

Designing an eDNA Sampler to Provide Health Surveillance through Detection & Identification of Bacterial Pathogens in Rural Communities

Introduction & Background

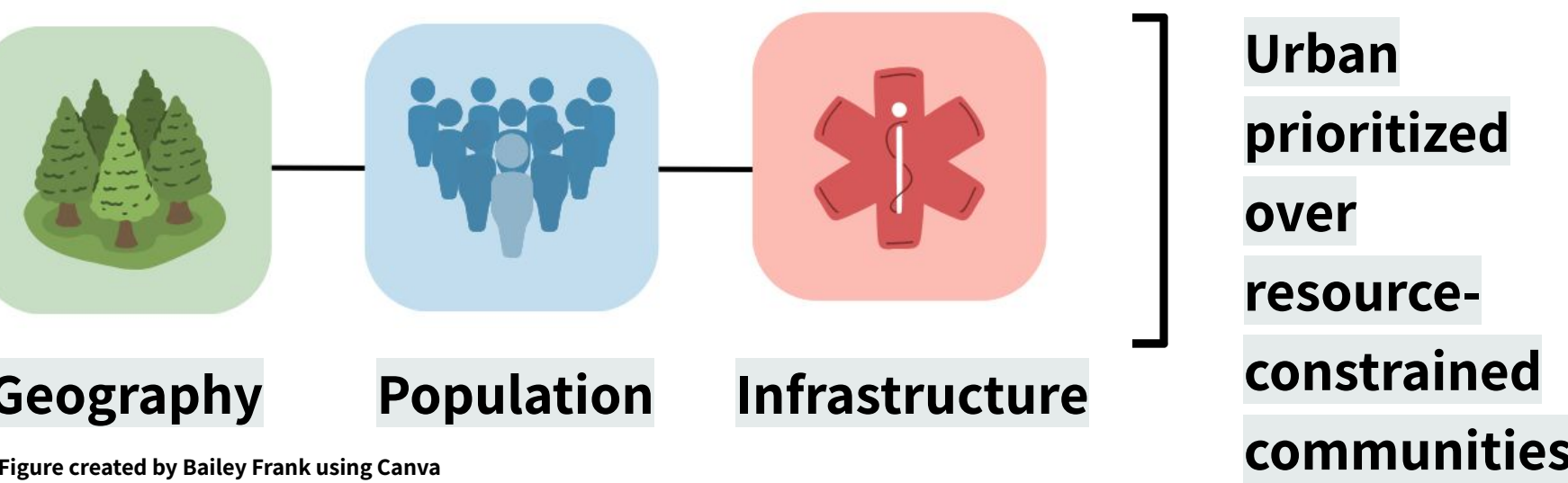
Health surveillance plays a critical role in public health through detection and analysis of health-related data, working to identify and prevent the spread of disease. The goal and purpose of health surveillance is to reduce human mortality and morbidity rates.

“Limited disease surveillance in remote rural areas is a crucial gap in global health security” (Worsley-Tonks, et al., 2022)

Current methods & drawbacks of rural health surveillance:

Collection:	Data:	Frequency:	Sources:	Analysis:	Limitations:
Mostly passive, routine reporting	Diseases, tests, deaths, vaccinations	Weekly, monthly, or annually	Clinics, labs, hospitals, community	Identifies disease trends and outbreaks	Underreporting, limited access, delays, incomplete

Low resource communities are at a natural disadvantage due to environmental factors:

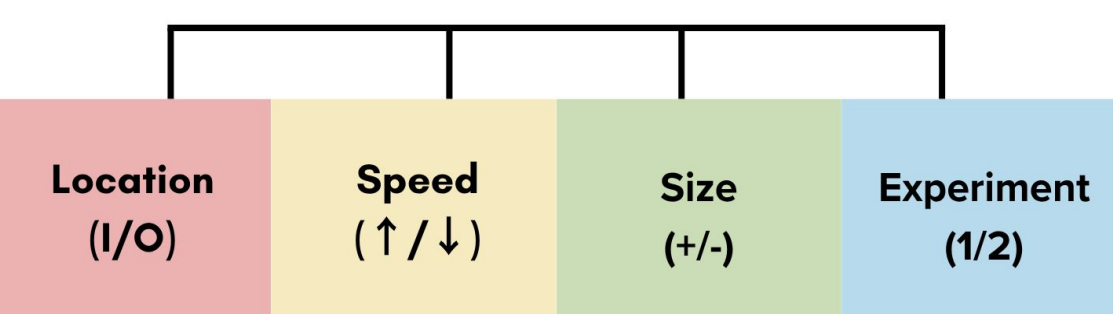


Potential solution: Use of eDNA as a substitute for case surveillance data to decrease cost, time, and resources

Research Question

Can an eDNA sampler created from simple and inexpensive parts collect sufficient DNA to determine if pathogenic bacteria are present?

10+ ↓, 10- ↓, 10- ↑, 10+ ↑, 11+ ↓, 11- ↓, 11+ ↑, 20+ ↓, 20- ↓, 20- ↑, 20+ ↑, 21+ ↓, 21- ↓, 21- ↑, 21+ ↑

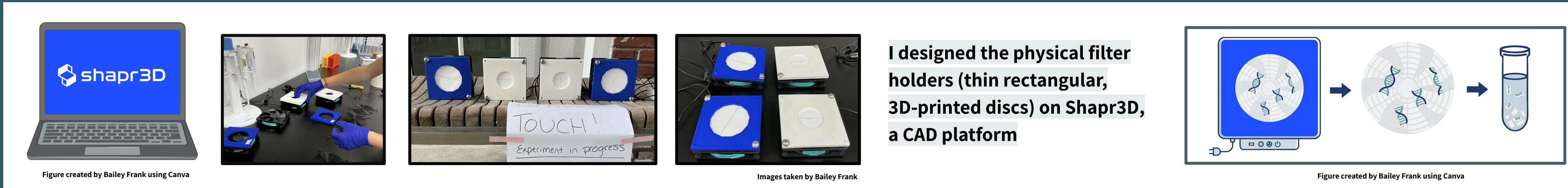


Controls:

- All samplers ran for 3 hours
- Brand and size of base fan
- Estimated amount of time between collection of data and DNA purification

I hypothesize that the eDNA sampler will successfully collect environmental DNA during a three-hour sampling period and that variables such as filter paper size and fan speed will influence DNA concentration, with larger filters and higher fan speeds producing higher yields. I also predict that outdoor samples will have lower eDNA concentrations than indoor samples due to environmental dispersion. Lastly, I expect that my 16S rRNA sequencing results will generally align with the bacterial culture findings at the genus, and potentially species, level.

Design of eDNA Collection Samplers & Sample Collection



Bacterial Culture & Colony Identification

PETRI DISH: BACTERIA PROGRESS AFTER ONE WEEK (OUTDOORS)				PETRI DISH: BACTERIA PROGRESS AFTER ONE WEEK (INDOORS)			
Sample Name:	Quantity of Colonies:	Quantity of Species:	Potential Species Identified (based on visual observations):	Sample Name:	Quantity of Colonies:	Quantity of Species:	Potential Species Identified (based on visual observations):
20+ ↓	4	3	Bacillus subtilis, Staphylococcus epidermis, Micrococcus luteus	21+ ↓	5	3	Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermis
20- ↓	2	2	Bacillus subtilis, Micrococcus luteus	21- ↓	2	1	Bacillus subtilis
20+ ↑	3	1	Bacillus subtilis	21+ ↑	9	1	Bacillus subtilis
20+ ↑	3	2	Staphylococcus aureus, Staphylococcus epidermis	21+ ↑	8	2	Bacillus subtilis, Staphylococcus epidermis

Average number of outdoor colonies ~ 3
*Staphylococcus aureus: Pathogenic, skin or bloodstream infections, pneumonia, etc.
*Staphylococcus epidermidis: Opportunistic pathogen (potential source of infection)

Average number of indoor colonies ~ 6
*Staphylococcus aureus: Pathogenic, skin or bloodstream infections, pneumonia, etc.
*Staphylococcus epidermidis: Opportunistic pathogen (potential source of infection)

SPECIES:	SIZE:	COLOR & OPACITY:	SHAPE:	TEXTURE:	EXTRA:
Bacillus subtilis	Large, 3-7mm or more post 24-48 hours	Off-white, cream, opaque	Irregular, uneven edges	Dry, irregular, rough, cracked, often matte	Powdery or frosted, can spread out over time
Staphylococcus epidermis	Small or Medium, 1-2 mm	White, off-white, opaque	Circular	Smooth, moist, slightly shiny or glossy	Colonies look neat and uniform, no pigment
Staphylococcus aureus	Medium (2-4 mm)	Creamy, golden colonies, opaque	Circular	Smooth, creamy, moist, shiny	Color is deeper than S.epidermis
Micrococcus luteus	Small, 1-2mm	Color ranges, reddish-brown	Circular	Smooth, buttery, dry or slightly shiny	Uniform surface

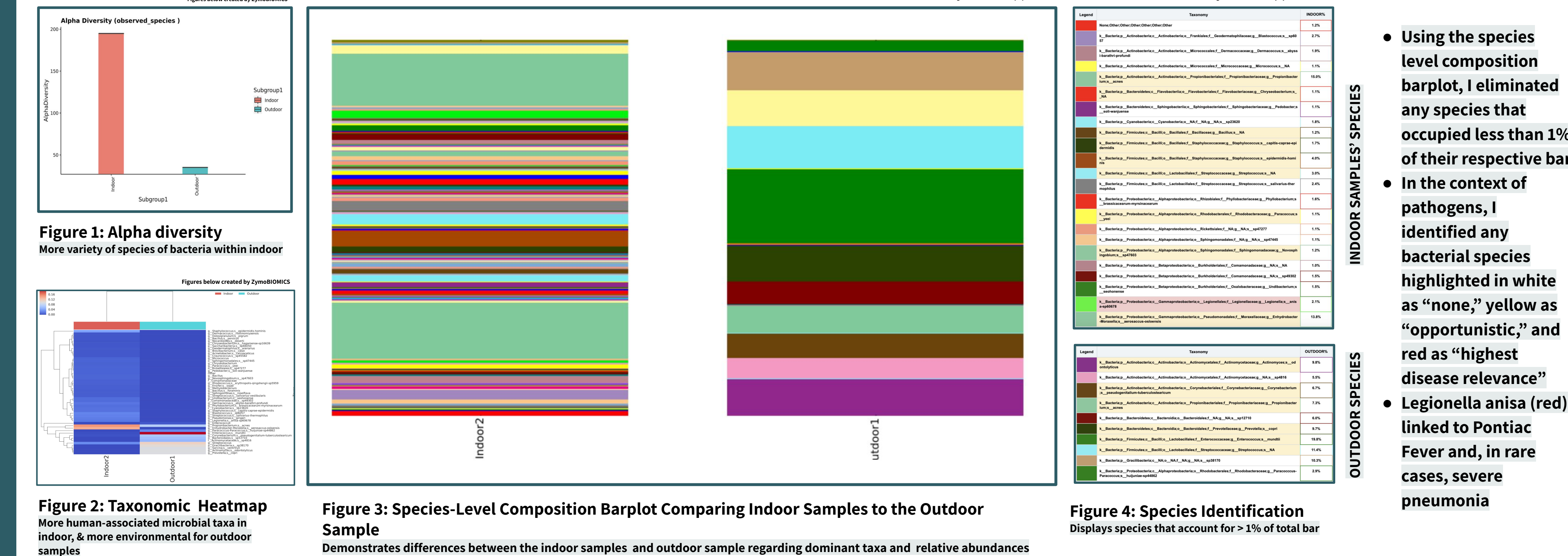
The table displayed above is a rough guide to the most commonly-occurring species of bacteria based on their visual characteristics and basic background / typical environment.

DNA Purification Methodology, Nanodrop Spectrophotometer, & 16S Sequencing Results

Methodology		Nanodrop Concentration Results			
Cut up the filter paper into quarters, cut up one quarter into tiny paper fragments	Placed paper pieces into tube with lysis solution and bashing beads, provided by kit	Put testing tubes containing samples & beads into Vortex Genie with Vortex Adaptor	Continued adding more solutions & chemicals, centrifuging after each step when needed	Pipetted solutions into test tubes, transferred liquid from one tube to another, mixed samples	Nanodrop Spectrophotometer added 1 µl of each sample at a time to test DNA Concentration
		Mailed off samples for external 16S rRNA Sequencing at ZymoBIOGENICS			

Sample Name:	DNA Quantitative Results:	260/280	260/230
10+ ↓	5.6 ng/µl	1.17	0.36
10- ↓	0.6 ng/µl	0.83	1.58
10- ↑	1.0 ng/µl	2.64	0.15
10+ ↑	0 ng/µl	0.07	-0.01
11+ ↓	3.0 ng/µl	1.19	0.2
11- ↓	4.0 ng/µl	2.15	0.16
11+ ↑	3.7 ng/µl	2.13	0.16
11+ ↑	2.6 ng/µl	1.77	0.14

16S rRNA Sequencing Results



Conclusion

- Samplers proved efficient and functional at physically collecting airborne eDNA from both test environments
- Airborne eDNA collected could be successfully extracted and sequenced for bacterial and pathogen identification
- Environmental conditions influenced eDNA collection, with indoor environments yielding higher concentration, purity, diversity, and human-associated pathogens
- 92.94% - 97.4% decrease compared to second surveillance system

	1. Use of eDNA for Health Surveillance in Rural Areas (My Experiment):	2. Typhoid Environmental Surveillance (Hagedorn, et al., 2023)	3. Community Biological Sampling in Rural Vietnam (Ding, et al., 2015)
Cost:	\$15.18**\$9.64 without one-time purchases	\$215-\$584	\$8.63
What Cost DOES NOT Account For:	Labor	N/A	DNA/Molecular Processing, Equipment/facilities

Limitations & Future Work

Variability of School Environment Compared to Target Setting: Project was tested in a school laboratory environment rather than a rural, low-resource community

Infrastructure Dependence: Sequencing was performed externally due to lack of sufficient medical equipment and resources in school; mirrors real world deployment model in which rural communities would send samples out

My project has the potential to be expanded or changed in many different ways due to its adaptability and global scalability:

Type of eDNA & Sequencing

- Viral sequencing: Laboratory process of detecting viruses, detects mutations to track new strains/ variants of concern, monitors how fast a virus spreads, and useful for design of vaccines
- Areas of real-world implementation: Agricultural hubs & farms, remote settlements, wastewater and sanitation outflows & drainage channels, etc.

References

Amman A O A I, Shauran, and Lorenzo Piccarini. "A Review of Pathogenic Airborne Fungi and Bacteria: Unveiling Occurrence, Sources, and Profound Human Health Implication." *Frontiers in Microbiology*, vol. 15, 19 Sept. 2024. <https://doi.org/10.3389/fmicb.2024.1429435>.

Bass, D., Christison, K. W., Stenford, G. D., Cook, L. S. J., & Hartikainen, H. (2023). Environmental DNA/RNA for pathogen and parasite detection, surveillance, and ecology. *Trends in Parasitology*. <https://doi.org/10.1016/j.pt.2022.12.003>

Chen, Li, et al. "Long-Term Variations of Urban-Rural Disparities in Infectious Disease Burden of over 8.44 Million Children, Adolescents, and Youth in China from 2013 to 2021: An Observational Study." *PLoS Medicine*, vol. 21, no. 4, 12 Apr. 2024, pp. e1004374–e1004374. <https://doi.org/10.1371/journal.pmed.1004374>

Childress, J., Faust, C. L., & Diener, K. (2023). Introduction to special issue: Advancing disease ecology through eDNA monitoring of infectious agents. *Environmental DNA*, 6(1). <https://doi.org/10.1007/s12580-023-00020-2>

"Colony Morphology." *Colony Morphology*, 1 Sept. 2007. www.colony-morphology.com/Gallery/Colony-Morphology

Ding, Yan, et al. "Measuring Costs of Data Collection at Village Clinics by Village Doctors for a Syndromic Surveillance System-A Cross Sectional Survey from China." *BMC Health Services Research*, vol. 15, 25 July 2015, p. 287. <https://doi.org/10.1186/s12913-015-0965-2>. PubMed Central. Accessed 29 Sep. 2026.

From, 2023, October 31. Kraken Sense. Kraken Sense. <https://bioinformatics.csi.cmu.edu/kraken-sense/kraken-sense-how-to-use-kraken-sense-bacteria/>

Graham, Bettie. "Bacteria." *Genome.gov*, National Human Genome Research Institute, 2019. www.genome.gov/genetics-glossary/Bacteria

Hagedorn, Brittany, et al. "Estimates of the Cost to Build a Stand-Alone Environmental Surveillance System for Typhoid in Low- and Middle-Income Countries." *PLOS Global Public Health*, edited by Habib Hagan Farooqui, vol. 3, no. 4, Public Library of Science (PLoS), Jan. 2023, p. e0093774. <https://doi.org/10.1371/journal.pgph.0093774>

James, Cara V. "Racial/Ethnic Health Disparities among Rural Adults – United States, 2012–2015." *MMWR. Surveillance Summaries*, vol. 66, no. 23, 2017. <https://www.cdc.gov/mmwr/preview/mmwrhtml/ss6623a1.htm>

Professor Dave Explains. "Taxonomy of Bacteria: Identification and Classification." *www.youtube.com*, 2 Dec. 2019. www.youtube.com/watch?v=81BwPC9ag

Reynolds, Jackie. "Bacterial Colony Morphology." *Biology LibreTexts*, 2016. bio.libretexts.org/Learning_Objects/Laboratory_Experiments/Microbiology_Labs/Microbiology_Labs/106%3A_Bacterial_Colony_Morphology

Singh, A. P., & Gouda, S. (2024). Unveiling Environmental DNA/RNA for Pathogen Detection & Ecology. 5(5), 27–32. <https://doi.org/10.1186/s12913-015-0965-2>

"Rising Rural Mortality Rates from Natural Causes for Working Age Adults Lead to Widening Gap with Urban Counterparts | Economic Research Service." *Usda.gov*, 2025. https://www.ers.usda.gov/amber-waves/2025/march/rising-rural-mortality-rates-from-natural-causes-for-working-age-adults-lead-to-widening-gap-with-urban-counterparts?utm_source=eschatology.com. Accessed 4 Dec. 2025.

Wildlife Surveys Out of Thin Air.pdf. (2019). Wildlife Surveys Out of Thin Air.pdf. Google Docs. <https://drive.google.com/file/d/1P666h-GmM4BtE9U7X2S6Y8bE8dYm/view>

Worsley-Tonks, K. E. L., Bender, J. B., Deem, S. L., Ferguson, A. W., Fèvre, E. M., Martins, D. J., Muloi, D. M., Murray, S., Mutinda, M., Ogada, D., Omendi, G. P., Prasad, S., Wild, H., Zimmerman, D. M., & Hessel, J. M. (2022). Strengthening global health security by improving disease surveillance in remote rural areas of low-income and middle-income countries. *The Lancet Global Health*, 10(4), e579–e584. [https://doi.org/10.1016/s2468-2667\(22\)00031-6](https://doi.org/10.1016/s2468-2667(22)00031-6)

ZymoBIOGENICS DNA Miniprep Kit DNA for microbiome or metagenomic analyses. (n.d.). https://files.zymo-bio.com/protocols/44309_44309_dna_miniprep_kit.pdf