The Feasilbility of Environmental and Wastewater Surveillance for Nipah Virus

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Why Nipah challenges conventional surveillance

- Nipah virus remains one of the most consequential zoonotic pathogens precisely because it does *not* behave like the viruses most surveillance systems are designed for.
- Outbreaks are:
  - Rare
  - Highly focal
  - Often detected late
- Transmission is:
  - Zoonotic
  - Environmentally mediated
  - Strongly shaped by ecology and human behavior

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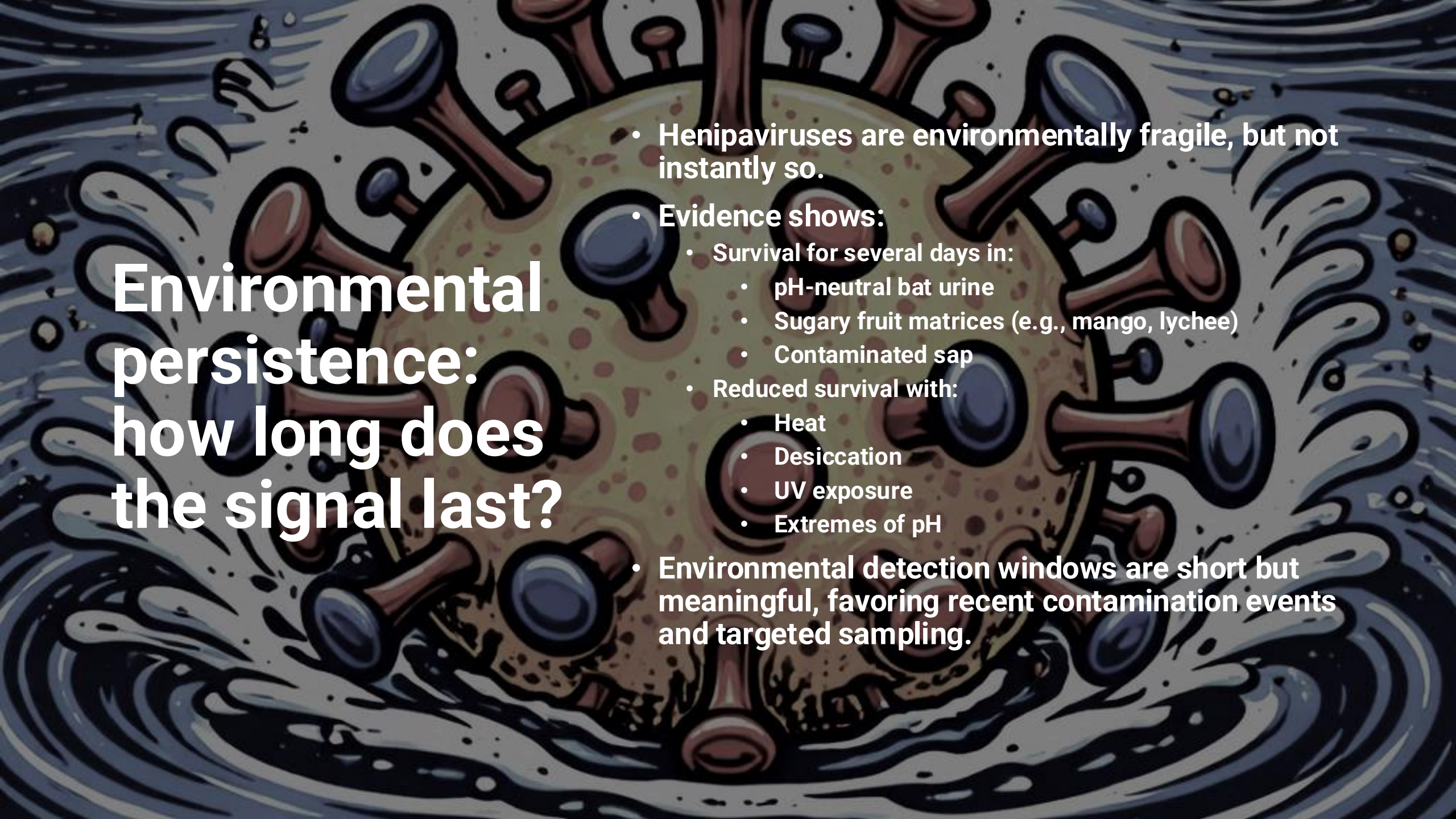
What we mean by environmental detection

- Environmental detection refers to identifying **Nipah virus RNA outside the host**, including:
  - Water and wastewater
  - Air and surfaces
  - Soil and urine-contaminated environments
  - Food items and food-contact surfaces
- Important framing:
  - Detection ≠ infectious virus
  - Detection ≠ human infection
  - Environmental signals are **contextual indicators of risk**
- Environmental surveillance is best understood as an **upstream early-warning layer**, particularly where clinical cases are rare or delayed.



Environmental persistence: how long does the signal last?

- Henipaviruses are environmentally fragile, but not instantly so.
- Evidence shows:
  - Survival for several days in:
    - pH-neutral bat urine
    - Sugary fruit matrices (e.g., mango, lychee)
    - Contaminated sap
  - Reduced survival with:
    - Heat
    - Desiccation
    - UV exposure
    - Extremes of pH
- Environmental detection windows are short but meaningful, favoring recent contamination events and targeted sampling.

Wastewater and Airline Waste Surveillance for Nipah Virus (Where could we look?)

Municipal & Facility Wastewater

- **Best settings**
  - Hospitals and referral centers
  - Encephalitis wards and isolation units
  - Small, defined catchments
  - Livestock interfaces (pig farms, slaughter facilities)
- **Why it could work**
  - Nipah virus RNA is shed in urine, saliva, feces, blood and tissue fluids, contaminated food matricies
  - Wastewater integrates signals from symptomatic and pre-symptomatic individuals
  - Livestock wastewater captures **spillover and amplification risk**

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Wastewater and Airline Waste Surveillance for Nipah Virus (Where could we look?)

Aircraft Wastewater

- **What it adds**
  - Captures signals from travelers before healthcare contact
  - Provides early indication of cross-border movement from outbreak regions
  - Non-invasive and independent of testing compliance
- **Best use**
  - Targeted sampling of flights from areas with known or suspected Nipah activity
  - Situational awareness during regional outbreaks

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But what do we need to detect Nipah in environmental samples?

Key requirements

- Highly sensitive, lineage-inclusive RT-qPCR
- Careful interpretation: detection is **probabilistic, not diagnostic**
- Sequencing confirmation when viral load permits

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Nipah genetic diversity: why lineage matters

Nipah virus is genetically diverse, with two major lineages of public health importance:

- Lineage 1 (Malaysia)
  - Historically associated with pig amplification
- Lineage 2 (Bangladesh)
  - More frequently linked to direct bat-to-human transmission
  - Associated with recent documented human-to-human transmission
- Critical point: Evidence indicates that both Malaysia-like and Bangladesh-like Nipah lineages circulate in Cambodian bat populations.
- Environmental and wastewater assays must:
  - Be lineage-inclusive
  - Avoid primer bias
  - Maintain sensitivity across genetic diversity

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Molecular detection: sensitivity is foundational

Environmental and wastewater samples typically contain **very low concentrations of viral RNA**.

- Effective detection therefore requires:
  - Highly sensitive RT-qPCR assays
  - Careful management of inhibitors
  - Lineage-inclusive primer design

For example:

- Assays capable of detecting **~5–50 genomic copies per reaction**
- Validated across both Bangladesh and Malaysia lineages

RT-qPCR Detection of Nipah Virus (NiV) Targeting the Nucleocapsid (N) Gene

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Sequencing: confirming and contextualizing detection

PCR detection alone is not sufficient.

Sequencing allows:

- Confirmation of specificity
- Lineage assignment
- Monitoring of viral evolution

Practical considerations:

- Amplicon-based sequencing performs well
- Reliable results typically require:
- Ct values ≤29–30
- Samples above this threshold often yield incomplete genomes

Sequencing capacity should be built into surveillance design from the outset.

Sequencing of Nipah Virus (NiV) Using Oxford Nanopore MinION

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Environmental metagenomics: powerful but not sufficient alone

Environmental metagenomics offers important advantages:

- Unbiased detection
- Ability to detect unexpected or novel viruses
- Insight into broader viral ecology

From Cambodia:

- Nipah virus RNA **has been detected in bat urine and environmental samples using metagenomic approaches**
- However:
  - Signal is typically very low
  - Reads are sparse
  - Detection is inconsistent

Metagenomics is excellent for:

- Discovery
- Contextualization
- Hypothesis generation

But for Nipah surveillance, it is best used **in combination with targeted PCR**, not as a standalone screening tool.

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Nipah Virus in Water/Liquids: Documented Detection vs Biologically Plausible Pathways

Documented

Raw date palm sap (Bangladesh) – Nipah virus RNA detected directly in sap collected from pots contaminated by bat saliva and urine

Sap collection vessels (Bangladesh) – Residual liquid inside pots positive for Nipah virus RNA after bat feeding

Surface water beneath bat roosts (Cambodia & Malaysia) – Nipah virus RNA detected in pooled urine-contaminated water under *Pteropus* roosts

Standing water at bat roost sites (Cambodia) – Repeated environmental contamination during peak shedding periods

Water and pasture contaminated by bat excreta (Australia – Hendra virus): Hendra virus detected in water-adjacent environmental materials contaminated by bat urine and feces

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Nipah Virus in Water/Liquids: Documented Detection vs Biologically Plausible Pathways

Biologically Plausible

Farm water and runoff: Virus introduced via bat-contaminated fruit with amplification in pigs, leading to contamination of shared water sources

Drainage water from animal holding and slaughter areas: Contamination via urine, blood, and tissue fluids during pig infection and processing

Human wastewater (outbreak settings): Nipah virus RNA shed in urine during human infection; targeted wastewater surveillance feasible

Mixed human–livestock wastewater systems: Convergence of bat, livestock, and human shedding into shared drainage


Will it work?

Thank You

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