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RESEARCH

The lethal effects of high-frequency ultrasound waves in *Pediculus humanus capitis* (Anoplura: Pediculidae) nymphs and adults

Pediculus humanus capitis (Anoplura: Pediculidae) nimf ve erginlerinde yüksek frekanslı ultrason dalgalarının öldürücü etkileri

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Abstract

Purpose: This study aimed to assess the efficacy of high-frequency ultrasound waves as a non-chemical alternative for controlling head lice (*Pediculus humanus capitis*) by evaluating mortality rates in adult and nymphal stages exposed to various ultrasound frequencies.

Materials and Methods: A total of 1,000 head lice (adults and nymphs) collected from infested children were exposed under controlled laboratory conditions to five ultrasound frequencies (0.5, 1.0, 1.5, 2.0, and 2.5 MHz) for up to 24 hours. A control group of 200 lice was maintained under the same laboratory conditions without ultrasound exposure. Mortality was recorded at 1, 2, 4, 8, 16, and 24 hours.

Results: Among the 1,000 head lice tested, ultrasonic frequencies of 1.5, 2.0, and 2.5 MHz caused the highest mortality, reaching ≥90% in adults and ≥98.3% in nymphs. In contrast, 0.5 MHz produced the lowest mortality (20–21.7%), while the control groups showed only 8.3% (nymphs) and 15.0% (adults) mortality. Most deaths occurred within 8–24 hours post-exposure. Correlation analysis revealed a strong positive relationship between mortality and exposure time ($r = 0.710$, $p < 0.001$). ANOVA confirmed significant effects of frequency on mortality ($F(5, 330) = 6.845$, $p < 0.001$), with mortality plateauing at 2.5 MHz. Probit regression estimated LT_{50} values of 12.1–13.4 hours for adults and 15.4–17.6 hours for nymphs at higher frequencies, compared to 22.2 and 27.3 hours, respectively, at 0.5 MHz.

Conclusion: This study is the first to demonstrate the direct effect of ultrasonic sound frequencies on *Pediculus humanus capitis*. High-frequency ultrasound (1.5–2.5 MHz) effectively controlled head lice without the need for

Öz

Amaç: Bu çalışma, baş biti (*Pediculus humanus capitis*) ile mücadelede kimyasal içermeyen bir alternatif olarak yüksek frekanslı ultrason dalgalarının etkinliğini, farklı frekanslara maruz bırakılan ergin ve nimf evrelerindeki ölüm oranlarını değerlendirerek araştırmayı amaçlamıştır.

Gereç ve Yöntem: Enfeste çocuklardan toplanan toplam 1.000 baş biti (ergin ve nimf) beş farklı ultrason frekansına (0.5, 1.0, 1.5, 2.0 ve 2.5 MHz) 24 saate kadar maruz bırakıldı. Kontrol grubunu oluşturan 200 bit, aynı koşullarda ancak ultrason uygulanmadan takip edildi. Ölümler 1, 2, 4, 8, 16 ve 24. saatlerde kaydedildi.

Bulgular: Test edilen 1.000 baş biti arasında, 1.5, 2.0 ve 2.5 MHz ultrasonik frekansları en yüksek mortaliteyi oluşturmuş, erginlerde ≥%90, nimflerde ise ≥%98,3'e ulaşmıştır. Buna karşılık, 0.5 MHz en düşük mortaliteyi (%20–21,7) göstermiş, kontrol gruplarında ise mortalite oranı nimflerde %8,3 ve erginlerde %15,0 olarak kalmıştır. En fazla ölüm 8–24 saatlik süre içinde kaydedilmiştir. Korelasyon analizi, mortalite ile maruziyet süresi arasında güçlü pozitif bir ilişki olduğunu göstermiştir ($r = 0,710$, $p < 0,001$). ANOVA sonuçları, frekansın mortalite üzerinde anlamlı etkisini doğrulamıştır ($F(5, 330) = 6,845$, $p < 0,001$) ve 2,5 MHz'de mortalitenin plato yaptığı gözlenmiştir. Probit regresyon analizinde, yüksek frekanslarda erginler için LT50 değerleri 12,1–13,4 saat, nimfler için ise 15,4–17,6 saat olarak hesaplanmış; bu değerler 0,5 MHz'de sırasıyla 22,2 ve 27,3 saat olarak bulunmuştur.

Sonuç: Bu çalışma, ultrasonik ses frekanslarının *Pediculus humanus capitis* üzerindeki doğrudan etkisini gösteren ilk çalışmadır. Yüksek frekanslı ultrason (1,5–2,5 MHz), ek kimyasal maddeye ihtiyaç duymadan saç bitlerini etkili bir şekilde kontrol etmiş ve bunun kimyasal olmayan bir

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additional chemicals, highlighting its potential as a non-chemical pediculosis management strategy. These findings provide a basis for future research and the development of portable devices, such as hair clips or necklaces, designed to repel or inactivate lice. Incorporating high-frequency ultrasound alongside routine combing may enhance head lice control, and further studies are warranted to optimize device design and exposure parameters.

Keywords:. *Pediculus humanus capitis*, head lice, ultrasonic frequencies, mortality, pediculosis control, non-chemical methods, lice control devices

pedikülozis yönetim stratejisi olarak potansiyelini ortaya koymuştur. Bu bulgular, saç bitlerini uzaklaştırmak, caydırmak veya etkisiz hâle getirmek amacıyla tasarlanmış saç tokaları veya kolyeler gibi taşınabilir cihazların gelecekteki araştırma ve geliştirme çalışmalarına temel sağlamaktadır. Yüksek frekanslı ultrasonun rutin tarama uygulamalarıyla birlikte kullanılması, saç biti kontrolünü artırabilir ve cihaz tasarımı ile maruz kalma parametrelerini optimize etmek için daha ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: *Pediculus humanus capitis,* baş biti, ultrasonik frekanslar, mortalite, pediküloz kontrolü, kimyasal içermeyen yöntemler, saç biti kontrol cihazları.

INTRODUCTION

Pediculus (P.) humanus (h.) capitis, the human head louse, is a wingless, six-legged, hemimetabolous, blood-sucking ectoparasite known for causing scalp pruritus, especially among children[1]. The life cycle of the head louse consists of three stages: egg, nymph, and adult[1]. Pediculosis typically involves fewer than ten live lice, and itching occurs if the individual becomes sensitized to antigenic components in the saliva injected during feeding[2].

Head lice are widespread in Türkiye as well as worldwide[3]. There is no established relationship between pediculosis and socioeconomic status, but infestations may cause socially unpleasant conditions[4]. Previous studies conducted with 578,938 individuals treated for head lice in Türkiye between 1982 and 2012 reported prevalence rates ranging from 0.3% to 34.1%[5]. Several risk factors influencing pediculosis prevalence include parental care, gender, and age[6]. The prevalence detected in Türkiye was higher than in Europe (0.48% to 22.4%) but lower than in many countries in the Americas, particularly Brazil (3.6% to 61.4%)[7].

Head lice are primarily transmitted through direct head-to-head (hair-to-hair) contact. Lice do not hop or fly but can crawl rapidly[2]. Therefore, it is unlikely that the infestation rate can be reduced, as children frequently have head-to-head contact. They also share personal belongings such as combs, brushes, and hats, which further increases transmission[8]. Treatment for pediculosis should not be initiated unless there is an accurate diagnosis of head lice. The ideal treatment should be safe, free of toxic chemicals, readily available without a prescription, simple to apply, effective, and affordable[9].

Despite the use of strong insecticides and the efforts of parents and healthcare organizations, head louse infestations cannot be effectively managed worldwide[10]. Leading causes of ineffective control include the sale of ineffective pediculicides, misuse, the use of inappropriate alternative therapies, development of pesticide resistance, difficulties in diagnosing infestations, and social stigma and shame[11]. Moreover, ineffective formulations and residual pediculicide concentrations on hair may expose lice to sublethal doses, potentially facilitating resistance development[12].

Some chemical compounds used in medical treatments include permethrin, malathion, piperonyl butoxide, and isododecane. The use of shampoos containing these compounds has been associated with health consequences, such as biochemical changes in the bodies of infected individuals and malathion residues in urine samples[13]. Consequently, mechanical control methods with proven efficacy have become increasingly important in head louse management[14]. High-frequency ultrasound waves have been proposed as a candidate mechanical control method to prevent infestations caused by ectoparasitic arthropods in humans and domestic animals. Ultrasonic sounds (>20 kHz) are used in daily life and technology, are inaudible to humans, but can harm some arthropod pests[15].

The effects of ultrasonic sound frequencies on arthropod vectors such as mosquitoes, ticks, and cockroaches have been extensively studied, primarily focusing on their repellent effects. However, despite this broad spectrum of research, there is a striking lack of studies investigating the direct impact of ultrasound on human ectoparasites, including head lice. To our knowledge, only one prior study has examined ultrasound in the context of lice, using it solely as a facilitative tool to improve combing efficiency rather than assessing its pure biological effects. Nevertheless, that study, along with a few related reports, suggested that ultrasound-assisted

combing systems may exert repelling or even lethal effects on lice. Some ultrasonic therapeutic agents have also been reported to achieve the highest efficacy against eggs, nymphs, and adults, with up to 100% mortality[14,16]. Therefore, the present study is, to our knowledge, the first to evaluate the direct biological effects of ultrasonic sound frequencies on *P. h. capitis*, highlighting the originality and novelty of our research.

Air bubbles within the dorsal tracheal trunk (DTT) of mosquitoes have been shown to resonate in response to ultrasonic waves, leading to larval mortality[17]. This mechanism represents a fundamental basis for the efficacy of acoustic larvicide applications. Similarly, microscopic examinations of *P. h. capitis* (head lice) have revealed the presence of small air bubbles in the head region[18].

The main aim of this study was to determine the effectiveness of ultrasound in killing head lice and to establish the corresponding effective exposure time. To provide a foundation for future development of ultrasonic-based devices, we investigated whether, and to what extent, high-frequency ultrasonic waves could reduce head lice populations. We therefore observed the pure lethal effects of high-frequency ultrasound on both adult and nymphal head lice obtained from infested children.

This study represents the first systematic evaluation of the direct biological effects of high-frequency ultrasonic waves on human head lice. Whereas previous research has been limited to ultrasound-assisted combing techniques, our investigation provides quantitative evidence of frequency- and time-dependent mortality in both adult and nymphal stages, thereby filling a critical gap in the literature. We hypothesized that exposure to higher-frequency ultrasonic waves would result in significantly greater and more rapid mortality compared to lower frequencies.

MATERIALS AND METHODS

Study area

Head lice screenings were conducted with four replications, without any treatment, between 2019 and 2021, in three schools located in rural areas of the Karaisalı district (Etekli, Çukur, and Eğlence Primary/Secondary Schools), 50 km from Adana, Türkiye (Figure 1). The study was approved by the Ethical Committee of Çukurova University Medical School (Approval No. 90, dated 07.05.2019). Additionally, official permission to conduct the studies in the schools was obtained from the district governorship, and informed consent was collected from all participants. All laboratory experiments on the collected lice samples were performed at the Çukurova University Karaisalı Vocational School and the Department of Medical Biology, Çukurova University Faculty of Medicine.

Screening students for head lice

In our four head lice detection studies conducted in three randomly selected schools, a total of 440, 429, 417, and 407 students, respectively, participated. Prior to the screening days, all students were informed about the scheduled head lice examinations and were asked to attend on the designated days. On the screening days, all students present at school were examined, without applying any exclusion criteria based on age, gender, or other characteristics. No participants were excluded due to missing data or other factors. To determine the prevalence of pediculosis, the studies were carried out in December (2019), March (2020 and 2021), and April (2021), which are known to be seasons with high infestation rates[19]. Screening for head lice was performed by an experienced doctor and/or parasitologists familiar with head lice detection methods and the various life stages of *P. h. capitis*. Students were examined using visual inspection, in which the examiners parted the hair into small sections using their fingers and thumbs over the entire scalp while looking for signs of live lice or eggs for three minutes. Plastic combs with a tooth spacing of 0.15 mm were used in the study. Each section of the scalp was combed three times. Following combing, suspected organisms on the comb were examined visually to confirm the diagnosis.

Control measures

In accordance with the protocol used in head lice detection studies conducted in schools in Türkiye, the parents of students identified with head lice were notified by the school via a formal letter. Families were instructed to initiate pediculosis treatment as recommended by the family physician. Additionally, parents were required to complete the first application of an approved treatment before the student returned to school.

Sound frequency

Sound frequency refers to the number of sound vibrations per second; therefore, higher frequencies produce greater vibration. Frequencies above 20 kHz are referred to as ultrasonic sound or ultrasound. These sounds are more easily directed than audible sounds and are therefore widely used in technology, while remaining inaudible to the human ear[20].



Figure 1. Study conducted areas in Adana.

Experimental design

In our experimental setup, 12 V–7 A batteries were used as the power supply. The ultrasound power applied in the replications was 0.15 W with 1.5 mA, and the device output voltage was 1.5 V. At these settings, the dB levels audible to humans could not be measured. Control units were used to turn the entire system on and off. Signals generated from the oscillator on the circuit board were amplified through electrical oscillations, which were further boosted by the amplifier in an integrated circuit. Different amplifier types were employed for the various high-frequency (ultrasound) values (0.5, 1.0, 1.5, 2.0, and 2.5 MHz) used in the experiments. Mechanical energy was converted into electrical energy, or vice versa, via the piezoelectric effect, a property of certain crystals and ceramics that allows them to generate electricity under mechanical compression and produce mechanical vibration when electricity is applied. In the experiment, square-wave electrical energy was applied to the piezo disc material (considered an electronic circuit element) at specific frequencies[21]. Glass cubic containers used to hold the collected lice were made of 4 mm double glazing with a 10.5 mm gap between panes and had dimensions of 12 × 12 × 12 cm. Additionally, a frequency meter was used to measure the sound frequencies obtained from all replications (Noise Measurement Device) (Figure 2).

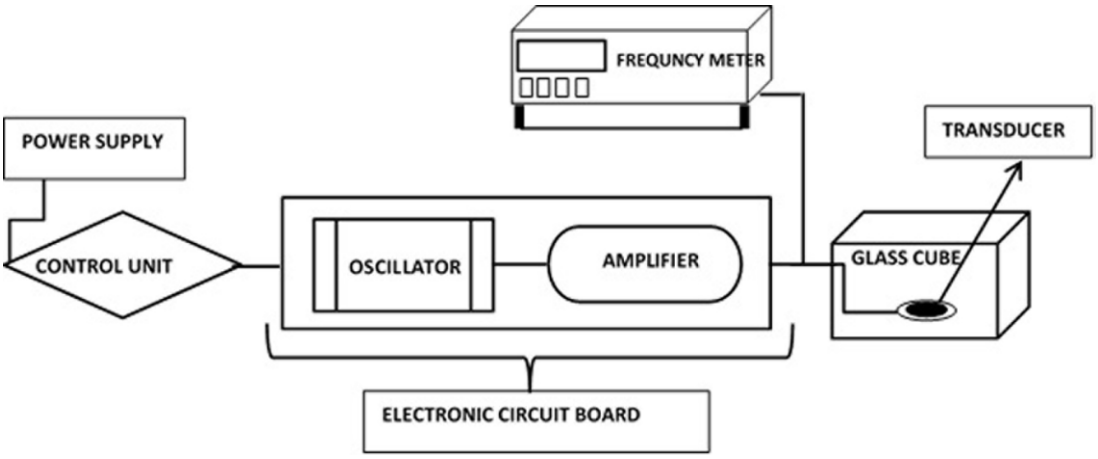


Figure 2. Graphical abstract of experimental design

P. h. capitis adults and nymphs, collected from students found positive after head lice detection combings in schools, were placed in petri dishes with a tuft of hair and transported to the sound laboratory at the institution as soon as possible. In the laboratory, the head lice were exposed to five different high-frequency ultrasound waves (0.5, 1.0, 1.5, 2.0, and 2.5 MHz, respectively) for 24 hours. The number of individuals affected by the pure effect of sound frequency on head lice, from both experimental and control groups, was recorded at 1, 2, 4, 8, 16, and 24 hours after the onset of ultrasound exposure. For each frequency, four replications were performed using different sets of 50 head lice (20 adults and 30 nymphs) per replicate. Additionally, 50 head lice (adults and nymphs) were placed in the control group, which was kept isolated in the laboratory (Figure 3). A total of 1,000 head lice (50 individuals × 5 frequencies × 4 replications) were exposed to high-frequency ultrasound waves across all trials, while 200 head lice (50 individuals × 4 replications) were used in the control groups.



Figure 3. Adults and nymphs head lice.

Binocular microscopy of ultrasonically exposed head lice

Head lice specimens were collected from individuals who tested positive for infestation. The specimens were exposed to ultrasonic waves at the designated frequencies and durations to assess the effects of the treatment. Following exposure, lice that had died were fixed in 4% formaldehyde to preserve their morphological structures.

After fixation, the specimens were rinsed with distilled water and dehydrated through a graded ethanol series (70%, 90%, and 100%) for 10 minutes at each concentration. Finally, the specimens were transferred to a Petri dish and examined under a binocular microscope for morphological analysis of the head region, with a particular focus on the presence of small air bubbles.

Statistical analysis

No previous studies have directly investigated the effects of ultrasonic frequencies on head lice. Existing research has focused primarily on mosquito larvae, where typically 20 larvae per container were used to assess ultrasound effects. In our study, 50 head lice were placed per container to increase the reliability and statistical robustness of the results, considering the expected variability in mortality among lice. As the methodology for head lice was specifically developed for this study, an a priori power analysis using G*Power 3.1 was conducted. The analysis indicated that a minimum of 216 lice would be required for a one-way ANOVA with six groups (five ultrasonic frequencies and one control), assuming a medium effect size ($f = 0.25$), $\alpha = 0.05$, and power = 0.8. Accordingly, a total of 1,000 head lice were included, ensuring sufficient statistical power to detect meaningful differences among the groups.

Statistical analyses were performed using SPSS 25.0 software. Descriptive statistics, including means and standard deviations, were first calculated to summarize the characteristics of the data. Corrected mortality rates were subjected to arcsine transformation to ensure normality and homoscedasticity prior to further analyses. The relationships between ultrasonic frequency, the number of dead individuals, and exposure time were evaluated using Pearson correlation, as all variables were continuous and normally distributed, allowing assessment of linear associations. Normalized mortality rates were then analyzed using one-way analysis of variance (ANOVA) to determine whether significant differences existed among frequency groups. ANOVA was chosen because it is suitable for comparing means across multiple independent groups. When ANOVA indicated significant differences, post-hoc tests were applied to identify which specific groups differed: the Tukey test was used when variances were homogeneous, and the Games–Howell test when variances were unequal, ensuring appropriate pairwise comparisons. A log-probit regression analysis was conducted according to the Finney method to calculate median lethal time (LT_{50}), 90% lethal time (LT_{90}), 95% confidence intervals, and slope values, which is a standard approach in toxicological studies and appropriate for modeling dose-response and time-to-event data. Additionally, a logistic regression analysis was performed to determine which ultrasonic frequency exhibited the highest repellency and lethal effect on *P. h. capitis*, as this method is suitable for modeling binary outcomes (dead/alive). For all analyses, a p-value of 0.05 was considered statistically significant.

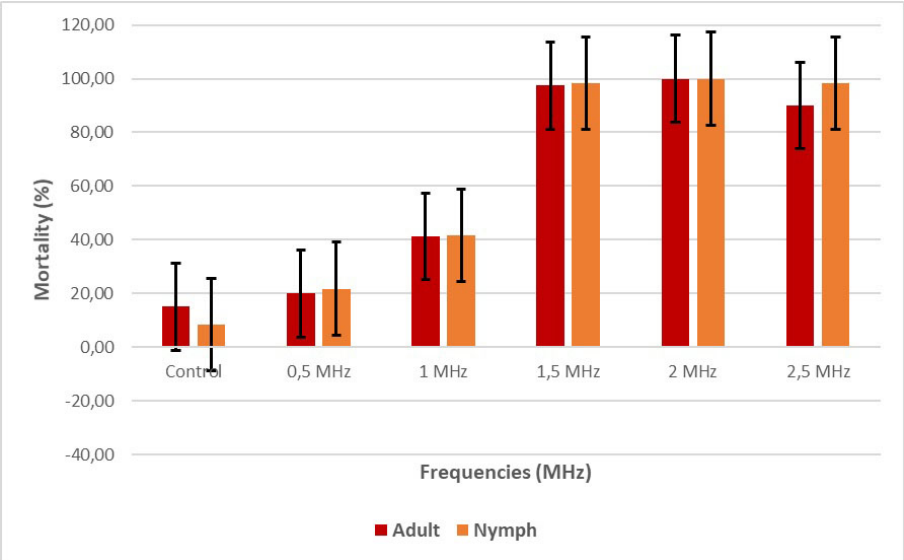


Figure 4. The rate of dead individuals detected in adults and nymphs of *P. h. capitis* at 0.5, 1.0, 1.5, 2.0, and 2.5 MHz ultrasonic sound frequencies.

RESULTS

We exposed 1,000 head lice to five different ultrasonic frequencies to assess their mortality. In high-frequency ultrasonic sound exposure trials, frequencies of 1.5, 2.0, and 2.5 MHz caused the highest mortality, especially in nymphs. At these three frequencies, a minimum of 90% mortality was observed in adults, while minimum mortality in nymphs reached 98.33%. Although mortality in nymphs and adults was 8.33% and 15.00%, respectively, in the control groups, 0.5 MHz was determined to be the frequency causing the lowest mortality (20–21.67%) among the five frequencies applied to head lice (Figure 4).

In addition, the highest number of deaths in *P. h. capitis* exposed to high-frequency ultrasound waves was recorded between 8 and 24 hours following exposure in all replications (Figure 5). In both adult and nymph groups, differences in mortality were observed for all frequencies except 1.5, 2.0, and 2.5 MHz.

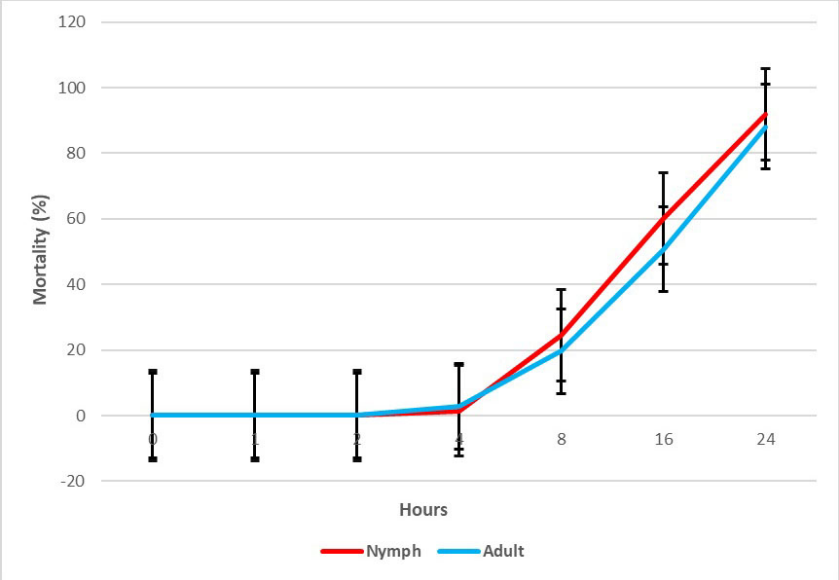


Figure 5. The rate of dead individuals detected in adults and nymphs of *P. h. capitis* at 1, 2, 4, 8, 16, and 24th exposure time in experiments.

Binocular microscopic examination of *P. h. capitis* exposed to ultrasonic waves revealed the presence of small gas vesicles specifically in the head region of the lice (Figure 6). These vesicles appeared as discrete, spherical structures within the cephalic tissue, consistent with the formation of intracellular bubbles induced by ultrasonic cavitation. The vesicles exhibited dynamic behavior, likely expanding and contracting under the influence of sound wave vibrations, consistent with cavitational processes that can lead to cellular rupture. This observation provides direct microscopic evidence supporting the mechanism by which ultrasonic treatment disrupts lice cells and contributes to mortality in both nymph and adult stages.

Correlation analysis revealed a strong and significant positive relationship between the number of dead individuals and exposure time ($r = 0.710$, $p < 0.001$), indicating that mortality increased markedly with longer exposure durations. A moderate positive correlation was observed between the number of dead individuals and applied frequencies ($r = 0.287$, $p < 0.001$), suggesting a less pronounced effect compared to exposure time. No significant correlation was found between frequencies and

exposure time (r = 0.000, p = 1.000). Furthermore, the developmental stage of the individuals (stage) did not show any significant association with the other variables (p > 0.05). These results suggest that mortality is primarily influenced by exposure time, with frequency exerting a limited effect, while the developmental stage appears to have no significant impact (Table 1).



Figure 6. Microscopic image of a *P. h. capitis* specimen after exposure to ultrasonic sound frequencies (4× magnification). Air bubbles are visible at the head region, indicating effects of the ultrasonic treatment.

Table 1. Pearson correlation coefficient between ultrasonic sound frequencies, number of dead individuals, exposure times and life stages of *P. h. capitis*

		Frequencies	Dead Individuals	Exposure Time	Stage
Frequencies	Pearson Correlation	1	,287**	,000	,000
	Sig. (2-tailed)		,000	1,000	1,000
	N	336	336	336	336
Dead Individuals	Pearson Correlation	,287**	1	,710**	,062
	Sig. (2-tailed)	,000		,000	,256
	N	336	336	336	336
Exposure Time	Pearson Correlation	,000	,710**	1	,000
	Sig. (2-tailed)	1,000	,000		1,000
	N	336	336	336	336
Stage	Pearson Correlation	,000	,062	,000	1
	Sig. (2-tailed)	1,000	,256	1,000	
	N	336	336	336	336

** Correlation is significant at the 0.01 level (2-tailed).

According to the ANOVA results presented in Table 2, frequency had a significant effect on the mortality of *P. h. capitis* adults and nymphs ($F(5, 330) = 6.845$, $p < 0.001$). The analysis showed that mortality rates increased markedly at 1.50 MHz and 2.00 MHz. Mortality in the control and lower-frequency groups remained relatively low and similar, whereas higher-frequency groups exhibited substantially higher mean mortality. At 2.50 MHz, mortality appeared to plateau, indicating stabilization of the effect at the highest frequency tested. These findings suggest that increasing frequency is associated with higher mortality, with the most pronounced effects observed between 1.50 and 2.00 MHz.

Table 2. Results of ANOVA testing the effect of frequency ranges and exposure time on *P. h. capitis*' adults and nymphs.

Frequencies	N	Mean	Std. Deviation	Sources	Sum of Squares	df	F	*P*
Control	56	2.0582	3.07914	Between Groups	1494.408	5	6.845	0.00
0.50	56	2.9864	4.24688	Within Groups	14410.154	330		
1.00	56	4.5755	5.37898	Total	15904.562	335		
1.50	56	7.1563	8.29016					
2.00	56	7.2530	8.37999					
2.50	56	7.0668	8.16096					
Total	336	5.1827	6.89031					

The values of d.f. = degree of freedom, significance level $P<0.05$.

In addition, there were no significant differences between the number of dead adults and nymphs. Logistic and probit regression analyses were performed to identify the most lethal frequencies and the corresponding LT_{50}–LT_{90} values. The logistic regression analysis presented in Table 3 was conducted to determine the most effective frequency and exposure period for killing *P. h. capitis*. The results indicate that frequencies of 1.5 MHz, 2.0 MHz, and 2.5 MHz had a statistically significant effect on mortality ($p = 0.008$, $p = 0.018$, and $p = 0.033$, respectively). In contrast, the control group and the 0.50 MHz and 1.0 MHz frequencies did not show a significant effect ($p > 0.05$). These findings suggest that higher frequencies are more effective in inducing mortality in *P. h. capitis*.

Probit regression analysis was conducted to estimate the median lethal time (LT_{50}) and 90% lethal time (LT_{90}) for *P. h. capitis* at different developmental stages and frequency levels (Table 4). For adults, LT_{50} decreased from 22.20 hours at 0.50 MHz to approximately 12.10–13.38 hours at 2.0–2.5 MHz, indicating faster mortality at higher frequencies ($p > 0.05$). Similarly, LT_{90} values decreased from 31.99 hours at 0.50 MHz to 22.61–25.46 hours at higher frequencies ($p > 0.05$). For nymphs, LT_{50} decreased from 27.27 hours at 0.50 MHz to approximately 15.39–17.61 hours at 1.5–2.5 MHz ($p > 0.05$), while LT_{90} decreased from 44.70 hours to 25.82–32.92 hours over the same frequency range ($p > 0.05$). Mean lethal times followed by the same letters within a column did not differ significantly ($p > 0.05$).

Table 3. Determining the most effective frequency and time period killing *Pediculus capitis* using logistic regression test.

Frequencies (MHz)	df	Sig.
Control		0.756
0.50	1	0.754
1	1	0.754
1.5	1	0.008*
2	1	0.018*
2.5	1	0.033*

DF.= Degree of Freedom, Sig.= Significant, *= $P< 0.05$

Table 4. Probit regression analysis of lethal time values (LT_{50} and LT_{90}) of *Pediculus capitis* in each developmental stage.

Louse Stage	Frequencies (MHz)	Lower Limit; [LT50]; Upper Limit (hours)	Frequencies	Lower Limit; [LT90] Upper Limit (hours)
Adult	0.50	20.67; [22.201]; 24.11	0.50	29.26; [31.99]; 35.91
	1	16.25; [20.23]; 27.62	1	28.82; [36.63]; 53.95
	1.5	10.94; [12.52]; 14.43	1.5	17.63; [20.04]; 23.62
	2	10.01; [12.10]; 14.65	2	19.22; [22.61]; 28.02
	2.5	10.66; [13.38]; 17.05	2.5	20.84; [25.46]; 34.03
Nymph	0.50	22.72; [27.27]; 35.85	0.50	36.06; [44.70]; 62.30
	1	18.78; [22.29]; 28.19	1	30.69; [37.33]; 50.11
	1.5	13.03; [15.39]; 18.53	1.5	23.39; [27.55]; 34.42
	2	13.70; [15.69]; 18.13	2	22.63; [25.82]; 30.67
	2.5	13.00; [17.61]; 27.46	2.5	24.45; [32.92]; 58.29

The mean of lethal times for each level of power followed by the same letter(s) within a column denotes no significant differences ($P>0.05$).

DISCUSSION

Ultrasonic sound frequencies have been extensively studied for their repellent effects against various arthropod vectors; however, their direct impact on human ectoparasites, such as head lice, has largely remained unexplored. To our knowledge, only one previous study has investigated the use of ultrasound in lice, employing it to facilitate combing rather than to assess its biological effects. Nevertheless, that study and related reports suggested potential repelling or lethal effects. Our findings confirm that the present study is the first to evaluate the direct biological effects of ultrasonic sound frequencies on *P. h. capitis*, demonstrating significant mortality. These results not only highlight the novelty of this approach but also provide a valuable contribution to the literature by establishing baseline data on the efficacy of ultrasound against head lice.

The presence of small air bubbles within the dorsal tracheal trunks of mosquito larvae has been previously documented, and these bubbles have been shown to resonate under ultrasonic exposure, becoming visible under microscopy. This resonance induces embolism, which damages surrounding tissues and obstructs respiration, ultimately leading to the death of larvae and pupae[17]. Similarly, the occurrence of small air bubbles in head lice has been reported[18]. In our study, we also observed bubble-like structures in head lice specimens that appeared to resonate under ultrasonic frequencies and became visible under a binocular microscope (Figure 6). We propose that such resonance-induced embolism may interfere with respiration, thereby contributing to the mortality of head lice. These findings suggest a plausible morphological mechanism underlying the lethal effects of ultrasonic waves and highlight the potential of ultrasound as a non-chemical alternative for head louse control.

Pediculosis is also considered a social problem for human beings and is often treated with chemical methods. However, researchers have suggested alternative physical methods for pediculosis treatment since some chemicals used to control *P. h. capitis* can adversely affect human health[14]. Pertaining to this study, we exposed *P. h. capitis* adults and their nymphs to high-frequency ultrasound waves. We observed that ultrasonic waves applied for 24 h at a frequency of 1.5–2.5 MHz caused the highest mortality. Similarly, the frequency range of 18–30 kHz has been reported to cause high mortality in mosquito larvae[15,22,23]. Although human audible sounds in the 8–20 kHz frequency range are known to cause hearing problems, sounds above this frequency range do not have this effect. Therefore,

the method used in vector control can also be applied to the control of head lice (Figure 4)[24].

In the present study, we evaluated the effects of ultrasonic sound exposure on *P. h. capitis* for durations ranging from 1 to 24 hours. Interestingly, we observed higher-than-expected mortality in the control group, which was not exposed to ultrasound. This phenomenon can be attributed to the inability of lice to feed after being transferred to the laboratory environment. Previous studies have reported that adult head lice can survive only 24–48 hours without a blood meal, while nymphs also have limited survival under these conditions[25]. Therefore, the elevated mortality observed in the control group likely reflects the physiological stress associated with starvation in a laboratory setting, rather than any experimental treatment effect.

To our knowledge, few studies have focused on the usage of high-frequency ultrasound waves to control head lice in the world. In addition, *P. h. capitis* adults and nymphs were also exposed to high-frequency ultrasound waves in only Cohen's patent study that assessed the effect of high-frequency ultrasound waves on head lice[26]. It was also reported that 1.7 MHz was the most effective frequency for head lice mortality rate among the applied frequencies. Furthermore, in another study that focused on determining the mortality resulting from applying shampoo made from *Cymbopogon citratus*, the high-frequency ultrasonic sound and permethrin were found to be effective in removing adult and nymph head lice. In addition, ultrasonic sound and shampoo containing *C. citratus* were determined to cause 100% mortality, while chemical insecticide caused a lower mortality, between 43% and 83%[16].

Furthermore, the findings of a study using ultrasonic brushes developed for both the detection and removal of head lice showed that the presence of lice in children participating in the study decreased from 95.6% to 40.9%[27]. The findings of our study, including the same physical factor, support the idea that head lice control could be achieved by integrating devices that produce high-frequency sound waves.

Some chemical insecticides (permethrin, malathion, piperonyl butoxide, and isododecane) used in head louse control could have toxic effects, especially on liver enzymes after dermal contact. Although the negative effects of ultrasonic sound frequencies in various kHz on living organisms have been determined, these effects of the MHz intervals used in our study have not yet been reported. It is likely that items such as special crowns, combs, and buckles which help integrate circuits with a frequency range of 1.5–2.5 MHz might be developed in the control of pediculosis in the near future[13,28]. Therefore, MHz-sized ultrasonic sound frequencies can be used as an alternative method in the treatment of pediculosis[29].

In a study conducted on cockroaches, another group of hemimetabolous insects, it was determined that frequencies of 0.40 and 0.75 MHz were the most effective in causing death. In our study, the frequencies that caused the highest mortality in head lice were in the range of 1.5–2.5 MHz. We suggest that the difference in the most lethal frequencies between the two groups is due to differences in the muscle structures of lice and cockroaches[30].

LT_{50} and LT_{90} values were calculated in mosquitoes exposed to ultrasonic sound frequencies delivered at 10, 15, 20, and 25 W power at a fixed frequency of 130 kHz. Accordingly, the ultrasonic sound frequency power between 20–25 W was determined to be the most lethal range, exhibiting the smallest LT_{50} and LT_{90} values. In our study, among the five different ultrasonic sound frequencies applied at a constant power of 0.15 W, the smallest LT_{50} and LT_{90} values were observed at frequencies in the range of 1.5–2.5 MHz[31].

When used at higher frequencies, the effect of ultrasound on tissue is primarily based on heating. The thermal effect usually becomes dominant as the frequency increases from kilohertz (kHz) to megahertz (MHz) levels[32,33]. In our study, we propose that the sound energy generated from vibrations transmitted at high frequencies causes significant tissue damage and mortality in lice. Furthermore, despite numerous animal studies, no human research to date has documented major physiological consequences of ultrasound exposure for imaging purposes.

More recently, Martinez and Harrison[34] reported that ultrasonic frequencies above 1.0 MHz significantly affected mortality in fleas and lice, highlighting the potential synergy between ultrasound and conventional control measures. In a parallel study, Rodriguez et al.[35] conducted a systematic review of ultrasonic pest control, concluding that efficacy depends on multiple factors, including the precise frequency range, exposure duration, and target pest physiology. These findings underscore the importance of optimizing ultrasound parameters to

maximize lethality while minimizing any unintended effects.

Although our study demonstrated significant mortality of *P. h. capitis* under ultrasonic exposure, several limitations should be considered. The natural environment of head lice on human hair differs substantially from laboratory conditions, including factors such as hair oils, sebum, and host temperature. These differences may influence the movement and survival of lice, and therefore, the efficacy of ultrasonic frequencies observed in vitro may not fully replicate in vivo conditions[25]. The frequencies used in this study (0.5–2.5 MHz) are generally considered safe for humans at low intensities. For instance, the American Institute of Ultrasound in Medicine[36] has established that inertial cavitation thresholds above 3.6 MPa at frequencies ≥0.5 MHz do not induce biological effects as long as the mechanical index (MI) remains below 1.9. Similarly, the International Electrotechnical Commission[37] has developed standards for the safe operation of medical ultrasound devices within the 0.5–2.5 MHz range. Nevertheless, potential risks associated with prolonged and direct exposure in real-world settings should not be overlooked. Future studies should address these variables and further evaluate the applicability and safety of ultrasonic devices under natural conditions.

Ultrasonic sound frequencies have been extensively studied for their effects on various vector arthropods; however, their direct impact on head lice has been largely unexplored, with previous applications limited to facilitating combing devices. This study is the first to demonstrate the pure biological effects of high-frequency ultrasound on *P. h. capitis*. Our results indicate that frequencies of 1.5–2.5 MHz effectively induce high mortality in both nymph and adult stages, achieving head lice control without the need for chemical agents.

These findings provide a foundation for the development of non-chemical, non-invasive approaches to pediculosis management. Future research should focus on designing practical devices capable of repelling, deterring, or inactivating head lice in real-world settings, such as portable hair clips, necklaces, or other wearable systems. Additionally, further studies are warranted to optimize exposure protocols, assess long-term effects, and compare ultrasonic methods with conventional pediculicides. Overall, high-frequency ultrasound demonstrates considerable potential as a safe and adjunct tool for head lice control, offering a promising avenue for chemical-free, public health interventions.

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Ethical Approval: Our study was conducted with the approval of the Çukurova University Ethics Committee, in accordance with the European Union legislation on pharmaceuticals, specifically directives 2001/20/EC and 2005/28/EC concerning Good Clinical Practice. In addition, informed consent to participate was obtained from the parents or legal guardians of any participant under the age of 16.
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