Knockdown Resistance Allele Frequencies in North American Head Louse (Anoplura: Pediculidae) Populations

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ABSTRACT The study examines the extent and frequency of a knockdown-type resistance allele (kdr type) in North American populations of human head lice. Lice were collected from 32 locations in Canada and the United States. DNA was extracted from individual lice and used to determine their zygosity using the serial invasive signal amplification technique to detect the kdr-type T917I (TI) mutation, which is most responsible for nerve insensitivity that results in the kdr phenotype and permethrin resistance. Previously sampled sites were resampled to determine if the frequency of the TI mutation was changing. The TI frequency was also reevaluated using a quantitative sequencing method on pooled DNA samples from selected sites to validate this population genotyping method. Genotyping substantiated that TI occurs at high levels in North American lice (88.4%). Overall, the TI frequency in U.S. lice was 84.4% from 1999 to 2009, increased to 99.6% from 2007 to 2009, and was 97.1% in Canadian lice in 2008. Genotyping results using the serial invasive signal amplification reaction (99.54%) and quantitative sequencing (99.45%) techniques were highly correlated. Thus, the frequencies of TI in North American head louse populations were found to be uniformly high, which may be due to the high selection pressure from the intensive and widespread use of the pyrethrins- or pyrethroid-based pediculicides over many years, and is likely a main cause of increased pediculosis and failure of pyrethrins- or permethrin-based products in Canada and the United States. Alternative approaches to treatment of head lice infestations are critically needed.

KEY WORDS Pediculus humanus capitis, human head louse, knockdown resistance, serial invasive signal amplification reaction

Pediculosis, caused by the human head louse, *Pediculus humanus capitis* (De Geer), is one of the most prevalent parasitic infestation of humans (Lee et al. 2000). Pediculicide sales in the United States were estimated at >US$240 million per year in 1997 (Gratz 1997) and increased to >US$350 million per year by 2003 (Jones and English 2003). The overall cost of louse infestations in the United States is estimated at ~US$1 billion annually. However, this cost estimate is overshadowed by the long-term impact of school absences by ~1 in every 10 school-aged children and the implementation of ineffective control measures that can lead to multiple trips to the hospital or health clinic (Gratz 1997, Williams et al. 2001, Frankowski and Weiner 2002, Lebwohl et al. 2007). Infestations also often cause intense itching, which can injure skin, allowing secondary infections and self-inoculation with disease-causing bacterial pathogens, such as *Staphylococcus aureus*. In addition, most people find lice intolerable and repeatedly and prophylactically apply pediculicides, many of which carry the risk of adverse effects, without realizing their potential for harm. Misapplication affects children in particular because of their small size and higher sensitivity.

Currently, the treatment of pediculosis depends primarily on the topical application of insecticides, including the natural pyrethrin esters (pyrethrum), synthetic “pyrethroids” (permethrin, phenthothrin), organochlorines (lindane), and the organophosphorus (malathion)- and carbamate (carbaryl)-based formulations (Durand et al. 2012). More recently, clinical demonstration of effectiveness against head lice has allowed the registration of three new treatments in the United States, namely, benzyl alcohol, spinosad, and ivermectin (Stough et al. 2009, Meinking et al. 2010, Pariser et al. 2012). However, the pyrethrins and pyrethroids continue to dominate the head lice treatment market as inexpensive and easily obtainable over-the-counter formulations.

Pyrethrum (natural pyrethrins) formulation for the control of head lice was first introduced in 1945 (Durand et al. 2012) and was then supplemented with the more environmentally stable pyrethroids (e.g., per-
methrin) during the 1980s (Carson et al. 1988). In the 1990s, permethrin was used in the Nix formulation and marketed worldwide as an over-the-counter product (Durand et al. 2012). This formulation has been used extensively and intensely for >20 yr. The pyrethrins and pyrethroids share a common target site in the nervous system, voltage-sensitive sodium channels (VSSC), and act as agonistic neuroexcitants by prolonging sodium current, leading to nerve depolarization and hyperexcitation, followed by muscle paralysis and death.

Both clinical and parasitological pyrethroid resistance to d-phenothrin was first reported in France in 1994 (Chosidow et al. 1994), with additional reports of clinical failures as follows: permethrin (2001) in the United States (Hipolito et al. 2001), phenothrin (2005) in the United Kingdom (Burgess et al. 2005), and permethrin (2005) in the United Kingdom (Hill et al. 2005). Also, parasitological resistance to pyrethroids has been reported in the Czech Republic (Rupes et al. 1995), the United Kingdom (Downs et al. 1999), Denmark (Kristensen 2005), Israel (Muncuguol et al. 1995), the United States (Pollack et al. 1999, Lee et al. 2000), Argentina (Picollo et al. 1998), Japan (Tomita et al. 2003), and Australia (Hunter and Barker 2003). There are multiple mechanisms that give rise to pyrethrin and pyrethroid resistance in insects, including reduced penetration, enhanced xenobiotic detoxification, and target site insensitivity, also known as knockdown resistance or kdr. The kdr phenotype (recalcitrant to knockdown) is a heritable trait associated with nerve insensitivity to dichlorodiphenyltrichloroethane, the pyrethrins, and pyrethroids and was first discovered in the house fly, Musca domestica L. (Farnham 1977). Point mutations within the α-subunit gene of the VSSC are functionally responsible for the nerve insensitivity to dichlorodiphenyltrichloroethane, the pyrethrins, and pyrethroids and result in the kdr, kdr-type, and super kdr traits (Williamson et al. 1993, Dong and Scott 1994, Knipple et al. 1994). Lee et al. (2000) first reported that head lice from Massachusetts and Florida were resistant to permethrin and exhibited in vivo responses in behavioral bioassays that were consistent with kdr. Subsequently, three point mutations (MS151I, T917I, and L920F) in the VSSC α-subunit gene of permethrin-resistant head lice were reported (Lee et al. 2000, 2003) and functionally validated as kdr-type mutations (Yoon et al. 2008). Of the three kdr-type mutations identified in permethrin-resistant head lice, only the T917I (TI) mutation resulted in complete insensitivity of the channel to permethrin when heterologously expressed. Recently, several genotyping techniques based on these mutations have been developed for determining the kdr-type allele frequency using genomic DNA extracted from individual head lice (Lee et al. 2010), and have established that although widespread, kdr-type resistance is not yet uniform worldwide (Hodgdon et al. 2010).

Although the presence of kdr-type mutations alone may not directly predict clinical failure, their increasing frequency in head louse populations coincides with reports of product failures in controlled studies.

Early reports of permethrin use from 1984 through 1995 consistently showed 96 to 100% effectiveness (Taplin et al. 1986, Carson et al. 1988, DiNapoli et al. 1988, Bainbridge et al. 1998). In 2001, a report described a reduced effectiveness of only 80%, and subsequent reports of effectiveness have ranged from 28 to 55%, even where treatments have been augmented by nit combing (Burkhart and Burkhart 2000, Hipolito et al. 2001, Stough et al. 2009, Meinking et al. 2010). Therefore, it seems likely that the prevalence of the kdr-type alleles can be an indicator of permethrin resistance and the probable cause of product failure for human head lice.

In this study, we used serial invasive signal amplification reaction (SISAR) to determine the extent and frequency of the TI mutation in individual lice. To date, the TI mutation has always been found in the presence of the other two kdr-type mutations and is the only mutation of the three that produces a VSSC that is completely insensitive to the agonistic action of permethrin (Yoon et al. 2008). An expanded sampling approach that includes 14 locations in Canada and 18 locations in the United States, some of which have been previously sampled, enabled us to analyze whether there were changes in the TI mutation frequency within North American populations of human head lice. The TI mutation frequency was also reevaluated using a quantitative sequencing (QS) method on pooled DNA samples from selected sites to validate this cheaper and faster molecular diagnostic method necessary to create a high resolution kdr-type allele frequency map for use in resistance monitoring and management of head louse populations worldwide. Although QS does not identify the genotype of individual lice as does SISAR, it allows the determination of kdr-type allele frequencies in head louse populations in an effective manner (e.g., cheaper and faster), and then to genotype individual lice by SISAR from only those populations where the kdr-type mutations are not yet at high levels as suggested by Lee et al. (2010).

Materials and Methods

Head Louse Collection. Head lice were collected by volunteers (school nurses and professional lice combers) supporting our research from 12 states within the United States (Table 1). Four of these locations (Arizona, California, Florida, and Texas, see Fig. 1, boxed location) had been previously sampled (Gao et al. 2003), allowing the determination of kdr-type allele frequency changes in those locations over time. Prospective volunteers were contacted by e-mail (contact list was supplied by Topaz Pharmaceuticals LLC, Jenkintown, PA) and provided an information sheet, which explained the study’s objectives, method for louse collection, sample preparation, labeling instructions, storage requirements, a collection key requesting pertinent information, and shipping instructions. Volunteers who enlisted in the study were then sent a package with sufficient materials to collect the required louse samples and an addressed return box with
prepaid postage. Each louse population consisted of 2–126 lice collected from 1–30 individuals. Lice were collected from healthy subjects of all races and genders, aged from 2 to 55 yr. The treatment history of subjects from whom lice were collected was unknown. Lice were stored in 70% ethanol upon collection and sent by FedEx to the Pesticide Toxicology Laboratory, University of Massachusetts, Amherst, for genotyping analysis. The protocols for all louse collections were approved by the University of Massachusetts Internal Review Board (# 104-1423 and # 109-1792) and the National Institute of Health (# 06-003).

Lice samples were also previously collected from three Canadian provinces, namely, British Columbia, Ontario, and Quebec. Details of this collection and study are given in Table 1 of Marcoux et al. (2010). In this initial study, only the \textit{kdr}-type allele frequency was reported. In the current study, we now provide the zygosity (homozygous susceptible [SS], heterozygous [RS], and homozygous resistant [RR]) of each sample for comparative purposes. In addition, DNA samples from individual lice that had been previously genotyped were pooled by province and used, in part, to validate the QS method discussed below.

### Genomic DNA Extraction and Amplification of 1.1-kb Polymerase Chain Reaction Fragment.

Genomic DNA (gDNA) was extracted from individual whole head lice after glass–glass homogenization with the Qiagen DNeasy blood and tissue kit (Qiagen Inc., Valencia, CA) according to the manufacturer’s instructions. A 1.1-kb length fragment of the head louse VSSC α-subunit gene, encompassing the TI mutation and four introns, was amplified using the polymerase chain reaction according to Hodgdon et al. (2010). The concentration of the fragment was quantified using PicoGreen dsDNA quantification kit (Invitrogen, Carlsbad, CA.), diluted to a final concentration of 27 \( \mu \text{g/liter} \), and SISAR (Hodgdon et al. 2010) and QS (Kwon et al. 2008) reactions were performed for the detection of TI mutation.

### SISAR Genotyping of the TI \textit{kdr}-Type Resistance Mutation.

SISAR protocols were as initially reported by Kim et al. (2004) and updated by Hodgdon et al. (2010). Net fold over zero (net-FOZ) values for each fluorescent fluorophore (resistant or susceptible) were calculated as determined by Kim et al. (2004), and a SISAR ratio was determined using Equation 1.

\[
\text{SISAR ratio} = \frac{\text{Net-FOZ T target}}{\text{Net-FOZ C target}} [1]
\]

### QS Genotyping of the TI \textit{kdr}-Type Resistance Mutation.

The QS protocols were as initially reported by Kwon et al. (2008). The nucleotide signal intensities of the resistant and susceptible alleles were determined and signal ratios calculated using Equation 2.

### Table 1. U.S. head louse collections and \textit{kdr} allele frequencies determined by SISAR analysis

<table>
<thead>
<tr>
<th>Geographical locations of louse collections (abbr.)</th>
<th>Collection month/yr</th>
<th>No. of subjects/no. of subject analyzed</th>
<th>No. of lice collected/no. of lice analyzed</th>
<th>\textit{kdr} genotype frequency (TI mutation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arizona</td>
<td></td>
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<tr>
<td>Pinon (PN-AZ)</td>
<td>May 2007</td>
<td>12/12</td>
<td>34/30</td>
<td>RR 0.50, RS 0.30, SS 0.20</td>
</tr>
<tr>
<td>Phoenix (PX-AZ)*</td>
<td>Dec. 2008</td>
<td>8/8</td>
<td>30/18</td>
<td>1.0</td>
</tr>
<tr>
<td>California</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>San Bernardino County (SB-CA)</td>
<td>Sept. 2001</td>
<td>–/–</td>
<td>34/34</td>
<td>RR 0.35, RS 0.35, SS 0.30</td>
</tr>
<tr>
<td>Daly City (DC-CA)*</td>
<td>Jan. 2008</td>
<td>1/1</td>
<td>4/4</td>
<td>1.0</td>
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<tr>
<td>Florida</td>
<td></td>
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<tr>
<td>South Florida (SFL)</td>
<td>July 1999</td>
<td>–/–</td>
<td>29/29</td>
<td>RR 0.97, RS 0.03, SS 0.00</td>
</tr>
<tr>
<td>West Palm Beach (WB-FL)</td>
<td>Dec. 2006</td>
<td>3/3</td>
<td>13/3</td>
<td>1.0</td>
</tr>
<tr>
<td>Ocklawaha (OC-FL)</td>
<td>Dec. 2006</td>
<td>1/1</td>
<td>19/7</td>
<td>1.0</td>
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<tr>
<td>Massachusetts</td>
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<tr>
<td>Natick (MA)*</td>
<td>Jan. 2009</td>
<td>8/8</td>
<td>34/24</td>
<td>RR 1.0, RS 0.0, SS 0.0</td>
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<tr>
<td>Michigan</td>
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<tr>
<td>Saginaw (MI)</td>
<td>July 2007</td>
<td>2/2</td>
<td>33/29</td>
<td>RR 0.97, RS 0.03, SS 0.00</td>
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<tr>
<td>Minnesota</td>
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<tr>
<td>Tracy (MN)</td>
<td>May 2007</td>
<td>1/1</td>
<td>2/2</td>
<td>1.0</td>
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<tr>
<td>New York</td>
<td></td>
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<tr>
<td>Oceanside (NY)*</td>
<td>Nov. 2008</td>
<td>10/6</td>
<td>39/7</td>
<td>RR 1.0, RS 0.0, SS 0.0</td>
</tr>
<tr>
<td>Ohio</td>
<td></td>
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<tr>
<td>Willoughby (OH)*</td>
<td>Nov. 2008</td>
<td>30/24</td>
<td>32/24</td>
<td>RR 1.0, RS 0.0, SS 0.0</td>
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<tr>
<td>South Carolina</td>
<td></td>
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<tr>
<td>Summerville (SC)</td>
<td>Jan. 2009</td>
<td>3/2</td>
<td>30/6</td>
<td>RR 1.0, RS 0.0, SS 0.0</td>
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<tr>
<td>Tennessee</td>
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<tr>
<td>Nashville (TN)*</td>
<td>Oct. 2008</td>
<td>7/4</td>
<td>32/9</td>
<td>RR 1.0, RS 0.0, SS 0.0</td>
</tr>
<tr>
<td>Texas</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mathis (MA-TX)</td>
<td>Jan. 2001</td>
<td>1/1</td>
<td>27/27</td>
<td>RR 0.11, RS 0.52, SS 0.37</td>
</tr>
<tr>
<td>San Antonio (SA-TX)*</td>
<td>Dec. 2006</td>
<td>1/1</td>
<td>20/9</td>
<td>RR 1.0, RS 0.0, SS 0.0</td>
</tr>
<tr>
<td>Missouri City (MC-TX)</td>
<td>Nov. 2008</td>
<td>15/3</td>
<td>30/7</td>
<td>RR 1.0, RS 0.0, SS 0.0</td>
</tr>
<tr>
<td>Wisconsin</td>
<td></td>
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<tr>
<td>Reeseville (WI)*</td>
<td>Dec. 2008</td>
<td>12/7</td>
<td>38/22</td>
<td>RR 0.96, RS 0.04, SS 0.0</td>
</tr>
</tbody>
</table>

* Samples analyzed by both SISAR and QS.
Signal ratio = \frac{\text{Resistant nucleotide signal}}{\text{Resistant nucleotide signal} + \text{Susceptible nucleotide signal}} \quad [2]

The signal ratios of template DNA mixtures were normalized by multiplying them with the normalization factor (signal ratio of the heterozygous DNA template by signal ratio of the 5:5 standard DNA template). A series of normalized signal ratios were plotted against the corresponding TI mutation frequencies, and standard regression equations together with lower and upper prediction equations were generated for the estimation of TI mutation frequencies of unknown samples. For validation purposes, gDNA samples extracted from individual lice and used in the SISAR analysis above were pooled within a collection location and reanalyzed using QS.

Results
SISAR Analysis of Head Lice Collected in the United States. In total, 115 subjects from 18 geographical locations in 12 U.S. states were examined for the presence of head lice and determined to be positive for an active infestation (Table 1). From these individuals, 480 lice of different developmental stages were collected, but only adults and third instars, which made up the majority of lice collected, were used in the determination of the TI mutation frequency and zygosity. Of the 115 subjects who provided lice for the study, 84 provided lice that were analyzed. The percentage of subjects analyzed per location ranged from 20 to 100% with a mean ± SE value of 83.9 ± 24.4%. Of the 480 lice that were collected, 291 were analyzed. The percentage of collected lice that were analyzed per location ranged from 20 to 100% with a mean ± SE value of 63.0 ± 32.2%. The 189 lice that were not analyzed came from collections where large numbers of lice were collected. In these cases, 20% of the total lice collected were analyzed as a representative subsample. Figure 1 presents the zygosity of head louse populations collected during 23 collections from 18 geographical locations in the United States over an 11-yr interval (1999–2009). Of the 291 lice collected and analyzed over this time interval, 227 were homozygous resistant (78% RR), 37 were heterozygous (12.7% RS), and 27 were homozygous susceptible (9.3% SS), with an overall TI mutation frequency of 84.4% within these 12 states. In louse populations collected before May 2007 (MA-TX, OC-FL, PN-AZ, SA-TX, SB-CA, SFL, and WB-FL), the overall TI mutation frequency was 70.4 ± 37.6%. In louse populations collected after May 2007 (DC-CA, OH, MA, MC-TX, MI, MN, NY, PX-AZ, TN, SC, and WI), the overall TI mutation frequency was 99.6 ± 0.9%. In the four states where lice were collected at multiple times (2–3 collections), the overall TI mutation frequency increased from 65% in PN-AZ (2007) to 100% in PX-AZ (2008), from 52.9% in SB-CA (2001) to 100% in DC-CA (2008), from 96.6% in SFL (1999) to 100% in WB-FL.
and OC-FL (2006) when combined, and from 37% in MA-TX (2001) to 100% in SA-TX (2006) and in MC-TX (2008). The overall increase in the TI mutation frequency in these four states from 1999 to 2009 was 37.1 ± 25.2%.

**SISAR Analysis of Head Lice Collected in Canada.** In total, 121 subjects from 14 geographical locations in three Canadian provinices were determined to be positive for active louse infestations in a previously published study conducted in 2008 (see Table 1 of Marcoux et al. 2010). Of the 421 lice collected from 121 subjects, 137 lice (32.5% of total lice collected) from 92 individuals (76.0% of total subjects providing lice) were analyzed by SISAR, their zygosity was determined, and the TI mutation frequency calculated.

Figure 2 presents the zygosity of the 137 head lice collected in Canada in 2008 (Marcoux et al. 2010). Of these, 132 were homozygous resistant (96.35% RR), 2 were heterozygous (1.46% RS), and 3 were homozygous susceptible (2.19% SS), with an overall TI mutation frequency of 97.1% within these three provinces. Of the 14 collection sites, only Central Toronto (2 SS, 20% susceptible allele frequency), Oakville (1 SS, 9.1% susceptible allele frequency), and Sudbury (2 RS, 7.7% susceptible allele frequency) had lice where the susceptible T917 allele was detected.

**SISAR Analysis of Head Lice Collected in Canada and the United States.** North American lice collected from 32 locations across Canada and the United States from 1999 to 2009 had an overall TI mutation frequency of 88.4% based upon SISAR data. These data included a population from a Navajo reservation, in Arizona (PN-AZ), where pediculicides were not frequently used and had a TI mutation frequency of 65%. SISAR data also included a Mathis, TX, population (MA-TX) that was collected in 2001 and had a TI mutation frequency of 37% and a San Bernardino, CA, population (SB-CA) collected in 2001 that had a TI mutation frequency of 53%. The remaining louse populations collected after 2006 had overall TI mutation frequency of 98.5 ± 4.3%.

**Correlation of the TI Mutation Frequencies Determined by SISAR Versus QS.** The TI mutation frequency, determined initially by SISAR, of 11 selected populations of North American lice collected after 2006 was redetermined by QS (Table 2). Samples in which both SISAR and QS were performed had TI mutation frequencies of 99.54 and 99.45%, respectively, indicating a high correlation between these two genotyping techniques. QS was not run on the previously mentioned, relatively susceptible, populations (PZ-AZ and MA-TX) or on the populations from...
Michigan (MI), Minnesota (MN), and Florida (WB-FL and OC-FL), explaining, in part, the difference in the TI mutation frequency between the two methods.

**Discussion**

Our current database of genotyping results obtained from Canada and the United States clearly substantiates the contention that TI mutations are frequently detected at high levels and are commonly found in North American head louse populations. The overall TI mutation frequency in the U.S. populations collected from 1999 to 2008 was 84.4%. When the U.S. populations were examined after 2007, the overall TI mutation frequency increased to 99.6%. In the four states where lice were collected at multiple times, the overall increase in the TI mutation frequency in these states from 1999 to 2008 was 37.1 ± 25.2%. In head louse populations collected in 2008 from three Canadian providences, the overall TI mutation frequency was 97.1%. These results indicate that the TI mutation frequency has rapidly increased to ≈100% within North American head louse populations and is likely a major reason for the treatment failures encountered with pyrethrins- and pyrethroid-based pediculicides in both Canada and the United States. The almost uniform finding of homozygosity of the TI mutation provides further support for believing that the repeated use of pyrethrins and pyrethroid products is selecting for head lice that are refractory to this class of pediculicides.

However, these above results should be interpreted cautiously in that the number of lice collected and analyzed was relatively small, collections were limited to only 12 states in the United States and to 3 Canadian providences, were biased to metropolitan and urban collection sites, and possibly to lice collected from subjects whose infestations had already been treated with permethrin or similar product. Because of these limitations, the actual level of susceptibility may be higher in North American head louse populations (higher occurrence of the susceptible T917 allele) than what we have reported here. Certainly the results obtained from the Indian reservation (PZ-AZ) and the Mathis, Texas (MA-TX) collections, both rural areas, supports the contention that more susceptible alleles may exist in more rural areas. Nevertheless, recent clinical studies on permethrin have suggested its effectiveness is decreasing (Durand et al. 2012), and additional studies should be carried out to sample from more geographical locations in more states and providences, and to sample in suburban and rural areas.

The strong agreement between the genotyping results obtained with QS when compared with those obtained with SISAR will allow us to use the QS technique for population genotyping. Although QS will not allow the determination of zygosity of individual lice, it is an accurate, rapid, easy, and cost-effective population genotyping technique amenable to handling a large number of populations. With this validated method, it should be possible to expand our present kdr-type allele frequency map to other Canadian providences and to states in the northwestern, western, and midwestern United States. The zygosity of those populations that do not have high levels of kdr-type mutations can then be determined efficiently using the SISAR technique.

Regardless of the exact kdr-type allele frequency across Canada and the United States, there is obviously a critical need for a reassessment of an approach to management of head lice infestations that balances effectiveness and safety with treatment expense and the need to use treatments that have novel modes of action, which do not elicit cross-resistance to the widely used pyrethrins- or pyrethroid-based products and organophosphorous- and carbamate-based products. The recent development and commercialization of topically applied pediculicides containing benzyl alcohol (Ulesía, Sciele Pharma, Atlanta, GA), ivermectin (Sklice, Sanofi Pasteur, Swiftwater, PA), and spinosad (Natroba, ParaPRO LLC, Carmel, IN) will certainly relieve some of the insecticide selection pressure of the pyrethrin- and pyrethroid-based pediculicides on louse populations and provide more effective control of pediculosis (Clark et al. 2013).

**Acknowledgments**

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**References Cited**


**Table 2. Comparison of kdr allele frequency (TI mutation) determined by either SISAR or QS**

<table>
<thead>
<tr>
<th>Geographical locations of louse collections (abbr.)</th>
<th>SISAR</th>
<th>QS</th>
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<tr>
<td>United States</td>
<td></td>
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<tr>
<td>Daly City, CA (DC-CA)</td>
<td>100</td>
<td>100</td>
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<td>Oceanside, NY (NY)</td>
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Evaluation of the Efficacy and Safety of 1% Sodium Chloride (LiceFreee Spray) against 1% Permethrin Crème Rinse on Head Lice Infested Individuals

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ABSTRACT

Head lice are a public health issue, and resistance available over-the-counter pediculicides is a concern. The objective of this randomized study was to evaluate the pediculicidal activity and safety of 1% Sodium Chloride spray (0.1709 M), (LiceFreee Spray®) compared to the current recommended treatment for head lice with 1% Permethrin Crème Rinse. Forty-two subjects were randomized equally into Sodium Chloride or Permethrin group. Products were applied to hair according to the label instructions. After application of the products at Day 1 and Day 8, pediculicidal efficacy and safety were assessed at Day 1, Day 8 and Day 15. Second treatment was only applied on Day 8 to individuals with observed live lice using the same products and protocols as Day 1. Proportion of lice free subjects per group, the reduction in number of live lice per head and adverse effects were recorded after each visit. The results showed significantly higher pediculicidal activity for Sodium Chloride spray (85%) as compared to Permethrin (45%) at Day 15 (p < 0.05). Similar numbers of lice per head (21.76 range 10 to 68 versus 21.29 range 10 to 60 for Sodium Chloride and Permethrin groups, respectively) were observed for individuals at Day 1. At Day 15, lice per head infested reduced to 0.55 ± 1.50 in Sodium Chloride spray group compared to 5.45 ± 7.91 in the Permethrin group (p < 0.01). No serious adverse effects were observed in both groups. Sodium Chloride spray had superior efficacy to 1% Permethrin Crème Rinse in treating head lice and is a safe and excellent alternative to the current recommended treatment.

Keywords: Head Lice; Sodium Chloride; LiceFreee Spray®; Nix®; Permethrin

1. Introduction

Pediculosis capitis caused by Pediculus humanus capitis has been recognized as an issue from the antiquity, and currently remains a common health concern. The traditional perception of head lice as a parasitosis exclusively associated with schoolchildren of low socioeconomic status is challenged by the facts that pediculosis capitis is widespread throughout the world and does not discriminate on socioeconomic status grounds [1,2]. It primarily occurs in pre-school or elementary school age children and those living in the same house, although it can be found in refugees, urban slums, jail inmates, orphanages, and fishing communities [1]. The prevalence worldwide varies from 0.7% to 59% and is higher in girls and women. Having a head lice infestation is annoying, can lead to pruritus, sleeplessness, and in extreme cases, anemia. Secondary bacterial infections can complicate the course of the infestation with Staphylococcus aureus the most commonly implicated pathogen in this setting and can lead to impetigo, cellulitis, pyoderma and abscess formation [3-5]. The consequences of a head lice infestation can also result in psychological frustration for both parents and children. In the USA alone, pediculosis capitis is the most prevalent parasitic infestation of children with 6 - 12 million children infested and needing treatment for head lice each year. Head lice infestation also accounts for 12 - 24 million lost school days and $4 - $8 billion in economic loss due to missed workdays by parents staying home with their children [6].

Parents or non-health care professionals diagnose a head lice infestation by observing a louse crawling on the scalp or more frequently, observation of the nits on the hairs at the nape of the neck or behind the ears [7]. Transmission of head lice is mainly by direct head-to-head
contact with infested persons, particularly with children in the same school class, team or sharing playground. Fomite routes are less frequent and involve combs, hats, towels, fabrics and warm air movements [8,9].

Removal of the head lice includes traditional physical methods (plucking off head lice etc.), the use of coating gels, oral medication and the application of chemicals to the hair. Using pediculicides is the most frequent method. Pediculicides have shown effectiveness in reducing head lice infestation in populations globally [5]. Normally, pediculicidal treatment has to be applied on two occasions with 7 - 10 days in between, as the first dose of pediculicides primarily kill nymphs and adult lice, while their ovicidal activity is generally poor. The time gap between treatments allows surviving eggs to hatch and the newly hatched nymphs subsequently be killed by the second application [5]. Until recently, topical agents such as permethrin, allethrin, lindane or benzyl benzoate were still among the favorites. However, resistance to pediculicides has been reported in many areas of the United States as well as all around the world [10-12]. For example, reported efficacy of permethrin to head lice was reduced from as high as 97% - 99% before 1999 to as low as 10% - 72% after 1999 [5]. Importantly, several recent studies have revealed an increasing trend of simultaneous resistance against multiple agents including lindane, phe-nothrin and permethrin suggesting over-the-counter commercially available insecticidal products may become ineffective [5,8,10]. Prescription-only products containing synthetic compounds, such as malathion and carbaryl, have not been established well by controlled trials in term of safety and effectiveness for children less than 6 years old (malathion) or raised the concerns of possible carcinogenic effects (carbaryl) [10,13]. However, it has been suggested that resistance is starting to develop to this group also [5,14]. Therefore, alternative drugs for treatment of head lice are needed having the require-ments of safety, high effectiveness, available OTC and easy to use.

The primary objective was to compare the efficacy and safety of a one percent solution of Sodium Chloride (0.1709 M), against 1% Permethrin Crème Rinse, the treatment of choice by the National Guideline Clearinghouse [15], on subjects with head lice immediately following the initial application at 7 (Day 8), and 14 (Day 15) days following the initial application. The study was to also examine the efficacy and safety of the two treatments following one or two applications without the use of the nit comb.

2. Methods
2.1. Study Population

The study was approved by The Investigational Review Board Inc. (IRB # Lf 001-0011) and was conducted in accordance with Good Clinical Practices and with the principles outlined in the Declaration of Helsinki. The study was registered with ClinicalTrials.gov Protocol Registration System ID number NCT01514513 and all study treatments with follow up assessments were performed by the authors listed residing at South Florida Family Health Research Center, 6971 West Sunrise Blvd., Suite 102, Plantation, FL 33,313.

Individuals, aged 4 years or older, having a single place of residence, diagnosed with an active head lice infestation of at least 10 live lice at the Screening visit and a presence of nits were eligible for inclusion in this study. The subjects agreed to not use a lice comb, or any other pediculicides or medicated hair grooming products for the duration of the study (through Day 15 visit). Furthermore, parents and other family members of a child were also screened for head lice. If other household members were found to have head lice and were eligible, they were either enrolled in the study or were treated with the same product and in the same manner as study participants. All the participants of the study and where appropriate their legal guardian were explained the study’s procedures and signed informed consents.

Subjects using any form of head lice treatment for at least four weeks or any topical medication for a period of 48 hours prior to the Screening visit (Day 1) were not eligible for inclusion in the study. Individuals were also not eligible for participation if taking systemic or topical medications (including antibiotics), or suffering from visible skin/scalp condition at the treatment sites, of which in the opinion of the investigative personnel would interfere with the evaluation of the test products. Anyone who was allergic or sensitive to ragweed or any ingredi-ent in either test product, pregnant or nursing, who did not understand the subject requirements for study participation and/or exhibited poor compliance with the re-quired visits were also excluded from the study.

Subjects could withdraw from study treatment at any time at their own request, or at the discretion of the inves-tigators for safety, behavioral or administrative rea-sons.

2.2. Study Design

Subjects were randomly assigned by sealed-envelop randomization to receive Sodium Chloride or Permethrin during the treatment period. An equal number of subjects were allocated into each arm of the study. This was an open-label study; as such, the investigators, site personnel and the subjects were aware of the product being used.

Test product was 1% Sodium Chloride (0.1709 M)
spray (LiceFreee Spray®, in 6 ounce bottles; Lot number J1821 by Tec Laboratories, Inc. Albany, Oregon), and reference product was 1% Permethrin Crème Rinse (Nix®, Insight Pharmaceuticals, LangHorne, PA, in 2 fluid ounce bottles, 280 mg/fluid ounce; Lot number NX0908—Expiration 3/2012). The doses used for test and reference products were strictly followed as outlined on product label directions.

Subjects had the products applied on Day 1 by investigative site personnel after determination of meeting study inclusion criteria. The time of application, the weight of the bottle before and after application, the time elapsed until the hair was considered dry (for Sodium Chloride spray), the start and end time of rinsing (for Permethrin) were recorded.

In the Sodium Chloride spray arm, the product was sprayed on the hair until the hair was completely saturated. The hair was then allowed to dry naturally. Efficacy evaluation was performed 1 hour after the application of Sodium Chloride or earlier if the hair was completely dry using a metal comb. The participants were asked to wait 24 hours before returning to their normal hair hygiene. For those in the Permethrin Crème Rinse treatment group, the hair was washed with baby shampoo (Johnson and Johnson, New Brunswick, NJ) without conditioner and then rinsed with water. The hair was towel dried. Permethrin Crème Rinse was then applied to saturate the hair, scalp, behind the ears and to the nape of the neck. After 10 minutes of treatment with Permethrin Crème Rinse, the hair was rinsed with warm water and towel dried and efficacy of the treatment was assessed using a metal comb. Subjects received treatment at the time they were in the clinic which varied based on appointment time. No nit combs were used during the study. If live lice were observed at the second visit (Day 8), the subjects received a second treatment with the assigned product used. Numbers of live lice and nits observed in the left, middle, and right side of the head were recorded for all time points but the number of nits was estimated only prior to the course of treatment.

Assessment of other symptoms such as pruritus, erythema, presence of secondary infection, and excoriation was recorded at each visit using a 4-point scale (none 0, mild 1, moderate 2, or severe 3). Vital sign measurements such as systolic, diastolic blood pressure and pulse rate, examined after the subject rested in a seated position for at least 5 minutes, and evaluation of existence of red eyes were performed at each visit. Urine pregnancy test was performed at the Screening Visit (Day 1) only to ensure all females of child-bearing potential were not pregnant.

2.4. End Points
The primary measurements were the safety and efficacy in the reduction of the total number of lice observed on the subject’s head and scalp after treatment assessed on Day 1, 8 and 15. An evaluation was performed at each subject’s visit to determine whether their eyes were clear (value recorded 0) or red (value recorded 1). Additional assessments during the study for frequency of pruritus, erythema, presence of secondary infection, or excoriation were performed at each subject’s visit using a four point scale detailed above [16-18].

2.5. Statistical Analysis and Expression of the Results
The sample size of 20 subjects per treatment group was a feasible sample size to provide descriptive statistics for the efficacy and safety parameters as no formal calculation of power or sample size was performed in this study. Fisher test and t-test were used when applicable to compare the efficacy of 1% Sodium Chloride spray product in eliminating head lice to that of Permethrin.

The following parameters were calculated: The proportion of subjects that were 100% free of live lice in each treatment group immediately post-dosing, and at the Day 8 and Day 15 visits and the proportion of subjects that required retreatment to be 100% free of lice at day 15 visit was recorded. For comparison, Fisher test and two sided 95% confidence interval were performed on the difference in proportion between treatment groups. The numbers of live lice per head before the treatment and after each visit were also counted for the comparison between treatments. A p-value < 0.05 was considered statistically significant.
3. Results

3.1. Demographic Characteristics of the Population

Forty-two subjects participated in the study were assigned equally into each treatment group (Sodium Chloride spray or Permethrin). All females of child-bearing potential had negative pregnancy tests on Day 1 prior to their participation in this study. One subject in each treatment group completed the first treatment and first assessments but dropped out of the study prior to Day 8, and twenty subjects in each treatment group completed the study.

As shown in Table 1, there were no differences in age, race, and gender in the two treatment groups. Nearly half of all subjects had average texture to the hair (47.6% in both treatment groups) but the curliness and length varied. The number of individuals with long and extra-long hair was higher in Sodium Chloride spray arm while curly hair occurred more in Permethrin arm.

Table 1. Each treatment group’s subject demographic characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Sodium chloride</th>
<th>Sodium chloride</th>
<th>Permethrin</th>
<th>Permethrin</th>
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<tbody>
<tr>
<td>n</td>
<td>21</td>
<td>21</td>
<td>20</td>
<td>20</td>
</tr>
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<td>85.71</td>
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<td>Male</td>
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<td>4.76</td>
</tr>
<tr>
<td>Age</td>
<td></td>
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</tr>
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<td>Mean</td>
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<td>4, 42</td>
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<td></td>
</tr>
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<td>White</td>
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<td>85.71</td>
<td>19</td>
<td>90.48</td>
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<td>Asian</td>
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<td>0.00</td>
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<td>9.52</td>
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<tr>
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<td>Hair length</td>
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<tr>
<td>Extra-long</td>
<td>5</td>
<td>23.81</td>
<td>3</td>
<td>14.29</td>
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<td>Long</td>
<td>7</td>
<td>33.33</td>
<td>6</td>
<td>28.57</td>
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<tr>
<td>Medium</td>
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<td>11</td>
<td>52.38</td>
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<td>Fine</td>
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<td>4</td>
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<td>Average</td>
<td>10</td>
<td>47.62</td>
<td>10</td>
<td>47.62</td>
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<tr>
<td>Coarse</td>
<td>3</td>
<td>14.29</td>
<td>7</td>
<td>33.33</td>
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<tr>
<td>Hair curliness</td>
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<tr>
<td>Curly</td>
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<td>4.76</td>
<td>5</td>
<td>23.81</td>
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<tr>
<td>Wavy</td>
<td>10</td>
<td>47.62</td>
<td>8</td>
<td>38.10</td>
</tr>
<tr>
<td>Straight</td>
<td>10</td>
<td>47.62</td>
<td>8</td>
<td>38.10</td>
</tr>
</tbody>
</table>

3.2. Efficacy Assessment

Immediately following treatment assessment (Figure 1) on Day 1, 79.2% Sodium Chloride Spray subjects (16 of 21) and 66.7% Permethrin subjects (14 of 21) were lice free (p > 0.05). At Day 8 visit, 25.0% subjects (5 out of 20) in Sodium Chloride Spray arm and 75.0% subjects (15 out of 20) in Permethrin arm required another course of treatment (p < 0.05). Of those requiring retreatment at Day 8 visit, 3 of 5 Sodium Chloride spray subjects (60.0%) were successfully treated as compared to 5 of 15 Permethrin subjects (33.0%) (p > 0.05). Figure 2 presents the percentage of subjects free of lice at the end of the course of treatment (Day 15 visit), 85.0% of the subjects in Sodium Chloride Spray arm and 45.0% of the subjects in Permethrin arm (p < 0.05).

The number of live lice presented prior to the initial treatment was comparable in both treatment groups (21.76 range 10 to 68 lice and 21.29 range 10 to 60, p > 0.05, for Sodium Chloride Spray and Permethrin Crème Rinse, respectively). Immediately after treatment on Day 1, treatment failure subjects treated with Sodium Chloride Spray had 1.95 ± 1.28 lice as compared to 2.95 ± 2.98 in the Permethrin group (p > 0.05). At Day 8, the number of lice in subjects of the Sodium Chloride Spray treatment failure group was 1.40 ± 2.19 as compared to 2.95 ± 2.98 in the Permethrin group (p > 0.05).

Figure 1. The percentage of subjects lice free after the 1st application and 2nd application for individuals needing a second treatment as assessed at Day 1 and 8 for Sodium Chloride Spray treatment arm of the study compared to the Permethrin treatment arm (p < 0.05).

Figure 2. The percentage of subjects lice free at Day 15 (completion of the study) for Sodium Chloride Spray treatment arm compared to Permethrin treatment arm (p < 0.05).
At Day 15, the number of lice observed in the treatment failure subjects of the Sodium Chloride Spray group was $0.55 \pm 1.5$ compared to $5.45 \pm 7.91$ in the Permethrin group ($p < 0.01$). The summary of the efficacy results is presented in Table 2.

### 3.3. Extent of Exposure

Sixteen of the twenty-one subjects in the Sodium Chloride Spray arm received only the first application on day one (all were free of lice on day eight assessment) while another 6 subjects received a second application on day eight. One subject failed to return for the day eight assessment. This compares to six subjects receiving one application (subjects were free of lice on day eight assessment) and another 15 subjects receiving a second application of 1% Permethrin Crème Rinse on day eight. Again one subject failed to return for the day eight assessment.

### 3.4. Adverse Events

As presented in Table 3, the secondary characteristics such as pruritus, erythema, secondary infection, and excoriation of the lice infestation from Sodium Chloride Spray treated group were comparable to the control group. However, the improvement in pruritus in subjects treated with Sodium Chloride was consistently greater compared to Permethrin Crème Rinse (day one $1.14 + 1.1$ versus $0.71 + 0.49$; day 15 $0.29 + 0.47$ versus $0.43 + 0.53$ for Sodium Chloride and Permethrin, respectively) however, these values were not statistically significantly different due to the number of subjects included in the study. One subject (white, Hispanic, 11 year old female with extra-long, fine, straight hair), with Sodium Chloride spray, reported a headache on Day 4 of the study, for which she self-administered one dose of ibuprofen (Motrin®). This adverse event was the only one reported in the study and she was a treatment failure.

### 3.5. Safety Assessment

No abnormal or significant differences in vital signs were measured for subjects in this study between the treatment groups. During the study, no serious adverse events were observed. Future studies are needed to fully assess adverse events as the number of subjects in the study was low.

### 4. Discussion

Traditional approaches to treat head lice infestations include using natural oils, nit combing and hair removal have resulted in low effectiveness or are undesirable [19]. Using OTC pediculicidal chemicals remains the most popular method for treating head lice infestations. Recent studies have shown a dramatic reduction in insecticidal activity because of increased resistance to the current insecticides [14,19]. With current pediculicides, it has been shown that resistance to popular “old” OTC products by head lice is genetically regulated [12,20], suggesting that the use of these agents may become impractical, and more effective products should be considered to eliminate the troublesome parasites.

<table>
<thead>
<tr>
<th>Table 2. Efficacy results observed for subjects in each treatment group at each treatment day.</th>
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<tr>
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<tr>
<td>Pre-treatment Lice—mean</td>
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<tr>
<td>Pre-treatment Lice—range</td>
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<tr>
<td>Day 1 Post treatment Lice—mean</td>
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<tr>
<td>Day 1 Post treatment Lice—standard deviation</td>
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<tr>
<td>Day 1 Post treatment Lice—95% confidence interval</td>
</tr>
<tr>
<td>Number subjects with NO Lice post-treatment</td>
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<tr>
<td>Day 8 Pre Lice—mean</td>
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<tr>
<td>Number subjects with NO Lice at Day 8 pre-treatment</td>
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<tr>
<td>Number subjects no show at Day 8</td>
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<tr>
<td>Number subjects retreated</td>
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<tr>
<td>Day 8 Post treatment Lice—mean</td>
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<td>Day 8 Post treatment Lice—standard deviation</td>
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<td>Day 8 Post treatment Lice—95% confidence interval</td>
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<td>Day 15 Lice—mean</td>
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<td>Day 15 Post treatment Lice—standard deviation</td>
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<td>Day 15 Post treatment Lice—95% confidence interval</td>
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Table 3. Subject evaluation values for pruritus, erythema, secondary infection, excoriation, and eyes during each treatment period of the study.

<table>
<thead>
<tr>
<th>Visit-1 Prea</th>
<th>Visit-1 post treatment</th>
<th>Visit-2 Preb</th>
<th>Visit-2 post treatment</th>
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<td>Sodium chloride (n = 21)</td>
<td>Permethrin (n = 21)</td>
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<tr>
<td>std dev</td>
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</table>

a: Day 1 pretreatment assessment; b: Day 1 post treatment; c: Day 8 pretreatment; d: Day 8 post treatment; e: Day 8 and 15 post treatment combined assessment for subjects completing study.

The effective treatment rate in this study of 1% Sodium Chloride Spray was superior to 1% Permethrin Crème Rinse immediately after the first application. This is a very important factor because parents, children, and infested individuals experience anxiety and stress upon observing live lice crawling on their scalp after treatment, which may lead to unexpected psychological trauma [1, 21]. The one hour treatment time of Sodium Chloride spray is a feasible length of time for an absence-of-lice condition to occur due to the highly effective results observed after one application. The study personnel commented regularly on the ease of application and use of the Sodium Chloride spray product over the 1% Permethrin Crème rise product though this was not evaluated during the study. The second application of Sodium Chloride spray a week later also provides an identical response leading to superior results in comparison to the reference product. The lower rate of retreatment in the Sodium Chloride Spray group may also be an indication that may be the result of the ovicidal capacity of the product clinically. The in vivo ovicidal efficacy of Sodium Chloride Spray has yet to be determined, however, in vitro data have shown that ovicidal activity of gelled 10% Sodium Chloride formulation is greater than that of Permethrin and other tested chemicals [22]. The superior efficacy of Sodium Chloride Spray to 1% Permethrin Crème Rinse is important because Permethrin Crème Rinse is to be applied to towel dried hair requiring additional effort and time on the part of the users, whereas 1% Sodium Chloride spray can be directly applied to hair without any preparation providing added convenience and less effort in treatment. One can question the possible interference of moisture on subjects’ hair in this study that may cause the reduction of Permethrin activity but in another Permethrin study [23], the product was applied to damp hair with 97% effectiveness, assessed two weeks post treatment, suggesting that the current poor activity of permethrin is most likely because of another reason [12,15,24,25].

One of the suggested alternatives for the treatment of therapy-resistant head lice is the use of prescription products [19]. In addition to a greater level of toxicity, the economic burden of prescription products, the waiting time and cost for physician visits add to the inconvenience of this treatment option making a simple and inexpensive OTC product a more feasible treatment option. Another alternative OTC product on the market recently evaluated for efficacy has its active ingredient dimethicone [26-29]. The reported mechanism of action of dimethicone on lice [27] is consistent with observations performed here. The mechanism of action of Sodium Chloro-
ride on lice is not fully known but similar rupture of the gastrointestinal tract in lice is observed with its application. Desiccation is suggested as the mode of action of Sodium Chloride which explains the one hour exposure to lice for its effects to occur. The ingredients in Sodium Chloride spray include Water, 1% Sodium Chloride and Poloxamer 188 (a surface active wetting agent to provide more efficient water and salt contact with the louse) as principle components.

The concentration of Sodium Chloride must be hypertonic to be effective against lice. Therefore, dilution of the active ingredient to hypotonic concentrations nullifies Sodium Chloride’s activity against lice. In addition, Sodium Chloride must remain in contact with the louse in liquid form for an extensive period of time to be effective. The Poloxamer 188 is included in the product to facilitate Sodium Chlorides extended residence on the louse.

The present study demonstrates that 1% Sodium Chloride Spray is a simple application, and supplies an excellent medical alternative to the current recommended treatment for head lice to 1% Permethrin Crème Rinse. With the increase in the resistance of head lice to Permethrin developing across the country, 1% Sodium Chloride Spray is a great option for the treatment of head lice. The application of either Sodium Chloride Spray or Permethrin provides no safety concerns in the population treated. The lack of using a metal nit comb during the study was designed to provide a direct assessment of the two products. The study also provides an indirect assessment of ovicidal activity due to the day eight and fifteen post-treatment head lice evaluations which showed minimal reinfection that would occur if nits survived the initial treatment. Use of a metal nit comb in therapy is likely to provide greater efficacy during therapy, but the extent of the improvement will need to be evaluated in the future.

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Evaluation of the Efficacy and Safety of 1% Sodium Chloride (LiceFreee Spray) against 1% Permethrin Crème Rinse on Head Lice Infested Individuals


Efficacy of the LouseBuster, a New Medical Device for Treating Head Lice (Anoplura: Pediculidae)

SARAH E. BUSH,1,2 ALEX N. ROCK,3 SHERRI L. JONES,3,4 JAEL R. MALENKE,1,3 AND DALE H. CLAYTON1


ABSTRACT Human head lice (Pediculus humanus capitis De Geer) occur worldwide and infest millions of children and adults every year. Head lice infestations, which are known as pediculosis capitis, are psychologically stressful, physically irritating, and are one of the leading causes of K-6 school absence. The prevalence of head lice in many countries is increasing rapidly because of resistance to chemicals used in many head lice treatments. We tested the efficacy of an alternative method for controlling head lice, the LouseBuster, a custom-built medical device designed to kill head lice and their eggs using controlled, heated air. A total of 56 infested subjects was treated with the LouseBuster, and the efficacy of the treatment was evaluated by comparing the viability of lice and eggs on randomly assigned pre- and posttreatment sides of each subject's scalp. We evaluate treatment efficacy in the hands of novice versus experienced operators. We also evaluate treatment efficacy on different hair types and at different ambient humidities. Overall mortality of lice and eggs was 94.8% after treatment by experienced operators. Novice operators also achieved good results after a short training session; their results did not differ significantly from those of experienced operators. No adverse events were associated with the LouseBuster treatment. The LouseBuster is efficacious for killing head lice and their eggs. The use of heated air is appealing because it is a fast, safe, nonchemical treatment. Head lice are also unlikely to evolve resistance to desiccation, which is the apparent mode of action.

KEY WORDS Pediculus humanus capitis, pediculicide, nonchemical treatment, heated air

Head lice (Pediculus humanus capitis De Geer) are a major problem for children and their parents throughout the world (Roberts 2002, Burgess 2004, Frankowski and Bocchini 2010). Symptoms of head lice infestations (pediculosis capitis) include itching, psychological stress, and the possibility of secondary bacterial infections (Meinking 1999). Millions of cases occur annually (Frankowski and Bocchini 2010), and it has been estimated that children in the United States miss 12–24 million days of school per year because of head lice (Roberts 2002). Unfortunately, the problem is increasingly difficult to treat because head lice have evolved resistance to some of the most common pediculicides in several regions of the world (Pollack et al. 1999, Burkhart and Burkhart 2000, Lee et al. 2003, Kwon et al. 2008). Moreover, some pediculicidal products have side effects, ranging from mild allergic reactions to severe seizures (Frankowski and Bocchini 2010), and many parents prefer not to treat their children with chemicals, even if they are safe (Burkhart 2004).

One strategy for dealing with resistant populations of head lice is to use chemicals to which head lice have not yet acquired resistance, such as oral ivermectin (Chosidow et al. 2010). Another strategy is to use a nonchemical approach; one possibility is heated air. Heated air has been shown to kill other medically important arthropods, such as ticks (Carroll 2003). In the case of lice, Kobayashi et al. (1995) reported that body lice (P. h. corporis) can be killed in vitro with air from a blow dryer at 50°C for 5 min, and that body louse eggs fail to hatch in vitro after exposure to hot air at 55°C for 90 s. More recently, Goates et al. (2006) evaluated several approaches for killing head lice and their eggs with large volumes of heated air. The most promising method was a custom-built, heated-air blower with a comb-like hand piece. The prototype device, dubbed the LouseBuster, was tested by experienced operators on a limited number of subjects in an arid climate (Utah, where it was developed). The prototype showed promise, but the comb-like hand...
piece was difficult to use on subjects with curly hair. Furthermore, it was not clear whether the machine would work in more humid geographic regions, nor whether novice operators could use the machine successfully. In this study, we report the efficacy of a modified LouseBuster with a diffuser-like applicator that can be used on subjects with any hair type. The device was tested on a sample of 56 subjects treated by experienced or novice operators in humid and arid regions of the country.

Materials and Methods

Recruitment. The study was conducted at three clinical sites: Larada Sciences, Salt Lake City, UT; Lice Solutions Resource Network, West Palm Beach, FL; and Lice Solutions Resource Network, Nashville, TN. Personnel at the three clinical sites recruited subjects from the community by means of outreach activities; 56 subjects were included in the study (Fig. 1). Subjects met inclusion criteria if at least one live, moving louse was detected on the scalp, as described below. We excluded children younger than 4 yr of age because the treatment takes 30 min, which is longer than young children can realistically remain seated (the device is not indicated for use on children under the age of 4). Subjects signed written consent forms approved by our Institutional Review Board after being fully briefed. Minors were given age-appropriate consent forms, and their consent was recorded in addition to the written informed consent provided by their parents or guardians.

LouseBuster Device. The LouseBuster device (U.S. patent no. 7,789,902) has received clearance from the Food and Drug Administration under 510(k) premarket notifications. A production quality LouseBuster (LB-3120; Fig. 2) is currently available on the market (Larada Sciences, Salt Lake City, UT; www.lousebuster.com). The LouseBuster prototype (Goates et al. 2006) required a 20 amp circuit; the modified device tested in this study operates on a standard 15 amp circuit (120V/15A; 240V/10A).

Study Design

Experienced Operators. Subjects were asked to arrive at treatment facilities with clean, dry, untangled hair. They were fitted with a disposable barber-style smock and seated in a chair over a white drop cloth (7 m²). One of five experienced operators recorded each subject’s hair type according to the following parameters: length, short (hair above chin line) versus long (hair extending below chin line); thickness, thick (hair ≥3 cm diameter in ponytail, or similar thickness if short) versus thin (<3 cm diameter in ponytail, or similar thickness if short); curliness, curly (=wavy or curly hair) versus straight.

To measure efficacy of the LouseBuster device, we used a paired sampling design in which each subject’s

![Fig. 2. The LouseBuster is a custom-built medical device designed to kill all life stages of head lice by delivering precisely controlled, heated air (59 ± 1.5°C) at an airflow two to three times greater than that of a hand-held blow dryer. A removable hose directs the air through a disposable, diffuser-like applicator that distributes air to the scalp and bases of hair shafts, where lice and their eggs congregate. During treatment the applicator is held in overlapping positions on the scalp (30 s per position). A specific pattern is followed to ensure that all areas of the scalp receive adequate coverage. The entire scalp can be treated in ~30 min, independent of hair length.](image-url)
scalp was randomly divided into two halves, as follows: a pretreatment side and a posttreatment side (Fig. 1). This study design is more powerful than a standard randomized controlled trial because each subject serves as his or her own control. The design minimized any influence of genetic or environmental variation in the subjects or their lice, including any effects introduced by previous head lice treatments. For each subject, the operator randomly selected a pretreatment side of the subject’s scalp and combed it using 20 careful swipes of a LiceMeister comb (National Pediculosis Association, Needham, MA) (Goates et al. 2006). If one or more live, moving lice were detected while combing the pretreatment side, the subject was considered to have an active infestation and was included in the study (Roberts 2002, Frankowski and Bocchini 2010). All lice and eggs removed during combing of the pretreatment side were placed in a petri dish that was kept in an incubator set at 29–32°C and >50% RH.

After combing the pretreatment side of the scalp, the operator treated the subject’s entire scalp with the LouseBuster, which required 30 min. Immediately after the treatment, the operator combed the posttreatment side of the scalp, again using 20 careful swipes of the LiceMeister comb. Lice and eggs from the posttreatment side of the scalp were placed in a separate petri dish in the same incubator. The subject’s smock and the drop cloth were carefully examined to recover any lice blown off the scalp during treatment. These lice were placed in a third petri dish in the same incubator. A new smock was used for each subject, and the drop cloth was cleaned thoroughly between subjects.

Within 3 h of treatment, the numbers of live versus dead lice in the pre- and posttreatment samples were tallied under a dissecting microscope, as described in Goates et al. (2006). Lice that showed any movement were considered live. Lice that showed no movement, even after being nudged with a forceps or dissecting needle, were considered dead. Goates et al. (2006) scored lice using these same criteria, then monitored dead individuals for up to 18 h to test for a “resurrection effect,” in which “dead” lice are not really dead (Burkhart 2004). Goates et al. (2006) found no cases of resurrection. The lice were scored as blindly as possible, although it was often obvious which ones had been treated; lice from the posttreatment side of the scalp often differed in appearance from lice on the control side. Lice from the posttreatment side were often shriveled and darker than lice from the pretreatment side, which tended to be plump and lighter in color.

Eggs were kept in the incubator for a minimum of 14 d to allow time for hatching (head lice hatch in 8.5–12 d; Lebwohl et al. 2007). After incubation, the hatching success of eggs was scored under a dissecting scope by one of two researchers, both of whom were blind to treatment. The percentage of eggs that hatched (%Hatch) was calculated as follows: N/(V+N), where N was the number of fully or partially emerged nymphs and V was the number of potentially viable eggs (those with intact opercula). Egg mortality was calculated as 100% − %Hatch. Efficacy was determined by comparing the percentage of mortality of lice and eggs from the pre- and posttreatment samples of lice.

Before treatment, subjects were instructed to give a “thumbs-down” signal to indicate any discomfort from the heated air (Goates et al. 2006). If this occurred, we immediately removed the applicator from the hair for a few seconds to allow the scalp to cool, then resumed treatment. Although subjects were informed to let us know whether they wanted to discontinue treatment because of any discomfort, none of the subjects found this to be necessary.

**Novice Operators.** We also tested the efficacy of the LouseBuster in the hands of novice operators. Potential novice operators were screened to verify that they were at least 18 yr of age and physically capable of delivering a treatment. They were excluded if they had previous experience with a LouseBuster device, any muscular or joint pain, carpal tunnel syndrome, upper extremity neurological deficits, or if they were unable to stand comfortably for at least 1 h. Novice operators represented a variety of professional backgrounds. All operators signed Institutional Review Board-approved informed consent forms.

The training of novice operators was multifaceted. Novice operators received the LouseBuster Operator’s Manual, viewed a multimedia presentation, and received interactive hands-on training in which they were given an opportunity to ask questions and perform practice treatments on mannequins. Training was typically completed in less than 2 h. After training, each novice operator took a short written and practical exam. An experienced operator reviewed and discussed incorrect answers and techniques with each novice operator. Each novice operator then administered a LouseBuster treatment to one subject with head lice. Treatments by novice operators took place 1–36 d (mean = 8.4 d) after they were trained.

Inclusion criteria for subjects treated by novice operators were the same as those treated by experienced operators. The treatment procedure was also the same. One of two researchers at each site, who were experienced operators, observed each novice operator and subject during the treatment, while completing a check sheet with questions regarding the quality of the novice operator’s technique. Researchers did not provide advice or answer questions during the treatment.

**Statistical Analyses.** We determined the efficacy of the LouseBuster by comparing data from pre- and posttreatment sides of the scalp using Wilcoxon signed-rank tests for matched pairs. To compare the efficacy of novice operators with experienced operators, we used Wilcoxon rank sum tests. We also used Wilcoxon rank sum tests to compare the efficacy of the LouseBuster between different hair types and ambient humidities. Values of P < 0.05 were considered to indicate statistical significance. Statistical analyses were performed using the JMP v.7.0 statistical package.
As before, the LouseBuster killed the majority of hatched lice (Fig. 3A, Experienced Operator). Posttreatment mortality was 98.2% (±2.2%), compared with 2.5% (±0.9%) on the pretreatment side of the scalp (Wilcoxon matched pairs $Z = 410.0, P < 0.0001, n = 40$). After treatment, about one-half of the lice (54.2 ± 4.6%) were still on the scalp; the rest had been removed, that is, blown onto the smock or drop cloth. Most of these lice (85.1 ± 4.4%) were dead. The rest were considered dead because head lice cannot survive off the host (Takano-Lee et al. 2003, Burgess 2004), particularly after exposure to heated air (Buxton 1946, Kobayashi et al. 1995).

The LouseBuster killed virtually all eggs (Fig. 3B, Experienced Operator). Egg mortality on the posttreatment side of the scalp was 99.2% (±0.3%), compared with 55.3% (±5.1%) on the pretreatment side (Wilcoxon matched pairs $Z = 279.5, P < 0.0001, n = 33$). Pretreatment (control) egg-hatching rates were consistent with those in other studies (Buxton 1946, Burgess et al. 1994, Goates et al. 2006). No eggs were blown off during treatment; they remained glued to the hair shafts.

Posttreatment mortality of lice and eggs combined was 94.8% (±1.0), compared with 42.6% (±4.7) pretreatment mortality ($n = 33$).

Novice Operators. Novice operators treated 15 subjects (Table 1; Fig. 1). Two subjects were excluded from the analysis of eggs because they did not have viable egg infestations (no pretreatment eggs hatched in the incubator). Consequently, the analysis of lice included data from 13 subjects, and the analysis of eggs included data from 13 subjects (Fig. 1). All of the novice operators used correct technique when treating subjects, according to evaluations by the experienced operators who observed them closely during the treatment procedure.

As before, the LouseBuster killed the majority of hatched lice (Fig. 3A, Novice Operator). Posttreatment mortality was 87.7% (±3.4%), compared with 2.6% (±1.6%) on the pretreatment side of the scalp (Wilcoxon matched pairs $Z = 60.0, P < 0.0001, n = 15$). The LouseBuster also killed the majority of eggs (Fig. 3B, Novice Operator). Egg mortality on the posttreatment side of the scalp was 97.4% (±0.9%) compared with 55.3% (±7.1%) on the pretreatment side (Wilcoxon matched pairs $Z = 45.5, P = 0.0002, n = 13$).

Posttreatment mortality of lice and eggs combined was 93.6% (±1.2), compared with 36.5% (±6.1) pretreatment mortality ($n = 13$). The efficacy of novice operators did not differ significantly from that of experienced operators (Wilcoxon rank sum $Z = -1.31, P = 0.19$; one-tailed power = 0.83 with effect size $d = 0.86$, capable of detecting ≥5% poorer efficacy by novices compared with experienced operators).

Hair Type. Efficacy was independent of hair type. Posttreatment mortality of lice and eggs did not differ significantly between subjects with long versus short hair, curly versus straight hair, or thick versus thin hair (all comparisons: Wilcoxon rank sum $P = 0.37$; two-tailed power ≥0.80 with effect size $d = 0.86$, capable of detecting a difference ≥5% between categories).

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### Table 1. Baseline characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Experienced Operator study</th>
<th>Novice Operator study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>Age: mean yr (range)</td>
<td>12.9 (6-60)</td>
<td>14.9 (4-43)</td>
</tr>
<tr>
<td>Sex: N (%)</td>
<td>24 (62%)</td>
<td>8 (53%)</td>
</tr>
<tr>
<td>Hair density: N (%)</td>
<td>24 (62%)</td>
<td>8 (53%)</td>
</tr>
<tr>
<td>Hair curliness: N (%)</td>
<td>15 (38%)</td>
<td>7 (47%)</td>
</tr>
<tr>
<td>Hair length: N (%)</td>
<td>16 (40%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Lice per subject: mean ± SE</td>
<td>53.4 ± 14.1 (40)</td>
<td>167.4 ± 65.4 (15)</td>
</tr>
<tr>
<td>Viable eggs per subject: mean ± SE (no. of subjects)</td>
<td>93.0 ± 24.1 (33)</td>
<td>93.1 ± 19.3 (13)</td>
</tr>
</tbody>
</table>

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(SAS Institute, Cary, NC). If $P = 0.05$, we determined statistical power using G*Power v.3 (Faul et al. 2007).

### Results

**Experienced Operators.** Experienced operators treated 41 subjects who met the inclusion criteria of having at least one live, moving louse on the pretreatment side of the scalp (Fig. 1). Baseline characteristics of these study subjects were recorded (Table 1). A single subject was excluded from the analysis of lice because no lice were recovered from the posttreatment side of the scalp, making it impossible to compare pre- and posttreatment mortality. Eight subjects were excluded from the analysis of eggs because, in seven cases, subjects did not have viable egg infestations (no pretreatment eggs from these subjects hatched in the incubator); no eggs were recovered from the posttreatment side of the eighth subject’s scalp. The analysis of hatched lice thus included data from 40 subjects, whereas the analysis of eggs included data from 33 of these subjects (Fig. 1).

The LouseBuster killed the majority of hatched lice (Fig. 3A, Experienced Operator). Posttreatment mortality was 88.2% (±2.2%), compared with 2.5% (±0.9%) on the pretreatment side of the scalp (Wilcoxon matched pairs $Z = 410.0, P < 0.0001, n = 40$).

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![Fig. 3. Efficacy of the LouseBuster device for treating head lice and their eggs.](image-url)

**A** Mortality of lice on the post-treatment side of the scalp (■) was much greater than that on the pretreatment side (□), both for experienced and novice operators. **B** Mortality of eggs on the posttreatment side of the scalp (□) was also greater than on the pretreatment side (■), both for experienced and novice operators.
Ambient Humidity. Efficacy was high in both humid and arid regions of the country. Posttreatment mortality of lice and eggs was 93.7% (±1.0%) for the 19 subjects treated in Florida and Tennessee (66–75% mean annual relative humidity) (NOAA 2002). It was 95.0% (±1.2%) for the 27 subjects treated in Utah (46–55% mean annual relative humidity) (NOAA 2002). There was a significant difference in efficacy between humid and arid regions; however, the difference was only 1.3% (Wilcoxon rank sum Z = -1.98, P = 0.048).

Adverse Events. No adverse events attributed to the LouseBuster were noted by experienced or novice operators or their subjects. Similarly, no adverse events were reported by any of several hundred subjects treated with earlier LouseBuster prototypes (Goates et al. 2006) (our unpublished data).

Discussion

The results of this study show that the LouseBuster device kills the majority of head lice and their eggs. After a single 30-min treatment by experienced operators, the combined mortality of lice and eggs was 94.8% (±1.0%). The high posttreatment mortality of eggs in our study is particularly noteworthy because eggs are impervious to most other methods of treatment, which typically require a second application or second dose once the eggs hatch (Burgess et al. 2007, Lebwohl et al. 2007, Chosidow et al. 2010). The timing of the second application is crucial; if the narrow window between hatching and reproductive maturity is missed, the cycle repeats itself and more than two treatments are needed over the course of several weeks (Lebwohl et al. 2007, Frankowski and Bocchini 2010). The LouseBuster kills most lice and virtually all eggs in a single 30-min treatment.

We did not do a follow-up study of cure rate because it is difficult, if not impossible, to account for cases of reinestation (Heukelbach et al. 2008). Goates et al. (2006) performed a small-scale follow-up study (n = 11 subjects) in which they reported elimination of 100% of viable head louse infestations 1 wk after treatment with the LouseBuster prototype. Given that the device does not kill 100% of hatched lice, the results of their study suggest there may be a delayed effect on the small number of lice or eggs not killed outright by the device.

The LouseBuster appears to work by desiccating lice and their eggs. Lice are particularly susceptible to desiccation because their small size and flattened shape give them a high surface area to volume ratio (Moyer et al. 2002, Goates et al. 2006). Buxton (1946) reported that dry, heated air also reduces the amount of anoxic fluid in louse eggs, making it more difficult for them to hatch, which may explain why heated air has such a devastating effect on eggs when applied correctly. It is unlikely that head lice can evolve resistance to desiccation because water is such a fundamental component of their physiology, and one that is already a limiting factor in the survival of small insects (Rudolph 1983, Moyer et al. 2002, Renault and Coray 2004). The evolution of resistance to many pediculicides, by comparison, is relatively simple (Kwon et al. 2008).

No adverse events attributed to the LouseBuster took place in this study. The efficacy of the LouseBuster is also independent of hair length, thickness, and curliness. The device is easy to use with any hair type because the applicator is not moved through the hair like a comb, but is held in place on each section of the scalp being treated. The results of this study also demonstrate high efficacy of the LouseBuster in both humid and arid regions of the country. Moreover, the device performed well in the hands of novice operators, whose results did not differ significantly from those of experienced operators.

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Study protocols were reviewed and approved by the University of Utah Institutional Review Board (Salt Lake City, UT) or by Sterling Institutional Review Board (Atlanta, GA). Trials were registered at ClinicalTrials.gov (NCT00731718, NCT00732134, NCT00732264).

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http://pediatrics.aappublications.org/content/118/5/1962.full.html
An Effective Nonchemical Treatment for Head Lice: A Lot of Hot Air

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Financial Disclosure: Dr Clayton, Mr Atkin, and Mr Wilding have US and international patents pending for methods of using heat to treat head lice.

ABSTRACT

OBJECTIVES. Head lice (Pediculus humanus capitis) are a major irritant to children and their parents around the world. Each year millions of children are infested with head lice, a condition known as pediculosis, which is responsible for tens of millions of lost school days. Head lice have evolved resistance to many of the currently used pediculicides; therefore, an effective new treatment for head lice is needed. In this study we examined the effectiveness of several methods that use hot air to kill head lice and their eggs.

METHODS. We tested 6 different treatment methods on a total of 169 infested individuals. Each method delivers hot air to the scalp in a different way. We evaluated how well these methods kill lice and their eggs in situ. We also performed follow-up inspections to evaluate whether the sixth, most successful, method can cure head louse infestations.

RESULTS. All 6 methods resulted in high egg mortality (≥88%), but they showed more-variable success in killing hatched lice. The most successful method, which used a custom-built machine called the LouseBuster, resulted in nearly 100% mortality of eggs and 80% mortality of hatched lice. The LouseBuster was effective in killing lice and their eggs when operated at a comfortable temperature, slightly cooler than a standard blow-dryer. Virtually all subjects were cured of head lice when examined 1 week after treatment with the LouseBuster. There were no adverse effects of treatment.

CONCLUSIONS. Our findings demonstrate that one 30-minute application of hot air has the potential to eradicate head lice infestations. In summary, hot air is an effective, safe treatment and one to which lice are unlikely to evolve resistance.

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Key Words
Pediculus humanus capitis, control, heat, desiccation, pediculosis, LouseBuster, nits

Abbreviation
CI—confidence interval

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275). Copyright © 2006 by the American Academy of Pediatrics
HEAD LICE (Pediculus humanus capitis) have been a ubiquitous problem throughout recorded human history. They are a major irritant to children and their parents around the world. Millions of cases of head lice (pediculosis) occur annually, including 6 to 12 million cases per year in the United States alone. It is estimated that children in the United States missed 12 to 24 million days of school in 1998 because of head lice. The number of cases of head lice is increasing, because lice are evolving resistance to pediculicides. Although head lice do not produce an illness per se, they are physically and psychologically unpleasant for the child and an exasperating problem for parents and school authorities.

The 3 general approaches currently in use for treating head lice infestations are chemical shampoos, specialized louse combs, and “home remedies.” Each approach has significant limitations. Chemical shampoos such as those containing pyrethroids or lindane are the most popular methods of treatment in the United States, with sales exceeding $160 million per year. However, in addition to the evolution of a resistance problem, chemical shampoos are not very effective at killing louse eggs. An additional treatment with shampoo is necessary 1 week after the first treatment to kill lice from newly hatched eggs. Moreover, many parents prefer not to treat their children with chemicals for fear of the adverse effects that have been reported for some of these insecticides, particularly lindane. None of the available pediculicides are considered safe for people who have asthma, a common childhood disease.

Another common method of treatment is the use of a louse comb. There are many varieties, usually involving thin metal or plastic tines that are designed to comb through the hair and pull out lice and their eggs. However, effective combing requires many hours over several days, and most parents do not have the time or patience to comb out all the lice and eggs.

The third group of treatments is home remedies, which parents often feel forced to use because shampoos fail to work and they do not take the time to use a louse comb effectively. Parents use an assortment of “treatments” ranging from bug spray to mayonnaise to kerosene. These remedies can harm the child, and there is little hard evidence to indicate that they are effective. In short, an effective new approach for treating head lice is sorely needed.

An ideal new treatment would be quick, safe, and effective at killing both lice and eggs. It should also be something to which lice cannot easily evolve resistance. Hot air is a promising solution that meets all of these criteria. Nearly 60 years ago, Buxton pointed out that body lice, Pediculus humanus corporis, which are closely related to head lice, die when exposed to 51°C air for 5 minutes. More recently, Kobayashi et al reported that body lice can be killed in vitro with air from a blow-dryer at 50°C for 5 minutes, and that body louse eggs fail to hatch in vitro after exposure to hot air at 55°C for 90 seconds. Hot air probably kills the lice and eggs by desiccating them. The high surface/volume ratio of small arthropods, such as lice, makes them vulnerable to control by desiccation, as has also been shown for lice on birds.

Although heat has the potential to kill both head lice and their eggs, we know of no studies that have tested the effectiveness of hot air on individuals infested with head lice. We experimented with this approach as a treatment for lice infestations on local schoolchildren using several forms of heat delivery. In this article we demonstrate that exposure to a large volume of hot air can result in 98% mortality of eggs and 80% mortality of hatched lice. We further show that this method is sufficient to eliminate viable head louse infestations from virtually all subjects, as determined by follow-up examinations 1 week after treatment. We suggest that heat is a preferred method for treating head lice because it is effective, safe, and requires only a single 30-minute treatment. Furthermore, it is unlikely that lice will evolve resistance to heat, because this would require fundamental changes in their water physiology.

METHODS

Study Design

Effect of Hot Air on Lice and Eggs

We tested 6 methods for delivering hot air to the scalps of infested individuals. With full University of Utah institutional review board approval, we solicited infested subjects from local elementary schools to enroll in our study. Before enrollment, the parents or guardians of these subjects were interviewed by telephone and asked nonleading questions about treatment history. To avoid residual effects from other treatments, we excluded subjects who had used pediculicidal shampoos or home remedies within the previous 2 weeks. We also excluded children younger than 6 years of age, because we felt it would be difficult to get them to sit still long enough for us to collect the necessary data. Note, however, that there is no reason why hot air cannot be used to treat head lice in children of any age. Parents and siblings of enrolled subjects were invited to participate in the study if they also had head lice. Treatment trials, which required ~1 hour, were conducted in the homes of infested subjects, providing a more secure and anonymous environment.

Informed consent procedures were followed, and all subjects regardless of age were asked to review and sign consent forms in English or Spanish (as appropriate). Forms tailored to children were easy to understand, and their parents reviewed and signed more-detailed consent forms. Each participant received a small honorarium (usually $10), free educational materials, a free Lice-
Meister comb (National Pediculosis Association, Needham, MA), and a free bottle of Nix shampoo (Insight Pharmaceuticals, Blue Bell, PA). They received detailed instructions on how to use these products effectively to eliminate lice not killed by our experimental treatment. Subjects and their parents were not informed of the honorarium or free materials they would receive until after completion of the treatment trial.

At the start of each trial, we carefully combed the subject’s hair with a LiceMeister comb (National Pediculosis Association, Needham, MA) until we confirmed the infestation by detecting 1 or more living, moving lice.20 Next, we thoroughly combed one side of the scalp (chosen at random) while removing all lice and eggs encountered and placing them in a portable incubator at 33°C. We continued combing, keeping track of time, until no additional lice or eggs were removed from that side of the scalp. Next we treated the subject’s entire scalp with 1 of 6 methods (see below). After treatment, we combed the other side of the scalp for the same amount of time as the first side, again placing all lice and eggs in the portable incubator. Hence, each subject served as his or her own control. Although the time spent combing each side of the scalp was equal, we did not necessarily get the same number of lice or eggs from the 2 sides. There was no consistent removal pattern; sometimes we got more lice and/or eggs from the first side of the scalp, other times we got more from the second side (see “Results”). Participants in our study had infestations varying in size from a few lice to hundreds of lice.

Lice and eggs collected from each side of the scalp were brought back to the laboratory within 3 hours of removal. The number of live versus dead lice from each side of the scalp was scored by carefully examining them under a dissecting microscope. Dead lice were reexamined for periods of up to 18 hours to check for the resuscitation effect, in which lice “killed” with pediculicides are not really dead.6 This was never a problem; all of our dead lice remained that way. Eggs were placed in a custom, stainless-steel lined Percival incubator set at 33°C and 75% relative humidity, and their hatching was monitored daily for 2 weeks.7 Effectiveness of the different treatment methods was assessed by comparing the percentage of dead lice and nonhatching eggs on the pretreatment and posttreatment sides of the scalp.

Before the start of treatment, each subject was instructed to give a “thumbs-down” sign to indicate any discomfort from the hot air. When this occurred, we immediately reduced the volume of air as described below for each method. Subjects were allowed to ask that we stop the treatment at any time. In summary, we took a conservative approach to comfort (see “Results”). We interviewed study participants and their parents at varying intervals after treatment (up to several months later) and no short- or long-term adverse effects of treatment were ever noted.

Follow-up Examinations
We used the most effective method, the LouseBuster with hand piece (see below), to test whether hot air can completely cure head louse infestations. Subjects with a high probability of reinfection, such as those with other infested family members or classmates, were excluded from these follow-up trials. We did follow-up examinations on 11 subjects with infestations ranging from several lice to >100 lice. The protocol was to (1) verify the infestation by directly observing living, moving lice in the scalp, (2) treat the entire scalp, and (3) return 1 week after treatment to reexamine the subject for head lice. Subjects and their parents were instructed not to use any head lice treatments for 1 week after our treatment. As an incentive, they were offered double the normal honorarium for participating in the follow-up examination on the condition that they refrained from using any form of treatment for that 1 week.

The follow-up examination was conducted by sampling the scalp with 20 careful swipes of the LiceMeister comb. The 20-swipe criterion was determined by using a repeat-sampling approach22 on an independent group of 21 subjects reported to have head lice. For 10 subjects with moderate infestations (≥6 lice), a mean of 2 swipes (range: 1–4) was required to detect the first live louse. For 6 subjects with light infestations (<6 lice), a mean of 14 swipes (range: 8–18) was required to detect the first live louse. The remaining 5 subjects did not, in fact, have active head louse infestations; no lice were found even after 250 swipes.

Treatment Methods
We tested the following 6 methods for heating the scalps of infested individuals.

Bonnet-Style Hair Dryer
We combined the airflow from 2 standard bonnet-style hair dryers (Belson, Miami, FL) by attaching the hose from each machine to a single plastic bonnet that enclosed the hair with an elastic band around its perimeter. One hose was attached to the bonnet near the crown of the head, and the other hose was attached near the nape of the neck. The entire scalp was treated simultaneously for a period of 30 minutes.

Handheld Blow-dryer: Diffuse Heating
The subject’s hair was divided into 10 sections, each consisting of a large tuft of hair held away from the scalp with a hair clip. Each section was heated with a standard handheld blow-dryer (Conair, Stamford, CT) by removing the clip and then gradually moving the nozzle of the dryer around the base of the section, where lice and eggs tend to congregate. Each section was heated for 3 min-
utes while moving the dryer to ensure uniform heating of the entire base of the section. Treating all 10 sections required ~35 minutes, including the time necessary to move between sections.

**Handheld Blow-dryer: Directed Heating**
The diffuse-heating method with the handheld blow-dryer was repeated with the following modifications: we divided the hair into 20 sections and treated each section for 60 seconds, holding the dryer in a stationary position for 30 seconds on one side of the section and then 30 seconds on the opposite side of the section. To heat all 20 sections in this manner required a total of 30 minutes, including the time necessary to move between sections.

**Wall-Mounted Dryer**
We used a detached wall-mounted blow-dryer (Excel Dryer Inc, East Longmeadow, MA) similar to those found in public restrooms for drying hands and hair. This device delivered far more than twice the volume of air as that delivered by the handheld blow-dryer (Table 1). We attached a 15-cm aluminum hose to the nozzle of the dryer. The dryer was placed on a table, and the hose was used to treat the hair in sections as described for the directed-heating method with a handheld blow-dryer.

**LouseBuster With Sections**
For this method we developed a custom-built, high-volume, hot-air blower called the LouseBuster (Fig 1; Dexterity Design, Salt Lake City, UT). The LouseBuster delivers hot air at a relatively constant temperature (modulated by an electronic feedback loop) and volume through a long flexible hose that can be aimed at the subject’s scalp. During trials we set it to a temperature slightly less than that of the handheld blow-dryer (Table 1). Like the wall-mounted dryer, the LouseBuster delivers more than twice the air volume of a handheld blow-dryer. We divided the hair into 14 to 20 sections depending on the amount of hair. We heated each section for 60 seconds, as described for the directed-heating method with a handheld blow-dryer. To heat all sections in this manner required ~30 minutes.

**LouseBuster With Hand Piece**
We again used the LouseBuster, together with a custom-designed hand piece, to facilitate exposure of the hair’s roots to hot air. The molded-plastic hand piece, which has coarse teeth, is pulled through the hair like a garden rake while hot air blows in the opposite direction (Fig 1). We slowly combed the entire scalp with the hand piece, ensuring that each region of the scalp was exposed to hot air for at least 30 seconds. This approach made sectioning of the hair unnecessary. The entire scalp required ~30 minutes to treat.

We monitored the temperature generated by each of the 6 methods. For the bonnet-style hair-dryer method, we measured temperature by clipping thermistors to the base of clumps of hair in 4 locations: top of the head, base of the scalp, and over each ear. We recorded the temperature from each thermistor every 5 minutes and then obtained the mean temperature for each of the 4 locations over the course of the trial. We used these means to calculate a grand mean temperature for the entire trial. For the handheld blow-dryer and wall-mounted dryer methods, we measured temperature by placing a thermistor near the scalp in the middle of the section of hair being treated. We calculated the temperature of the treatment for each subject by averaging the temperatures across the sections. For the LouseBuster methods, air temperature exiting the hose was recorded continuously, and the temperature data was automatically downloaded to a laptop computer. The LouseBuster produced more even heating than the other methods (Table 1) because of its feedback mechanism.

We also measured the air volume produced by each method (Table 1).

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**TABLE 1**

Demographic Characteristics and Treatment Data

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Median Age (Range), y</th>
<th>Gender, Female/Male</th>
<th>% Louse Mortality</th>
<th>% Egg Mortality</th>
<th>Mean (Range) Temperature, °C</th>
<th>Air Volume, cu ft/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonnet-style hair dryer</td>
<td>54</td>
<td>11 (6–33)</td>
<td>51/3</td>
<td>10.1</td>
<td>88.8</td>
<td>54.8 (41–63)</td>
<td>9c</td>
</tr>
<tr>
<td>Handheld blow-dryer: diffuse heat</td>
<td>26</td>
<td>9 (6–44)</td>
<td>24/2</td>
<td>20.8</td>
<td>96.7</td>
<td>60.8 (50–67)</td>
<td>41</td>
</tr>
<tr>
<td>Handheld blow-dryer: directed heat</td>
<td>27</td>
<td>9 (6–32)</td>
<td>25/2</td>
<td>55.3</td>
<td>97.9</td>
<td>58.5 (52–67)</td>
<td>41</td>
</tr>
<tr>
<td>Wall-mounted dryer</td>
<td>15</td>
<td>10 (6–13)</td>
<td>15/0</td>
<td>62.1</td>
<td>96.5</td>
<td>58.4 (53–65)</td>
<td>103</td>
</tr>
<tr>
<td>LouseBuster with sections</td>
<td>18</td>
<td>10 (9–23)</td>
<td>18/0</td>
<td>76.1</td>
<td>94.0</td>
<td>58.4 (56–60)</td>
<td>88</td>
</tr>
<tr>
<td>LouseBuster with hand piece</td>
<td>18</td>
<td>10 (6–33)</td>
<td>16/2</td>
<td>80.1</td>
<td>98.0</td>
<td>58.9 (58–59)</td>
<td>88</td>
</tr>
<tr>
<td>LouseBuster with hand piece: follow-up examinations</td>
<td>11</td>
<td>11 (6–61)</td>
<td>10/1</td>
<td>NAa</td>
<td>NAa</td>
<td>58.9 (58–59)</td>
<td>88</td>
</tr>
<tr>
<td>Total</td>
<td>169</td>
<td>10 (6–61)</td>
<td>159/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Percentage of lice dead within 3 hours of treatment.
b Percentage of incubated eggs still unhatched after 2 weeks.
c Decreases rapidly with distance from input hoses under bonnet.
d Not calculated (see "Methods").
Statistics

The effectiveness of each method was determined by comparing the percentage of viable lice and eggs removed from the pretreatment and posttreatment sides of the scalp. Absolute differences were tested for statistical significance by calculating the 95% confidence of the difference and taking nonoverlap with zero as an indication of significance. Data for lice and eggs were analyzed separately. Statistical power was calculated by using G*POWER: we were able to detect a "medium" effect size ($d = 0.50$) with a power of $0.83$ for all 6 of the methods tested. Indeed, in all cases except the first handheld blow-dryer method (Table 1), we had power of $0.97$.

RESULTS

Population Characteristics

Between September 2001 and February 2005 we received >300 calls from parents of children with head lice. Of these, 169 individuals met our inclusion criteria (see “Methods”) and were enrolled in the study. Demographic characteristics of the participants are shown in Table 1. The predominant gender was female (94.1%), and the median age was 10 years.

Treatment Groups

All 6 methods had an impact on lice and/or their eggs (Table 1; Fig 2). However, the percent killed varied considerably, particularly for hatched lice, as described below.

Bonnet-Style Hair Dryer

We treated 54 subjects using this method. Although a majority of subjects completed the treatment without incident, 13 (24%) indicated some discomfort during treatment. When this happened, we turned the dryer from “high” to “low” for 2 to 3 minutes, resulting in no further discomfort in 12 of 13 cases. In 1 case (2%), the subject asked to stop the treatment. From the remaining 53 subjects, a total of 108 control lice were combed out before treatment, 3 (2.8%) of which were dead. After treatment, 138 lice were combed out, 14 (10.1%) of which were dead. Despite this low mortality rate, the percent of dead treated lice was significantly higher than the percent of dead control lice, with an absolute difference of 7.3% (95% confidence interval [CI]: 1% to 13%).

In 13 of the 53 trials, no control eggs hatched, indicating that they were from old infestations; these 13 trials were excluded from the egg-hatch analysis. A total of 586 control eggs were combed out from the remaining 40 subjects before treatment, and 546 eggs were combed out after treatment. The control–egg-hatch rate was 30.4%, and the treated–egg-hatch rate was 11.2%, a significant absolute difference of 19.2% (95% CI: 15% to 24%). In 19 (47.5%) of the 40 subjects, none of the treated eggs hatched. In summary, this method killed very few hatched lice but a larger proportion of eggs (Fig 2).

Handheld Blow-dryer: Diffuse Heating

We treated 26 subjects using this method, of which 13 (50%) indicated some discomfort. When this happened, we pulled the blow-dryer away for a few seconds, and in 12 of 13 cases the subject had no further discomfort. One subject (4%) asked to stop the treatment. From the remaining 25 subjects, a total of 101 control lice were combed out before treatment, 12 (11.9%) of which were dead. After heating, a total of 53 lice were combed out, 11 (20.8%) of which were dead. Although the percent of
dead treated lice was higher than the percent of dead control lice, with an absolute difference of 8.9%, the effect did not differ significantly from zero (95% CI: −4% to 21%).

In 7 of the 25 trials none of the control eggs hatched, and these trials were excluded from egg-hatch analysis. From the remaining 18 subjects, a total of 835 eggs were combed out before treatment, and 582 eggs were combed out after treatment. The control–egg-hatch rate was 48.7% and the treated–egg-hatch rate was 3.3%, a significant absolute difference of 45.4% (95% CI: 42% to 49%). In 8 of the 18 subjects (44.4%), none of the treated eggs hatched. In summary, this method killed few hatched lice but large numbers of eggs (Fig 2).

Handheld Blow-dryer: Directed Heating

We treated 27 subjects using this method, 9 (33%) of which indicated some discomfort. Pulling the blow-dryer away for a few seconds resolved the issue in 8 of 9 cases. One subject (4%) chose to stop the treatment. From the remaining 26 subjects, a total of 263 control lice were combed out before treatment, 28 (10.6%) of which were dead. After treatment, a total of 179 lice were combed out, 99 (55.3%) of which were dead. The percent of dead treated lice was significantly higher than the percent of dead control lice, with an absolute difference of 44.7% (95% CI: 36% to 53%).

Eight of the 26 trials were excluded from egg-hatch analysis because none of the control eggs hatched. From the remaining 18 subjects, a total of 1217 eggs were combed out before treatment, and 863 eggs were combed out after treatment. The control–egg-hatch rate was 44.3% and the treated-egg–hatch rate was 2.1%, a significant absolute difference of 42.2% (95% CI: 39% to 45%). In 8 (44.4%) of the 18 subjects, none of the treated eggs hatched. In summary, this method killed more lice than the previous 2 methods and most of the eggs (Fig 2).

Wall-Mounted Dryer

We treated 15 subjects using this method, 4 (27%) of which indicated some discomfort. Pulling the air hose away for a few seconds resolved the issue in 2 of 4 cases; however, 2 subjects (13%) chose to stop the treatment. From the remaining 13 subjects, a total of 174 control lice were combed out before treatment, 26 (14.9%) of which were dead. After treatment, a total of 235 lice were combed out, 146 (62.1%) of which were dead. The percent of treated lice that were dead was significantly higher than the percent of dead control lice, with an absolute difference of 47.2% (95% CI: 39% to 55%).

One of the 13 trials was excluded from egg-hatch analysis because none of the control eggs hatched. From the remaining 12 subjects, a total of 518 eggs were combed out before treatment, and 647 eggs were combed out after treatment. The control–egg-hatch rate was 51.0% and the treated–egg-hatch rate was 3.5%, a significant absolute difference of 47.5% (95% CI: 43% to 52%). The fraction of subjects on which no treated eggs hatched was 5 (41.7%) of 12. In summary, this method killed a slightly higher proportion of lice than the directed-heating handheld blow-dryer method and a similar proportion of eggs (Fig 2).
LouseBuster With Sections
We treated 18 subjects using this method, 3 (17%) of which indicated some discomfort. Briefly moving the air hose away resolved the issue in all 3 cases, with no requests to stop the treatment. From the 18 subjects, a total of 422 lice were combed out before treatment, 35 (8.3%) of which were dead. After heat treatment, a total of 578 lice were combed out, 440 (76.1%) of which were dead. The percent of dead treated lice was significantly higher than the percent of dead control lice, with an absolute difference of 67.8% (95% CI: 62% to 72%).

Two of the 18 trials were excluded from egg-hatch analysis because none of the control eggs hatched. From the remaining 16 subjects, a total of 839 eggs were combed out before treatment and 969 after treatment. The control–egg-hatch rate was 52.0% and the treated–egg-hatch rate was 6.0%, a significant absolute difference of 46.0% (95% CI: 42% to 50%). The egg-hatch rate was zero in only 6 (37.5%) of 16 treated subjects. In summary, this method killed more lice than any of the previous methods and an appreciable number of eggs (Fig 2).

LouseBuster With Hand Piece
We treated 18 subjects using this method, 2 (11%) of which indicated some discomfort. Briefly moving the air hose away resolved the problem, with no requests that the treatment be stopped. From the 18 subjects, a total of 217 lice were combed out before treatment, 17 (7.8%) of which were dead. After heat treatment, a total of 287 lice were combed out, 230 (80.1%) of which were dead. The percent of dead treated lice was significantly higher than the percent of dead control lice, with an absolute difference of 72.3% (95% CI: 66% to 78%).

Six of the 18 trials were excluded from egg-hatch analysis because none of the control eggs hatched. From the remaining 12 subjects a total of 309 eggs were combed out before treatment and 439 after treatment. The control–egg-hatch rate was 46.9% and the treated–egg-hatch rate was 2.0%, a significant absolute difference of 44.9% (95% CI: 39% to 51%). The proportion of subjects on which no treated eggs hatched was double that of the previous methods (10 of 12 subjects [83.3%]). In summary, this method killed the largest proportion of lice of any of our other methods and nearly all of the eggs (Fig 2).

Follow-up Examinations
We treated another 11 subjects using the LouseBuster with hand piece. In the case of these subjects we did not comb out lice or eggs on the day of treatment because we wanted to test whether this method could eradicate entire infestations of head lice (see “Methods”). None of the 11 subjects indicated that the treatment was uncomfortably hot, and none asked to stop treatment. At the 1-week follow-up, 10 (91%) of 11 had no lice. The eleventh subject had a single live male louse, which is not a viable breeding population.

DISCUSSION
The initial goal of this study was to test the effect of heated air on head lice and their eggs. We tested 6 methods for delivering hot air to the scalp. The best of these methods, the LouseBuster with hand piece, resulted in 98% mortality of eggs and 80% mortality of hatched lice. The LouseBuster was effective at killing lice and their eggs when operated at a slightly cooler temperature than a standard blow-dryer. Few subjects found it to be uncomfortable, and none asked for the treatment to be stopped.

The second goal of our study was to test whether the best method has the potential to cure children of head lice. This proved to be the case; follow-up examinations 1 week after treatment showed that 10 of 11 subjects were completely cured of lice, and the eleventh subject had just 1 live male louse. The infestations were eliminated by a single 30-minute treatment with the LouseBuster with hand piece. No household cleaning or other preventive measures were taken. Such measures are not essential for curing head lice, which cannot survive for more than a few hours off the host’s head (unpublished data).

All 6 treatment methods had a minimum mean temperature of 55°C (Table 1), which is the temperature that Buxton1 and Kobayashi et al14 found to be lethal to body lice in vitro. Despite the high temperature, however, none of our methods killed 100% of hatched lice. The reason may be that it is difficult for hot air to penetrate the entire scalp and reach all of the hair bases, where lice tend to hide. This problem underscores the importance of in situ trials when testing antipediculosis agents. Because we did not achieve a 100% kill rate of hatched lice, how do we explain the fact that 10 of 11 of our infested subjects were free of lice 1 week after treatment? In addition to stochastic extinction resulting from small population size, there may be a delayed effect of hot air on lice that are not killed outright. We plan to test this hypothesis in the future.

The first method we tested was the bonnet-style hair dryer. This early in our study we had not perfected procedures for harvesting and incubating intact louse eggs. For this reason, the control–egg-hatching rates in our tests of this method were less than those in subsequent tests of the other 5 methods; these later tests involved control–egg-hatching rates more typical of other studies.1,27 Low rates aside, the bonnet-style dryer caused a significant reduction in hatch rate, resulting in an overall egg mortality rate that approached 89%. However, this method killed very few hatched lice (Table 1). Therefore, we do not consider it to be a viable...
means of controlling head lice. The reason for the poor effect of the bonnet-style dryer on lice may be that airflow beneath the bonnet was uneven, as suggested by highly variable air temperatures (Table 1). Lice typically move ~2 mm/second when exposed to heat; however, they are capable of moving 7 mm/second.18 Lice may well have escaped to cooler microhabitats under the bonnet, which would explain why the bonnet had a stronger effect on immobile eggs.

The next 2 methods we tested used a handheld blow-dryer to apply hot air to sections of hair diffusely or more directly. Diffuse heating for 3 minutes per section resulted in an egg mortality rate that approached 97%, but the mortality rate of hatched lice was only 21%. Directed heating killed nearly all eggs while increasing the mortality rate of hatched lice to 55%. Hot air probably kills head lice by desiccating them, and effective desiccation may require the sudden onset of hot air, as in the directed heating methods. More diffuse heating may provide the lice with an opportunity to acclimate.

The fourth method involved a wall-mounted dryer of the type found in public restrooms. The air volume of this machine was more than twice that of the handheld blow-dryer (Table 1). A 60-second application per hair section, as in the third method, killed most eggs while increasing the mortality rate of hatched lice to 62%. Although the machine was detached from the wall, it was cumbersome to use and, like the previous methods, was incapable of maintaining a very constant air temperature (Table 1).

The final 2 methods included use of a custom-built machine, the LouseBuster (Fig 1), which was less cumbersome and had the ability to maintain a reasonably constant air temperature (Table 1). In the first method, we used the LouseBuster for 60 seconds per hair section. This method killed most eggs while increasing the mortality rate of hatched lice to ~76%. In the other method we added a molded-plastic hand piece with coarse teeth that were designed to lift the hair slightly, exposing the roots, where lice and eggs congregate. In this case we did not section the hair but simply pulled the hand piece slowly through the hair like a garden rake while the air blew in the opposite direction. Each region of hair was exposed to the airflow for a minimum of 60 seconds. This method proved to be the most successful, resulting in 98% mortality of eggs and 80% mortality of hatched lice. Ten of 11 subjects were also cured of head lice when examined 1 week after treatment.

User comfort was an important consideration in our study. Although most of our subjects completed the various treatments without complaint, a few indicated discomfort at some point during the treatment, especially in the case of the non-LouseBuster methods. A small number of subjects even asked to stop the treatment. In comparison, the 2 LouseBuster methods caused very little discomfort and no requests to halt the treatment. Fortuitously, the most effective methods we tested were the ones that caused the least discomfort.

The effectiveness of hot air was independent of subject age, hair length, or hair thickness.28 It worked equally well on people of diverse ethnic backgrounds, including those of African, European, Hispanic, and South Pacific Island descent. Effectiveness of this approach was also not dependent on ambient humidity. Although most of our trials were conducted in the arid environment of Salt Lake City, Utah, preliminary trials with the LouseBuster in humid South Florida (n = 12) show nearly identical results to those in Utah (unpublished data).

The proximal mechanism by which hot air kills lice is uncertain, although we think desiccation is the most likely candidate. Lice are highly susceptible to desiccation because their small size and flattened shape give them a high surface area/volume ratio.19 High temperature could conceivably also cause conformational changes in cuticular molecules, promoting rapid desiccation and death.29 Buxton1 reported that hot, dry air reduces the amount of amniotic fluid in louse eggs, which makes it more difficult for them to hatch; this could explain why hot air has such a devastating effect on egg-hatch rates. Although we hope to determine the exact proximal effect of hot air on lice in the future, this was not the purpose of the current study.

Our study is one of the few to measure the impact of a treatment regime on actual infested subjects. In a previous such study, Burgess et al27 reported that 1% permethrin creme rinse (Nix) leads to nearly 60% mortality of eggs in situ. In comparison, we report 98% mortality of eggs and 80% mortality of hatched lice treated in situ with the LouseBuster and hand piece. Our method requires just one 30-minute treatment, unlike permethrin/pyrethroid-based chemical shampoos or suffocation-based pediculicides, which require at least 2, and often 3, treatments 1 week apart.26 Our method is safe, and it is unlikely that lice will evolve resistance, because that would require fundamental changes in their water physiology. In summary, hot air is a significant improvement over other therapies used to treat head lice.

We envision the LouseBuster to be an institutionally based machine operated by health care providers, school administrators, or trained parents and other volunteers. Although effective use of the LouseBuster is not difficult, it does require a little practice to perfect. The advantage of an institutionally based device, particularly for schools, is that it could be used to simultaneously treat all children with head lice, minimizing the problem of reinestation. In our experience, this would be particularly useful in the case of children with parents who cannot afford the time, expense, or discipline required to treat head lice effectively in their home.
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