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Q&A – Asia PGI Webinar Series: Enhancing Nipah virus surveillance with wastewater and environmental surveillance?

Speakers: Dr. Farah Ishtiaq, Dr. Erik Karlsson; Moderator: Dr. Vincent Junxiong Pang

1. **Are protocols standardised to detect the (Nipah) virus in environmental samples?**

   Thank you for the question. As far as I understand, there is no standardised protocol for environmental samples yet. Asia PGI aims to facilitate this, as much as possible. Through our collaboration with Dr. Erik Karlsson from IPC, there is now a protocol for detecting Nipah virus using RT-QPCR (https://www.protocols.io/view/rt-qpcr-detection-of-nipah-virus-niv-targeting-the-36wgq1mwkvk5/v1) and NGS (https://www.protocols.io/view/sequencing-of-nipah-virus-niv-using-oxford-nanopor-6qpvryr5pgmk/v1).

2. **What is the shedding rate or replication of this virus in GI in the case of humans?**

   The basic reproduction number (as a measure of transmissibility) of Nipah virus is R_0 = 0.48 (in contrast with COVID-19, with an R_0 of 2.5 -3.5 and up to 7 for variants), with a low potential for self-sustaining viral transmission in human populations. A reproduction number (i.e., the average number of secondary cases per case patient) of 0.33 was shown in this study with 248 cases infected between 2001-2014 (Transmission of Nipah Virus — 14 Years of Investigations in Bangladesh | New England Journal of Medicine). There is very limited evidence on the shedding rate of Nipah virus in human cases. However, a recent systematic review of 717 cases highlighted that 20% and 48% of the cases had diarrheal and vomiting, respectively (Interpreting the natural history and pathogenesis of Nipah virus disease through clinical data, to inform clinical trial design: a systematic review - The Lancet Microbe).

3. **Have there been any studies done using SISPA?**

   SISPA is an attractive approach because it is sequence-independent, unbiased, and well suited for exploratory work, but in practice it is fundamentally constrained by what is present in the sample. When viral RNA is abundant, SISPA can perform very well. However, in samples where viral material is scarce, degraded, or mixed with large amounts of host and environmental nucleic acids—as is often the case for bat, urine, fecal, or environmental samples—the method can quickly become dominated by background signal. This is not a limitation of the technique itself, but rather a reflection of the underlying biology

and sample complexity. For this reason, once PCR positivity is established, amplicon-based sequencing is typically the most effective strategy. At that point, the goal shifts from broad detection to targeted genome recovery, where primer-based enrichment provides substantially greater sensitivity and reliability.

4. **How can we understand selection pressure on viruses detected from wastewater?**
   We need to sample and perform genomic sequencing of Nipah virus from various geographical hotspots (with previous outbreaks) and bat roosts and environmental sampling of nearby areas to understand selection pressure. A study using the time-scaled phylogenetic analysis showed that the root of the phylogenetic tree originated in 1947 when the virus entered in southeastern Asiatic regions. At the phylogenetic analysis the nucleocapsid gene sequences segregated in two main clades, indicating two different introductions: one in 1995 and the other in 1985 (Molecular epidemiology and phylogeny of Nipah virus infection: A mini review - ScienceDirect).

5. **Did you have experience using a concentration-extraction-identification (CEID) device to detect viruses? If so, what were the results? Is it applicable to remote environments?**
   There are a number of CEID device prototypes that have been developed for wastewater surveillance to address the challenges of transportation and sample quality from remote settings. However, more implementation research is needed to evaluate the sustainability of these CEID [Enabling SARS-CoV-2 Wastewater Surveillance Using an Integrated Microfluidic Chip - PubMed, Paper microfluidic sentinel sensors enable rapid and on-site wastewater surveillance in community settings: Cell Reports Physical Science ; Towards ultra-sensitive and rapid near-source wastewater-based epidemiology | Nature Communications]. Beside CEID, there are many buffers/media available on the market which can be used to preserve the samples in the field/remote environments. Nevertheless, biosafety risks will need to be evaluated, including the need for personal protective equipment (PPE) for personnel handing this outside the laboratory.

6. **Has Nipah virus been found in other bat species in addition to Pteropus bat?**
   Pteropus bats (flying foxes) are the primary reservoir of Nipah virus. Studies have identified the virus in non-*Pteropus* bats, indicating potential for spillover or exposure.
   Sharing a link to this webinar for more insights: Environmental and behavioral Drivers of Cross-species Nipah Virus Transmission… by Clifton McKee https://www.youtube.com/watch?v=MOgTl1EUSes&t=5491s

7. **Can you shed some light on the sampling strategy for wastewater surveillance for PCR and sequencing, like the amount of sample, number of samples, site within the waterbody, etc.?**
   Sampling needs to be consistent and regular (e.g. weekly/bimonthly) to establish a baseline before we can use environmental surveillance as an early warning system to identify unusual surges in signal. We collect 500ml-1000ml water – this can be collected from various collection points in high-risk areas where there are bat-pig-human interfaces. Composite samples can be used for RNA extractions and screening.

8. **For the surveillance design, especially for environmental surveillance, it's only once a year sampling? Eg in slaughterhouses, livestock farms and/or primary medical centres**
   Sampling needs to be consistent and regular (e.g. weekly/bimonthly) to establish a baseline before we can use environmental surveillance as an early warning system to identify unusual surges in signal. In addition, targeted sampling strategies near bat roosts in high-risk areas are also recommended (Nipah Virus Detection at Bat Roosts after Spillover Events, Bangladesh, 2012–2019 - Volume 28, Number 7—July 2022 - Emerging Infectious Diseases journal - CDC).

9. **If only limited labs are able to test NiV, how to manage the sample storage from remote areas to reference lab?**
   Inactivated environmental samples can be stored for transportation to the reference laboratory. Some methods for consideration are listed here, although more evidence is needed to assess the effectiveness of inactivation of NiV in wastewater & environmental samples: Inactivation Methods for Experimental Nipah Virus Infection - PMC; Effective inactivation of Nipah virus in serum samples for safe processing in low-containment laboratories - PubMed; Detection, fate and inactivation of pathogenic norovirus employing settlement and UV treatment in wastewater treatment facilities - ScienceDirect

10. **Can bioaerosol sampler be utilised to detect Nipah?**
    In Bangladesh and India, contagion occurs mainly by airway transmission (via aerosols and droplets), and generally close physical contact with the infectious patient or their secretions is required for infection acquisition. Even though there is a possibility of NiV transmitted via aerosols and droplets, air sampling using bioaerosol samplers for the detection of NiV has been very limited. More research can be implemented with the use of bioaerosol samplers in hospitals, livestock farms, and slaughterhouses to assess the detection of NiV in the air of high-risk areas.

11. **What kind of viral deactivation methods have you tried for suspected patient samples (respiratory or blood)? And where would you do it if you didn't have a BSL-3+?**
    For suspected Nipah patient samples (respiratory specimens or blood), the core principle is that samples should be rendered non-infectious as early as possible, ideally at the point of initial processing, while preserving RNA for diagnostics. In practice, this means relying on validated chemical inactivation approaches that are already embedded in routine molecular workflows. Chaotropic-agent–based lysis buffers used for nucleic acid extraction are widely accepted to inactivate enveloped viruses like Nipah when used as intended, and this approach has been applied safely across multiple high-consequence pathogens. Heat or solvent-based methods may also inactivate virus, but these are generally less compatible with downstream molecular sensitivity and are not preferred for diagnostics. More recommendations can also be found in the responses to Qn 9.

12. **Is the consumption of fruits with signs of scratches and cuts by bats or birds a cultural habit in Southeast Asia?**
    No. Only date palm sap is a delicacy that is consumed in winter season. Sharing a link to this webinar for more insights: Environmental and behavioral Drivers of Cross-species Nipah Virus Transmission… by Clifton McKee https://www.youtube.com/watch?v=MOgTl1EUSes&t=5491s

13. **Why don't the bats show any clinical signs of Nipah? Are they immune to this virus?**
    It is very hard to find a bat infected with Nipah virus as shedding rate is very low. There are many groups working on this question of why bats are immune to the viruses they carry. Some insights may be gathered from this perspective piece: Lessons from the host defences of bats, a unique viral reservoir | Nature.

14. **Can event-based or syndromic surveillance realistically outperform passive reporting, since the signs are quite similar to those of other diseases?**
    These surveillance systems are equally useful to suggest potential emerging or imminent outbreaks, but these signals need to be cross-validated with laboratory surveillance systems as a form of outbreak verification. Therefore, strengthening laboratory capabilities is a critical step to strengthen pandemic preparedness.

15. **What percentage of all Nipah viruses isolated from the environment are from the total number of Nipah viruses isolated?**

There is currently limited evidence of NiV isolated from the environment as compared to from human cases, bats, infected pigs and other animals. Nipah Virus Disease: Epidemiological, Clinical, Diagnostic and Legislative Aspects of This Unpredictable Emerging Zoonosis - PMC; Nipah Virus Detection at Bat Roosts after Spillover Events, Bangladesh, 2012–2019 - Volume 28, Number 7—July 2022 - Emerging Infectious Diseases journal - CDC

16. **Could you please send us widely used qPCR primers and probe sequences for Nipah virus?**
    Through our collaboration with Dr. Erik Karlsson from IPC, there is now a protocol for detecting Nipah virus using RT-QPCR (https://www.protocols.io/view/rt-qpcr-detection-of-nipah-virus-niv-targeting-the-36wgq1mwkvk5/v1) and NGS (https://www.protocols.io/view/sequencing-of-nipah-virus-niv-using-oxford-nanopor-6qpvryr5pgmk/v1) for your consideration and reference.

17. **I get the impression our responses to the spillover of Henipah viruses are primarily reactive in nature. Where does environmental surveillance need to go to support a more proactive framework to preventing spillover events? Even if it is just more frequent sampling, how do we assess the threat potential if we were to detect something in the environment? Or are we better off investing more in managing the social dimensions of human interactions with the environment?**
    Adopting a One Health approach will help to strengthen our surveillance system for the early detection of and response to zoonotic spillover events. The investment case to build environmental surveillance for priority pathogens, including wastewater surveillance, requires building evidence for the public health value. Sampling needs to be consistent and regular (e.g. weekly/bimonthly) to establish a baseline trend before we can use environmental surveillance as an early warning system to identify unusual surges in signal for appropriate responses. Investing in social dimensions of human behaviour with the environment is equally important. For example, most spillover has been reported in winter when date palm sap consumption is most prevalent and so public health advisory and community engagement may be increased during this period to raise the awarenss of the potential risks.

18. **Can the primer-probe from in-house RT-PCR by Institut Pasteur detect recent strains (2023 and 2025) from the outbreak in India?**
    Yes, it should. Targeted to the N gene.

19. **In your experience, under what sample conditions have metagenomic sequencing successfully recovered near-complete or complete Nipah virus genomes, and where does it typically fail?**
    Due to the limited transmission of NiV in the human population as well as the potential low shedding rate of NiV from bats, there is still limited detection of NiV from metagenomics. In order to gather more evidence, environmental surveillance of Nipah virus will need to be targeted at outbreak sites and other potential areas to understand bat ecology and identify roosts, and more human behavioural data is also needed to understand viral shedding patterns and metagenomic approaches (Nipah Virus Detection at Bat Roosts after Spillover Events, Bangladesh, 2012–2019 - Volume 28, Number 7—July 2022 - Emerging Infectious Diseases journal - CDC).