Tehran University of Medical Sciences Publication
http://tums.ac.ir

Iran J Parasitol

Open access Journal at
http://ijpa.tums.ac.ir

Iranian Society of Parasitology
http://isp.tums.ac.ir

Original Article

Prevalence of *Pseudomonas aeruginosa* and *Acanthamoeba* spp. Co-Infection in Dust from Public Parks in Eastern Thailand

***Pattakorn Buppan, Chonrachart Tuptaintong, Toranus Takamwong, Araya Charoenworawong, Rattiporn Kosuwin, Sunisa Krainara, Pakarang Srimee**

Department of Health promotion, Faculty of Physical Therapy, Srinakharinwirot University, Ongkharak Campus, Nakhon Nayok 26120, Thailand

Received 21 Feb 2025
Accepted 14 May 2025

Keywords:
Pseudomonas aeruginosa;
Acanthamoeba spp.;
Co-infection;
Dust,
Eastern Thailand

***Correspondence**
Email:
Pattakorn@g.swu.ac.th

Abstract

Background: We investigated the prevalence and environmental factors associated with co-infection of *Pseudomonas aeruginosa* and *Acanthamoeba* spp. in dust from public parks in Eastern Thailand, where industrial activity and air pollution may enhance pathogen survival and pose public health risks.

Methods: A cross-sectional study was conducted from May to September 2023, with 336 dust samples collected from public parks in seven provinces of Eastern Thailand. Samples were analyzed using microscopy and PCR for *Acanthamoeba* spp. detection, and co-infection with *P. aeruginosa* was confirmed by PCR. Environmental data, including temperature, humidity, $PM_{2.5}$ and PM_{10}, were analyzed for associations with infection rates.

Results: The prevalence of *Acanthamoeba* spp. was 22.32%, with 75 samples positive microscopically and 43 confirmed by PCR. Of these, 57.33% showed co-infection with *P. aeruginosa*. Prachinburi province had the highest co-infection rate (75%). Temperature was significantly associated with co-infection rates (P= 0.02), while humidity, $PM_{2.5}$ and PM_{10} showed no significant correlations.

Conclusion: This study highlights the prevalence of *P. aeruginosa* and *Acanthamoeba* spp. co-infection in Eastern Thailand's public parks, emphasizing its link to temperature. The findings underscore the role of dust as a pathogen reservoir and the need for public health strategies to reduce exposure risks.

Introduction

Co-infection with parasites and bacteria like *Pseudomonas aeruginosa* is a public health concern due to increased disease severity and treatment complexity (1). *P. aeruginosa*, a Gram-negative bacterium found in the environment (2), causes infections in immunocompromised patients (3, 4), forms bio-

films and exhibits multidrug resistance, complicating management (5, 6).

On the other hand, *Acanthamoe*ba spp. are free-living amoebae found in various environments, including soil, freshwater, seawater, and dust (7, 8). These amoebae can cause severe diseases, such as Acanthamoeba keratitis, an eye infection that can lead to vision loss if not promptly treated (9). Additionally, they can cause Granulomatous Amoebic Encephalitis (GAE), a severe central nervous system infection with a high mortality rate (10). Although infections caused by *Acanthamoeba* spp. are relatively rare, they are challenging to treat due to the amoebae's ability to thrive in various environmental conditions, making them pathogens of particular concern in medical and public health research (11). Given these factors, *Acanthamoeba* spp. should receive considerable attention in medical and public health research due to its potential to cause significant infections.

Airborne particulate matter, especially $PM_{2.5}$, poses serious health risks in Thailand due to its ability to penetrate deep into the respiratory system, causing lung and cardiovascular diseases (12). Particulate matter can also harbor pathogenic microorganisms, including *P. aeruginosa* and *Acanthamoeba* spp., raising public health concerns. In Eastern Thailand, where rapid industrialization increases dust pollution, these pathogens may spread easily, especially affecting vulnerable groups such as children, the elderly, and those with chronic illnesses. This underscores the urgent need for preventive measures in high-risk areas.

Co-infection of *P. aeruginosa* and *Acanthamoeb*a spp. in PM2.5-rich areas like Eastern Thailand suggests a strong epidemiological link between air pollution and pathogen transmission. Dust particles may carry these microbes, increasing respiratory infection risks, especially among vulnerable populations in rapidly industrializing and polluted environments.

This study highlights the infection risks of *P. aeruginosa* and *Acanthamoeba* spp. co-infection from dust in Eastern Thailand, where pollution is high. The findings offer vital insights for public health officials to guide prevention strategies and hygiene measures aimed at reducing dust-related health risks at both local and national levels.

Materials & Methods

Study Design and Environmental Monitoring for Dust Sampling

A cross-sectional study was conducted from March to May 2023 across seven provinces in Eastern Thailand (Chonburi, Rayong, Chanthaburi, Trat, Chachoengsao, Prachinburi, and Sa Kaeo). Public parks were selected as sampling sites due to accessibility and potential as dust-borne pathogen reservoirs. Environmental data, including average temperature (34 °C), relative humidity (63.57%), wind speed (13.23 km/h), PM2.5 (35.09 μg/m³), and PM10 (59.28 μg/m³), were collected using standardized meteorological equipment (13, 14). A total of 336 dust samples were obtained from three parks per province (48 per park), using sterile brushes to collect approximately 0.1 g of dust from surfaces such as benches, pathways, and playground equipment (15, 16). To preserve microbial integrity, samples were immediately sealed in sterile containers, labeled, and stored under controlled conditions. Sunlight and moisture were avoided during collection. All samples were then transported to the Faculty of Physical Therapy, Srinakharinwirot University, for microbiological analysis.

Cultivation of Acanthamoeba spp.

The dust samples were prepared and inoculated onto 1.5% non-nutrient agar (NNA) plates pre-covered with heat-inactivated *Escherichia coli* (ATCC 25922) (7). The *E. coli* cultures were heat-inactivated at 60 °C for 1 hour before being applied to the agar. A 0.1 mL

aliquot of the *E. coli* suspension was inoculated onto each NNA plate. The plates were then incubated at 25 °C to grow Acanthamoeba trophozoites (17). Observations for the presence of trophozoites were carried out every 48 hours for up to one week using an inverted microscope (Olympus IX71, Tokyo, Japan) [8]. Once trophozoites were identified, the samples were considered positive for *Acanthamoeba* spp. Positive samples were subcultured by transferring a portion of the original sample onto fresh NNA plates, following the same inoculation and incubation procedures [10]. The amoeba genera were identified based on the morphological characteristics of cysts and trophozoites, noting key factors such as the type of movement and specific morphological features (18). Each isolate was classified into its corresponding morphological group according to established taxonomic criteria (19).

Detection of Acanthamoeba spp. Using PCR

DNA extraction from *Acanthamoeba*-positive samples, confirmed via microscopy, was carried out using the Qiagen Amp DNA Micro Kit (Qiagen, Germany), following the manufacturer's protocol. The process began by placing each sample into Eppendorf tubes, where 200 µL of lysis buffer was added, followed by 20 µL of Proteinase K. The samples were incubated in a dry block at 56°C for 10 minutes. After incubation, 200 µL of absolute ethanol was added to each tube, and the mixture was centrifuged at 8,000 x g for 1 minute and 30 seconds. The supernatant was discarded, and 500 µL of RW1 buffer was introduced into each sample. After centrifugation, the supernatant was discarded again, and 500 µL of RW2 buffer was added. The final centrifugation was carried out, after which the supernatant was discarded, and 50 µL of elution buffer was added. DNA was then eluted by centrifugation and stored at -20°C.

The concentration and purity of the extracted DNA were measured using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). Specific primers targeting the 18S ribosomal RNA gene of *Acanthamoeba* spp. were used for PCR amplification. The forward primer, JDP1 (5'-GGCCC ATC GTTTA CCGT GAA-3'), and reverse primer, JDP2 (5'-TCTC ACAA GCTGCTAGGG-GAGTCA-3'), amplified a 490 bp fragment specific to *Acanthamoeba* spp. (20).

PCR reactions were performed in a total volume of 20 µL, which included Taq DNA polymerase, 10 ng of template DNA, 10 pmol of each primer, and distilled water. Amplifications were conducted using a BIO-RAD PCR thermocycler under the following conditions: initial denaturation at 96 °C for 2 minutes, followed by 35 cycles of denaturation at 96 °C for 1 minute, annealing at 60 °C for 1 minute, and extension at 72 °C for 1 minute. A final extension was performed at 72 °C for 5 minutes (20).

The PCR products were separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide (0.5 ng/mL), and visualized under ultraviolet light to detect specific bands confirming the presence of *Acanthamoeba* spp.

Detection of Co-Infection with Pseudomonas aeruginosa

Samples that tested positive for *Acanthamoeba* spp. PCR analyses were subsequently prepared for further detection of co-infection. These samples were also examined for the presence of *P. aeruginosa* to assess the potential for co-infection between the two pathogens. The presence of *P. aeruginosa* was confirmed through a PCR assay using specific primers designed to target a unique region of the bacterium. The forward primer PA-SSF (5'-GGG GGA TCT TCG GAC CTC A-3') and the reverse primer PA-SSR (5'-TCC TTA GAG TGC CCA CCC G-3') were employed to amplify a 956 bp fragment specific to *P. aeruginosa* (21).

PCR amplification was performed in a 20 µL reaction mixture containing Taq DNA polymerase,

10 ng of DNA template, 10 pmol of each primer, and distilled water. The amplification followed a specific program using a PCR thermocycler, starting with an initial denaturation step at 95 °C for 2 minutes. This was followed by 35 cycles of denaturation at 95 °C for 1 minute, annealing at 58 °C for 1 minute, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 5 minutes (21).

Following PCR amplification, the products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide (0.5 ng/mL). Visualization under ultraviolet light allowed for the identification of specific bands corresponding to *Acanthamoeba* spp. and *P. aeruginosa*. The detection of both bands in the same sample confirmed the co-infection of *Acanthamoeba* spp. and *P. aeruginosa*, indicating the presence of both pathogens simultaneously in the analyzed samples.

Statistical Analysis

The prevalence of *Acanthamoeba* spp. and co-infection with *P. aeruginosa* was calculated as proportions and expressed as percentages of the total number of dust samples analyzed. Descriptive statistics, including means and standard deviations, summarized environmental data (temperature, relative humidity, wind speed, and particulate matter concentrations). These environmental factors were correlated with *Acanthamoeba* spp. and co-infection rates using Pearson's correlation coefficient. The dependent variables included the presence of *Acanthamoeba* spp. and co-infection status, while the independent variables comprised environmental factors such as temperature, humidity, $PM_{2.5}$ and PM_{10} levels. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the strength of these associations. The chi-square (χ^2) test evaluated the relationship between the detection of *Acanthamoeba* spp. and *P. aeruginosa* and categorical variables, including dust sample location (public parks) and surface type (benches, pathways, playground equipment). Statistical significance was set at $P < 0.05$.

Results

Prevalence of Acanthamoeba spp. in Different Provinces of Eastern Thailand

We examined various environmental factors influencing the presence and distribution of pathogens in dust across seven provinces in Eastern Thailand. The temperature ranged from 29 to 37 °C, averaging 34 °C, while humidity levels varied from 29% to 100%, averaging 63.57%. Wind speeds averaged 13.23 km/h. Additionally, the average levels of $PM_{2.5}$ and PM_{10} were notable, at 35.09 μg/m³ and 39.29 μg/m³, respectively. Particulate matter can serve as a vehicle for microbial pathogens, with $PM_{2.5}$ posing a particular respiratory risk due to its ability to penetrate deeper into the lungs in Table 1.

Table 1: Summary of environmental conditions measured in eastern Thailand during the study period.

Category	Minimum	Maximum	Average
Temperature (°C)	29	37	34
Humidity (%RH)	29	100	63.57
Wind speed (Km/h)	9.3	18.1	13.23
PM 2.5 (μg/m³)	21.1	56.3	35.09
PM 10 (μg/m³)	35	103	39.29

From a total of 336 dust samples collected from public parks in each province, the study found an overall prevalence of *Acanthamoeba* spp. at 22.32%. PCR tests confirmed the pathogen in 43 samples (57.33%). This high confirmation rate through PCR demonstrates the method's

effectiveness for detecting pathogens in environmental samples. Among the provinces, Chanthaburi reported the highest prevalence of *Acanthamoeba* spp. at 25%, followed by Trat and Chonburi at 16.67% each. Lower prevalence rates were found in Chachoengsao, Prachin Buri, and Sa Kaeo, with Rayong exhibiting the lowest prevalence at 2.08% in Fig. 1.

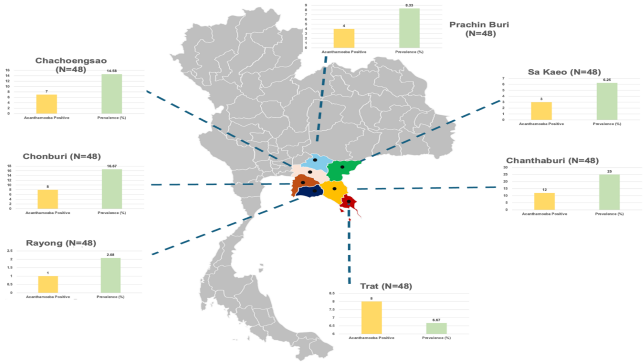

Fig. 1: Prevalence of *Acanthamoeba* spp. in Different Provinces of Eastern Thailand.

The co-infection rate of Acanthamoeba spp. and P. aeruginosa in each province of eastern Thailand was assessed using polymerase chain reaction (PCR)

The presence of *P. aeruginosa* was confirmed using a PCR assay rather than culture methods. In order to confirm the presence of *Acanthamoeba* spp. and *P. aeruginosa* in the environmental dust samples, PCR amplification was performed using species-specific primers targeting 490 bp and 956 bp gene fragments, respectively. The gel electrophoresis image in Fig. 2 illustrates representative samples including positive and negative controls, as well as samples showing single and co-infection patterns.

Bands at approximately 490 bp confirm the presence of *Acanthamoeba* spp., while bands at 956 bp confirm the presence of *P. aeruginosa*. Samples showing both bands indicate co-infection. The results of PCR amplification were consistent across replicates, supporting the validity of the assay.

The results showed significant geographical variation in co-infection rates across the regions. Prachin Buri had the highest co-infection rate at 75%, followed by Sa Kaeo at 66.67%. Chonburi and Trat recorded co-infection rates of 62.50% in Fig. 3. In contrast, Rayong reported no co-infection (0.00%), highlighting notable differences in pathogen detection among the provinces.

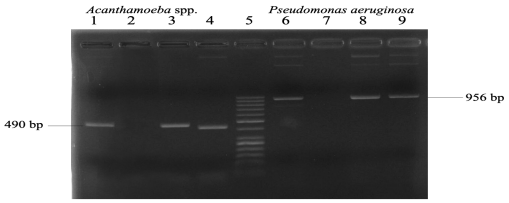

Fig. 2: Gel electrophoresis analysis of PCR amplification products for the detection of *Acanthamoeba* spp. and *P. aeruginosa*

Note: Lane 1 = *Acanthamoeba* spp. Positive control; Lane 2 = *Acanthamoeba* spp. Negative control; Lane 3 = sample DCBT 9-2; Lane 4 = sample DCTT 13-1; Lane 5 = 50 bp marker; Lane 6 = *P. aeruginosa* Positive control; Lane 7 = *P. aeruginosa* Negative control; Lane 8 = sample DCBT 9-2 (co-infection); Lane 9 = sample DCTT 13-1 (co-infection)

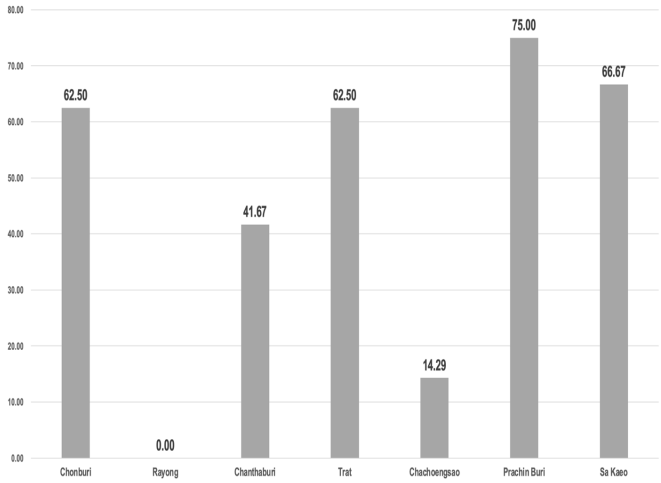

Fig. 3: Co-Infection Rates of *Acanthamoeba* spp. and *P. aeruginosa* in Dust Samples from Eastern Thailand

The relationship between environmental factors and co-infection between Acanthamoeba spp. and P. aeruginosa

The Table 2. displays the chi-squared test results, which examined the relationship between environmental factors and the co-infection rate of *P. aeruginosa* and *Acanthamoeba* spp. Among the factors analyzed, only temperature demonstrated a statistically significant association with co-infection rates (χ^2 = 7.79, *P*= 0.02).

Table 2: Statistical analysis of environmental factors associated with co-infection rates

Category	χ^2	*p*-value
Temperature (°C)	7.79	0.02
Humidity (%RH)	4.96	0.08
PM 2.5 (µg/m³)	1.01	0.60
PM 10 (µg/m³)	2.40	0.30

Environmental factors and co-infection rates of Acanthamoeba spp. and P. aeruginosa in the provinces of eastern Thailand

According to the graph depicting co-infection rates between *Acanthamoeba* spp. and *P. aeruginosa* across various provinces and temperature ranges, Chonburi, Chanthaburi, and Chachoengsao experienced the highest temperature range (35-37°C). Chonburi and Chanthaburi reported the highest co-infection rates among these provinces at 23.81%. In contrast, in Trat, co-infection rates were distributed across different temperature ranges, with 4.76% found in the 29-31°C range and 19.05% in the 32-34°C range. This suggests that temperature may influence the co-infection rate.

When analyzing relative humidity, Prachin Buri and Chachoengsao exhibited the highest levels (86-100%), with co-infection rates of 14.29% and 4.76%, respectively. In contrast, Chonburi and Trat had the same co-infection rate of 23.81% within a relative humidity range of 50-70%. Additionally, Chonburi dis-

played the highest co-infection rate of 23.81% at a lower relative humidity range of 25-40%.

Regarding wind speed, the highest wind speed range (17.8-20.0 km/h) was observed in Sa Kaeo, where a co-infection rate of 9.52% was recorded. In Trat, the co-infection rate was distributed across different wind speeds, with a rate of 19.05% in the 9.0-11.2 km/h range and the highest rate of 4.76% in the 11.2-13.4 km/h range.

When examining co-infection rates between *Acanthamoeba* spp. and *P. aeruginosa* based on $PM_{2.5}$ levels (µg/m³), Chanthaburi, Chonburi, and Trat had the highest rates at 23.81%, corresponding to $PM_{2.5}$ ranges of 20-28 µg/m³ and 44 µg/m³, respectively. In contrast, Chachoengsao exhibited a lower co-infection rate of 4.76% despite having the highest PM_{10} concentration in the 78-93 µg/m³ range. It was also noted that Rayong, Chanthaburi, and Chonburi had the highest co-infection rates (23.81%) within the PM_{10} range of 30-35 µg/m³ and 46-61 µg/m³, respectively in Fig. 4.

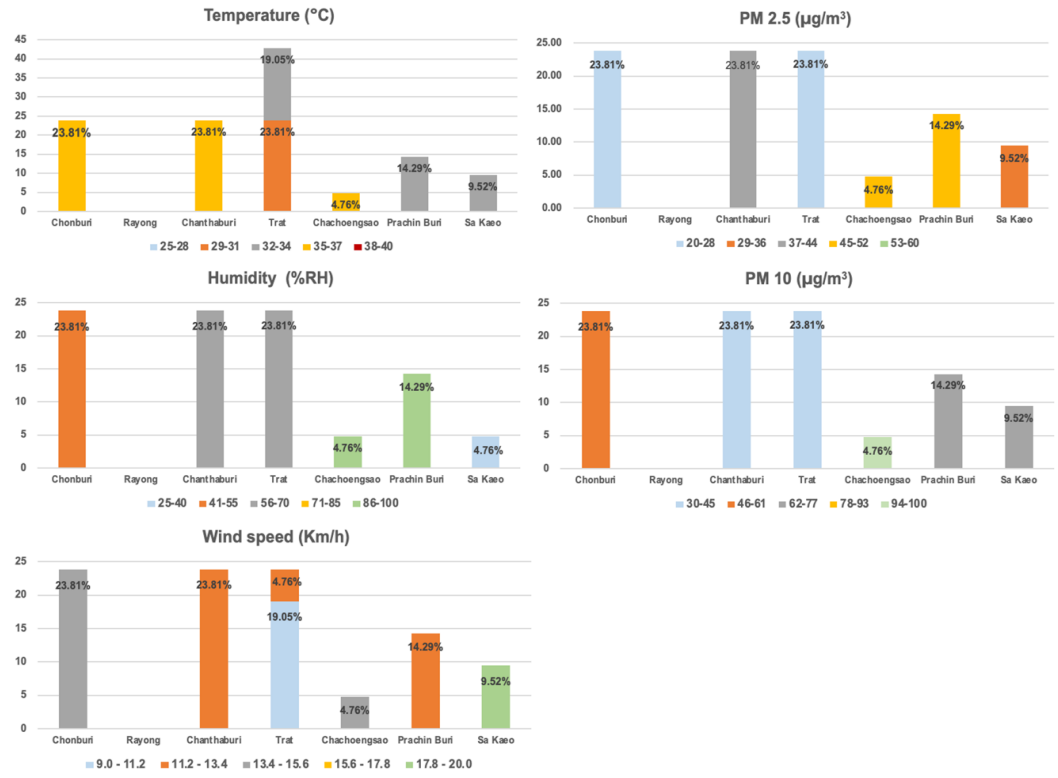

Fig. 4: Environmental Factors and Co-Infection Rates of *Acanthamoeba* spp. and *P. aeruginosa* in Eastern Thailand

Discussion

This study detected *Acanthamoeba* spp. and co-infection with *P. aeruginosa* in dust samples collected from public parks across seven provinces in Eastern Thailand, indicating a potential public health risk. Notably, high co-infection rates were observed in Prachinburi (75%), Sa Kaeo (66.67%), Chonburi, and Trat (62.50%). This finding is significant, as *Acanthamoeba* spp. is an opportunistic pathogen that can lead to severe infections, particularly in immunocompromised individuals, such as meningitis and keratitis (9, 10). Meanwhile, *P.*

aeruginosa is a well-known pathogen responsible for infections in the respiratory and urinary tracts, as well as wound infections (3). Co-infection with both pathogens could complicate treatment and increase disease severity, underscoring the need for effective environmental monitoring and control to minimize public health risks.

The results align with previous studies that identified *Acanthamoeba* spp. in environmental samples such as dust, soil, and water, particularly in tropical regions (7, 8). However, limited data exist regarding co-infection with *P. aeruginosa* in dust in Thailand. Our study found a statistically significant positive correlation between co-infection rates and average provincial temperature, supporting the hypothesis that higher temperatures may increase the prevalence of co-infection. In contrast, humidity, $PM_{2.5}$, and PM_{10} levels showed no significant associations with co-infection rates, suggesting these factors may not critically influence co-occurrence of these pathogens. This highlights temperature as a key environmental factor, although future studies should also consider variables such as air pollution, dust concentration, and urban activity to better understand microbial persistence.

The presence of these pathogens in publicly accessible areas poses risks of community-acquired infection, particularly among vulnerable populations such as people with diabetes, HIV, and the elderly (4, 18). These findings emphasize the need for proactive, area-specific public health strategies based on contamination levels in each province. Continued environmental surveillance is essential to guide effective intervention and prevention planning.

From a microbiological perspective, *Acanthamoeba* spp. can act as both host and nutrient source for *P. aeruginosa*, promoting bacterial survival under unfavorable environmental conditions (22, 23). This interaction protects *P. aeruginosa* from stressors such as nutrient depletion and disinfectants and may enhance its antibiotic resistance and ability to evade immune responses (24). Moreover, *Acanthamoeba* spp. may increase the pathogenicity of *P. aeruginosa* by promoting biofilm formation and toxin production (11, 18), consistent with our findings in warmer provinces.

Several case reports have documented co-infections involving these pathogens, particularly in contact lens–associated keratitis. One such case involved a 20-year-old female who presented with eye pain, photophobia, and mucopurulent discharge. Initially diagnosed with *P. aeruginosa*, further examination confirmed the presence of both pathogens in corneal scrapings and contact lens storage solution. The patient was treated with topical antibiotics and anti-amoebic agents, resulting in full recovery within seven weeks (25). Additional reports describe atypical co-infections with clinical signs such as perineuritis and epitheliitis, stressing the importance of early differential diagnosis to prevent long-term complications (26). A case from South Africa further highlights the environmental relevance, documenting co-infection with *Acanthamoeba polyphaga* and *P. aeruginosa* in a contact lens wearer (27). These cases reinforce the clinical significance of environmental exposure and its potential to cause severe disease, particularly in immunologically vulnerable tissues such as the cornea.

This study has some limitations. Dust samples were collected from only one or two public parks per province, which may not represent the overall environmental conditions of each area. Additionally, species-level identification of *Acanthamoeba* spp. and *P. aeruginosa* was not conducted, which may be important in understanding pathogenic diversity and antimicrobial susceptibility. Future studies should expand the sampling area to include residential zones, schools, and healthcare facilities, and incorporate molecular typing techniques to better assess pathogen diversity and potential risk (8, 13).

Conclusion

This study reveals a high prevalence of *Acanthamoeba* spp. and co-infection with *P. aeruginosa* in dust samples from public parks in Eastern Thailand, with the highest co-infection rate observed in Prachin Buri. Temperature was significantly associated with co-infection rates, suggesting it plays a key role in the survival and transmission of these pathogens. In contrast, humidity and particulate matter showed no significant correlation. These findings highlight environmental dust as a potential reservoir for pathogenic co-infections, posing public health risks especially for immunocompromised individuals. Continued environmental surveillance and expanded research, including molecular typing, are recommended to inform effective prevention strategies.

Acknowledgements

This research was supported by the Department of Health Promotion, Faculty of Physical Therapy, Srinakharinwirot University, for providing research resources and laboratory facilities. We gratefully acknowledge the Strategic Wisdom and Research Institute, Srinakharinwirot University, for financial support (Contract No. 230/2566), which enabled the project's implementation. Sincere thanks also go to the team involved in data collection and analysis, as well as our colleagues for their valuable feedback and encouragement throughout the study.

Conflict of Interest

The authors declare that there is no conflict of interests.

References

1. Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage *Pseudomonas aeruginosa* infections. Drugs Context. 2018; 7:212527.
2. Silby MW, Winstanley C, Godfrey SAC, Levy SB, Jackson RW. *Pseudomonas* genomes: Diverse and adaptable. FEMS Microbiol Rev. 2011; 35(4):652-80.
3. Poole K. *Pseudomonas aeruginosa*: Resistance to the max. Front Microbiol. 2011; 2:65.
4. Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. Clin Microbiol Rev. 2002; 15(2):194-222.
5. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Science. 1999; 284(5418):1318-22.
6. Breidenstein EB, de la Fuente-Núñez C, Hancock RE. *Pseudomonas aeruginosa*: All roads lead to resistance. Trends Microbiol. 2011; 19(8):419-426.
7. Khan NA. *Acanthamoeba*: Biology and increasing importance in human health. FEMS Microbiol Rev. 2006; 30(4):564-595.
8. Nuprasert W, Putaporntip C, Pariyakanok L, Jongwutiwes S. Identification of a novel T17 genotype of *Acanthamoeba* from environmental isolates and T10 genotype causing keratitis in Thailand. J Clin Microbiol. 2010; 48(12):4636-4640.
9. Lorenzo-Morales J, Khan NA, Walochnik J. An update on *Acanthamoeba keratitis*: Diagnosis, pathogenesis and treatment. Parasite. 2015; 22:10.
10. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. FEMS Immunol Med Microbiol. 2007; 50(1):1-26.
11. Siddiqui R, Khan NA. Biology and pathogenesis of *Acanthamoeba*. Parasit Vectors. 2012; 5:6.
12. Kim KH, Kabir E, Kabir SA. A review on the human health impact of airborne particulate matter. Environ Int. 2015; 74:136-43.
13. Tong H, Lighthart B. The annual bacterial particle concentration and size distribution in the ambient atmosphere in a rural area of the Willamette Valley, Oregon. Aerosol Science and Technology. 2000; 32(5):393–403.

14. Buttner MP, Stetzenbach LD. Monitoring airborne fungal spores in an experimental indoor environment to evaluate sampling methods and the effects of human activity on air sampling. Appl Environ Microbiol. 1993;59(1):219-26.
15. Chen C, Zhao B. Review of relationship between indoor and outdoor particles: I/O ratio, infiltration factor and penetration factor. Atmospheric Environment. 2011; 45(2):275-88.
16. Lee T, Grinshpun SA, Martuzevicius D, Adhikari A, Shukla R, Reponen T. Culturability and concentration of airborne fungi in six diverse indoor environments. Environ Health Perspect. 2006; 114(5):750-757.
17. Schuster FL, Visvesvara GS. Free-living amoebae as opportunistic and non-opportunistic pathogens of humans and animals. Int J Parasitol. 2004; 34(9):1001-1027.
18. Marciano-Cabral F, Cabral GA. *Acanthamoeba* spp. as agents of disease in humans. Clin Microbiol Rev. 2003;16(2):273-307.
19. Pussard M, Pons R. Morphologie de la paroi kystique et taxonomie du genre *Acanthamoeba* (Protozoa, Amoebida). Protistologica. 1977; 13(4):557-598.
20. Schroeder JM, Booton GC, Hay J, et al. Use of subgenic 18S ribosomal DNA PCR and sequencing for genus and genotype identification of *Acanthamoeba* from humans with keratitis and from sewage sludge. J Clin Microbiol. 2001; 39(5):1903-1911.
21. Spilker T, Coenye T, Vandamme P, LiPuma JJ. PCR-based assay for differentiation of *Pseudomonas aeruginosa* from other *Pseudomonas* species recovered from cystic fibrosis patients. J Clin Microbiol. 2004; 42(5):2074-2079.
22. Greub G, Raoult D. Microorganisms resistant to free-living amoebae. Clin Microbiol Rev. 2004; 17(2):413-433.
23. Wang X, Ahearn DG. Effect of bacteria on survival of *Acanthamoeba castellanii* in saline solution. Curr Microbiol. 1997; 34(4):212-215.
24. Matz C, Bergfeld T, Rice EW, Kjelleberg S. Microcolonies, quorum sensing and cytotoxicity determine the survival of *Pseudomonas aeruginosa* biofilms exposed to protozoan grazing. Environ Microbiol. 2004; 6(3):218-26.
25. Sharma R, Jhanji V, Satpathy G, et al. Coinfection with *Acanthamoeba* and *Pseudomonas* in contact lens–associated keratitis. Optom Vis Sci. 2013;90(2):e53–e5.
26. Chuang YH, Wang YC, Yen CY, et al. Unusual presentation of *Acanthamoeba* coinfection in the cornea. Optom Vis Sci. 2022;99(7):605–611.
27. Dini LA, Cockinos C, Frean JA, Niszl IA. *Acanthamoeba polyphaga* and *Pseudomonas aeruginosa* keratitis in a contact lens wearer. J Clin Microbiol. 2000;38(2):826–829.