given product.[64] The first study to report resistance was that head lice from Massachusetts and Florida were resistant to permethrin and that the lice tested in bioassays

seemed to have genes consistent with what was termed knockdown resistance (kdr).[54] kdr was first discovered in the house fly[21] as a heritable trait associated with nerve insensitivity to DDT, pyrethroids, and pyrethrins (for example, permethrin). kdr and kdr-like genes were later found to be linked to 3-point mutations in human lice.[32,54] Permethrin was first used in the Nix formulation (Prestige, Tarrytown, NY) as a commercial over-the-counter product and extensively used for over 20 y. Loss of activity of the Nix product over the period from 1998 to 2013 was correlated with the increase of kdr and kdr-like mutations over that time.[32] While the presence of kdr-type mutations may not directly predict product clinical failure, their increasing frequency in louse populations coincides with reports of product failures in controlled studies.[32] The literature contains many reports describing the genetics of the kdr trait and bioassays used to track distribution of kdr and the mean percent resistance allele frequency in louse populations.[20,22,26,32,43,54]

Immunity to human lice and vaccine potential. The potential of vaccines for louse control has prompted study of the immunogens found in louse salivary and digestive products and louse feces.[8,65-68,70,71] In the process of biting and imbibing of blood, lice inject vasodilators, anesthetics, and anticoagulants (for example, thrombin inhibitor) to facilitate the process.[47,65] Human clinical literature is also replete with reports that infested humans develop pruritus and dermatitis as clinical symptoms of untreated infestation.[14,63] The sequence of skin lesion development has been characterized as a series of phases: 1) initially, no lesion; 2) 3 to 4 wk later, papules and moderate pruritus; 3) wheal development (so-called immediate hypersensitivity); and 4) delayed papular development and intense pruritus (delayed hypersensitivity). When healed hosts were reinfested, lesion development went directly to phase 2, indicative of an allergic immune response.[63] The literature commonly asserts that the clinical allergic responses are a consequence of saliva injection during louse feeding, but, while plausible, no laboratory research directly attributes the allergic condition to saliva injection. Another indicator of parasitic allergy is the recently noted hematologic eosinophilia that occurs during pediculosis.[14] The host allergic symptomology has no significant effect on louse populations (that is, infection-induced immunity is not very effective).[7,14]

The digestive process in the human louse and the assorted digestive enzymes have recently been reviewed.[88] Antigens of the louse midgut, including aminopeptidase,[71] have been studied and characterized.[67] When rabbits were immunized with extracts of louse midgut, they developed antibodies to all major midgut proteins.[70] When lice fed on rabbits immunized to midgut extracts, they took smaller blood meals, had higher mortality, laid fewer eggs, and took longer to develop.[8] Similarly, when rabbits were immunized with extracts of louse feces, they showed the same indicators of immunologic resistance.[66] The immunogenic antigens of the louse midgut were located in the outer epithelial cells of the midgut.[67] Unlike the salivary proteins, these midgut antigens are not injected at feeding and therefore are not normally presented to the host immune system and have been termed hidden antigens.[88] The hidden antigens include the gut proteases, components of the peritrophic membrane, and midgut symbiotic bacteria. Immunization of rabbits with recombinant hidden antigens has not been tested but presents a potential for vaccine development as an alternative to chemical pediculicides for louse control.[86,88]

Symbionts of *Pediculus* lice. Human lice are deficient in the gene-enabled synthesis of most B vitamins, including pantothenate (vitamin B_{12}), a necessary nutrient that is provided by the resident obligatory symbiont *Candidatus* Riesia pediculicola. These symbiotic bacteria are located in specialized cells of the mycetome, a visible disk-shaped organ located on the ventral aspect of the midgut.[13,18,49,50,88] The symbionts are transmitted to the next louse generation transovarially from the maternal mycetome to the developing oocysts and undergo a complex transfer cycle to 4 subsequent mycetomes as the lice go through their nymphal molt stages to the adult form.[73,88] The genomes of the symbionts and their louse hosts have been studied extensively.[39,49] A given louse clade can be identified by determining its particular subset of symbionts.[39] Further, the digestive tract of human lice and their digestive processes, including elucidation of the enzymes needed for the digestion of blood, have been investigated and recently summarized.[88]

Lice as vectors of human disease. The human health issues of epidemic typhus, trench fever, and relapsing fever are currently somewhat quiescent but globally continue to occur as important infectious diseases.[14,77] In fact, the collection and examination of lice from infested populations are effective surveillance mechanisms for the detection of these bacterial pathogens.[77] Laboratory studies of the respective rickettsial, bacterial, and spirochete organisms and their louse vectors have required substantial numbers of timed louse cohorts at various stages, provided predominantly by using the rabbit host system.[9,28,45,46,82] Infected bacteremic rabbits have been shown experimentally to effectively transmit *Rickettsia prowazekii* to body lice.[46] The study of the dynamics of louse infestation has shown that although infective rickettsia were discharged in the louse feces, infected lice did not transmit the bacteria to their progeny.[46] Similarly, infected rabbits were used to establish body lice as potential vectors of *R. rickettsii* and *R. conorii*[45] and proliferation and excretion of *Bartonella quintana*.[81] Isolation of *R. prowazekii* from infected rabbit midgut cells enabled ultrastructural studies.[82]

The potential for head lice as vectors of *R. prowazekii* and *B. quintana* has been investigated. Although head lice can be experimentally infected with these organisms and later void virulent forms in their feces, they do not transmit the organisms to rabbit hosts.[61] Under field conditions, human bacterial infection by head lice in the absence of body lice has not been reported, leading to the conclusion that head lice were poor biological vectors of human disease.[61]

Ethical Considerations

Important final topics are the ethical considerations of using sentient unsedated rabbits as the current standard model for periodic feeding of body lice at sequential stages of their life cycle. The discomfort (distress?) of rabbit hosts has scarcely been noted or commented on in the literature describing their use. Moreover, except for one affirmative comment in the 1940s,[24] no descriptions have been reported regarding the development of pruritus or dermatitis in rabbits that are used repeatedly as subjects for louse feeding. For at least the last 30 y or so, institutional IACUCs have approved these studies seemingly without questioning whether sedated alternatives exist and should be used.

Human volunteers used as subjects for body or head louse feeding subjects unquestionably find the practice uncomfortable. Researchers have difficulty in using humans as a sustainable and repetitive source of lice. Based on the author's several years' experience in maintaining a human body louse colony fed on rabbits for evaluation of commercial pediculicide formulations, rabbits clearly seemed to find successive episodes of feeding to be upsetting and they struggled after removal from the cage on their way to recumbency, as if they

knew what was coming. Whether the distress was due to the expectancy of lice feeding on their abdomen or merely to the process of being turned upside down and having their legs strapped down in dorsal recumbency was not clear. Once in place with the lice feeding, the rabbits were calm, seemingly tolerant of the situation, and not in pain. Furthermore, we did not observe rabbits developing pruritus or inflammatory skin lesions after repetitive use for feeding lice. Although the duration of use of a rabbit as a host for feeding lice is not part of the literature, the author suggests that good practice might be to discontinue the use of a given rabbit after 4 to 5 wk because the antibodies that develop in response to saliva injection might inhibit the production of healthy lice.

Long-standing institutional policy as promulgated by 1) the NIH Principles for the Utilization and Care of Vertebrate Animals, 2) the terms of accreditation by AAALACI as recommended in the *Guide for the Care and Use of Laboratory Animals*, and 3) the requirements of compliance with the Federal Animal Welfare Act regulated by the USDA all recommend with authority or require by statute that procedures causing more than momentary pain and distress be performed with appropriate sedatives, analgesics, or anesthetics. These converging obligations are also summarized in the AALAS policy on this issue. The AALAS Position Paper[1] on what seems like a gray zone of uncertainty can bring some clarity to this discussion. The Paper states that "AALAS supports live animal research when it is performed in an ethical and humane manner. That is, anyone working with laboratory animals has the moral obligation to explore, consider, and implement any means for avoidance and minimization of pain or distress in laboratory animals, whenever possible." Further, the Paper outlines that

1) The ability of vertebrate animals to feel or perceive pain is not fully understood and the assessment of pain in nonverbal individuals is often difficult. Therefore, any event, procedure, or situation known to cause pain or distress in humans must be expected to cause pain or distress in nonhuman vertebrate animal species, as well, unless proven otherwise.
2) The avoidance and minimization of pain and distress in laboratory animals is an ethical obligation that preserves the welfare of animals used in research, teaching, and testing, and optimizes the interpretation of scientific data collected during experiments.
3) Any experimental, husbandry, or other procedure that has the potential to produce more than slight or momentary pain or distress (for example, in excess of an injection of an innocuous substance) requires the consideration and implementation of pain-relieving measures, including but not limited to, the use of anesthetic and analgesic drugs, supportive care associated with surgical/painful procedures, social housing, acclimatization to stressful procedures, environmental modifications, and training to perform particular tasks allowing the animal some control over the situation. Preemptive measures should also be considered.

The use of rabbits for feeding lice is highly relevant to the AALAS policy and other regulatory or accrediting organizations. In the author's experience and opinion, the use of rabbits in this manner causes them distress. Whether the discomfort is caused by the simple anticipation of either being placed in dorsal recumbency, having the lice feed, or both, the evident stressful effects of the feeding process are sufficient to recommend giving consideration to the use of sedating anesthetics to ameliorate or prevent the distress that rabbits experience when used in this manner.

Some limited experience has been reported regarding the use of anesthetics to sedate rabbits being used for louse feeding in the standard model.[46,88] One study[46] used an intramuscular injection of a combination of 17 mg chlorpromazine and 67 mg ketamine chlorohydrate over a period of years, seemingly without (reportable?) ill effect on louse propagation. If any labs have used anesthetics, they should report this in the literature. The issue of whether sedation of the rabbit host has an adverse effect on the feeding lice is an open question at present, as no published information is available on the subject. Laboratories that use rabbits for feeding lice might investigate this question. IACUCs should ask investigators proposing the use of rabbits for the propagation of lice to consider the use of distress-alleviating sedation if that is not included in the protocol and to justify the need to omit sedation by citing scientific reasons.

IACUCs should also question whether the proposed use of rabbits could be accomplished by using an in vitro membrane feeding method, which is already a process well-established method. In short, "why use rabbits at all?" As an interesting aside, a similar evolution in accepted methodology took place about 25 y ago when the then-standard producing monoclonal antibodies in mice was replaced by the use of in vitro in cell cultures. The answer to the question of why rabbits are used is in a state of evolution based on 2 considerations. The first is historic. From about 1950 until 2015, virtually all nonclinical research and testing using human lice was conducted by using body lice produced in rabbits, even though reports of using in vitro membrane feeding technique to propagate human lice date back to 1949, this approach produced only small numbers of somewhat physiologically impaired lice. Although in vitro membrane feeding methodology is currently well established in principle for the production of both head and body lice, the assembly and use of the apparatus requires training and experience and until recently produced only small numbers of lice. More recently, perhaps in the last 5 to 10 y, improved membrane-feeding technology has been developed, and more recent research reports have used lice produced by an in vitro technique. For many types of research, in vitro methodology can replace the standard rabbit model.

The second rationale for the need to use live-rabbit hosts is that some types of testing and research (for example, pesticide testing) require larger numbers of same-age cohort lice, which can currently only be produced through the use of live rabbits. Furthermore, studies of the transmission, immunology, and pathobiology of infectious organisms carried by lice also require the use of a live host.

In conclusion, the need for a reliable source of lice continues to require a live host under some circumstances. Nonetheless, sedating the rabbits is a possible option that should be evaluated with regard to the amount and condition of the harvested lice. Such considerations are essential to minimizing animal distress, and because lice are vectors of serious human diseases and cause discomfort and possible allergic responses in human hosts, research on lice and therefore need for a live rabbit host may be necessary for many years.

Conflict of Interest

The author has no competing interest to declare.

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