

Clinical Presentation and Molecular Diagnosis of Monkeypox Virus and Varicella Zoster Virus Co-infection in an Adult Immunocompetent Filipino: A Case Report

April Keith Balingit

Research Institute for Tropical Medicine

Phoebe Grace Grande (✉ phoebegrace.grande@gmail.com)

Research Institute for Tropical Medicine <https://orcid.org/0000-0001-7366-0673>

Amalea Dulcene Nicolasora

Research Institute for Tropical Medicine

Francisco Gerardo Polotan

Research Institute for Tropical Medicine

Roslind Anne Pantoni

Research Institute for Tropical Medicine

Maria Yna Joyce Chu

Research Institute for Tropical Medicine

Nicole Rivera

Research Institute for Tropical Medicine

Marie Socouer Oblepias

Research Institute for Tropical Medicine

Jemelyn Garcia

Research Institute for Tropical Medicine

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Abstract

BACKGROUND

Following the detection of the first laboratory-confirmed Monkeypox (MPXV) infection in the Philippines, guidelines on Monkeypox diagnosis, treatment, and prevention have been strengthened to further help healthcare providers in differentiating it properly from other diseases with similar clinical presentation, one of which is Varicella zoster (VZV) infection. Interestingly, co-infection with Monkeypox and Varicella has been previously reported in Monkeypox endemic countries. We then report the first travel-related case of MPXV-VZV co-infection in the Philippines, a country that is endemic for Varicella but non-endemic for Monkeypox.

CASE PRESENTATION

A 29-year-old Filipino, female, with a travel history to Switzerland and with no prior history of VZV infection consulted due to rashes. She presented with multiple papular, pustular, and vesicular skin lesions, some with umbilication and with irregular borders, on the face, neck, trunk, inguinal area, upper extremities, and right leg. She also had bilateral submandibular and post-auricular lymphadenopathies. Tzanck smear exhibited viral cytopathic effects. She was confirmed to have Monkeypox infection from Clade II and Varicella infection via quantitative real-time polymerase chain reaction (qPCR) tests. Shotgun metagenomic sequencing (mNGS) successfully recovered sequences from the Varicella zoster virus which corroborated with the high viral load detected using qPCR. In contrast, shotgun mNGS showed too few reads mapped to the Monkeypox virus reference sequence. Systemic and topical acyclovir was given to the patient. She was discharged and continued home isolation for 30 days from the rash onset.

CONCLUSION

Strategies have been formed by the country's healthcare facilities to properly identify monkeypox infection. However, Monkeypox co-infection with other viral diseases presented a challenge in the proper diagnosis of our patient. This prompted a high index of suspicion and the usage of suitable diagnostic tests. The qPCR tests confirmed the presence of both Monkeypox and Varicella zoster virus infections in the patient. Shotgun metagenomic sequencing (mNGS) successfully recovered sequences from the Varicella zoster virus, while there were too few reads mapped to the Monkeypox virus reference sequence. With proper clinical evaluation and utilization of appropriate diagnostic tests, we were able to diagnose the first Filipino patient with Monkeypox and Varicella zoster virus co-infection.

Background

Monkeypox is a re-emerging infectious disease known to be endemic in Central and West Africa but unexpectedly created new outbreaks worldwide in May 2022. It is caused by the Monkeypox virus

(MPXV), an Orthopoxvirus in the *Poxviridae* family, and acquired via zoonotic and human-to-human transmission through respiratory secretions or direct contact with skin lesions of infected animals or individuals (1). The classic Monkeypox rash usually starts from macular lesions developing into papules and pustules which eventually form central umbilication and then crusts (2). The rash is usually accompanied by lymphadenopathy and preceded by a prodromal period. A Monkeypox patient is considered infectious until all scabs have fallen off. Meanwhile, reports from the recent global Monkeypox outbreak described infected patients who presented with atypical symptoms and rash characteristics (3).

There are currently around 65,000 monkeypox cases reported worldwide, with four cases found in the Philippines (4). Following the detection of Monkeypox cases in Filipinos, guidelines on monkeypox diagnosis, treatment, and prevention have been strengthened to help healthcare providers in differentiating it from other disease conditions with similar clinical presentation. One of these is chickenpox caused by the Varicella zoster virus (VZV). In regions of the world where both viruses are present, there is confusion in the diagnosis of Monkeypox and Varicella-zoster (VZV) (5). VZV is also a DNA virus-like MPXV, but it belongs to the *Herpesviridae* family and is only transmitted among humans (6). Contrary to Monkeypox, the typical Chickenpox rash presents simultaneously at different stages on the skin, with lymphadenopathy being an uncommon occurrence and the appearance of fever more commonly seen before or during rash onset (6). VZV is contagious beginning one to two days before rash onset until all lesions have crusted. It is known to occur worldwide, but it is mostly seen in children living in temperate regions and in adults living in tropical countries such as the Philippines (7). Without knowing these key characteristics, MPXV is often misdiagnosed for VZV or vice versa. Moreover, cases of co-infections of the two viruses have only been reported by surveillance studies in Africa (8). We then report the first travel-related case of MPXV-VZV co-infection in the Philippines, a country that is endemic for Chickenpox but non-endemic for Monkeypox.

Case Presentation

This was a case of a 29-year-old, female, Filipino, who consulted due to multiple pustular and vesicular rashes on the face, neck, trunk, inguinal area, bilateral upper extremities, and right leg. The patient had no known comorbidities, no history of varicella or measles infection, and no known allergies to food or drugs. She had a complete primary childhood immunization and was fully vaccinated against COVID-19. She was a nonsmoker, an occasional alcoholic beverage drinker, and a non-illicit drug user. She only had one long-term male sexual partner.

Travel history revealed the patient's significant work-related trip to Geneva, Switzerland from February to July 2022. She did not visit any other countries during her stay in Geneva. She made use of public transportation, mainly buses and trains, to go to work daily. She left Geneva on July 31, 2022, and arrived in the Philippines on August 1, 2022, with no reported symptoms. Ten days after her arrival, she noticed small pruritic macular rashes erupting on both of her arms. She did not seek consultation nor received any intervention. Thirteen days after her arrival, she noted an increase in the number of her skin lesions

which progressed to maculopapular rashes. She also noted the appearance of an erythematous pustule on her nape. No other associated signs and symptoms were noted. Fifteen days after her arrival, her skin lesions progressed to vesicular pruritic rashes on her face, chest, back, and lower extremities with accompanying undocumented fever and myalgia. Sixteen days after arrival, she went for a consultation at a local clinic due to the persistence of her rashes. She was advised to contact the city health office which referred her to a local hospital for evaluation. Figure 1 shows the timeline of the patient's symptom progression.

The patient was seen at the emergency room with blood pressure of 100/70 mmHg, heart rate of 89 beats/min, respiratory rate of 20 cycles/min, body temperature of 38.1°C, and oxygen saturation of 99% at room air. Pertinent physical examination findings were multiple papular, pustular and vesicular skin lesions, some with umbilication, some with irregular borders, presenting at different stages on the face, neck, trunk, inguinal area, bilateral upper extremities and right leg. She also had bilateral submandibular and post-auricular lymphadenopathies (Fig. 2). Other physical examination findings were unremarkable.

Following the recent guidelines for screening patients presenting with rashes at the emergency room, she satisfied the criteria for Monkeypox Suspect hence she was admitted in the isolation room for further evaluation. Varicella zoster virus (VZV) infection was also considered due to the presentation of skin lesions at different stages of development. Initial blood tests showed a white blood cell count of $4.5 \times 10^9/L$, neutrophils 75%, lymphocytes 15%, and a normal urinalysis result. Measles infection was ruled out with a negative measles polymerase chain reaction (PCR) test result. The patient was referred to the Dermatology service who conducted a Tzanck smear test which showed neutrophils with rare atypical round cells exhibiting viral cytopathic effects, suggesting a viral etiology (Fig. 3).

The patient's plasma tested positive for VZV using a real-time quantitative polymerase chain reaction (qPCR) test with 5,350 copies/mL detected. Meanwhile, a total of nine specimens (three samples of skin scrapings and six vesicle fluid swabs) were obtained and sent to the Special Pathogens Laboratory for a confirmatory probe-based monkeypox qPCR test. Nucleic acid extraction from dry swab and tissue samples were performed using QiaAmp DNA Mini Kit (QIAGEN, Hilden, Germany, Cat No: 51306) according to the manufacturer's instructions (9). The PCR primers and probes were developed from the sequences described by Li, et al (Table 1S **of the supplementary file**). Probe-based real time PCR assay was performed using Applied Biosystem's AgPath-ID One Step PCR kit (4387424) (10) and Bio-Rad CFX96 Touch real time PCR machine as PCR platform. RNase P was the assays' internal target control.

Two sets of the lesion dry swab and lesion roof were confirmed to be positive for monkeypox RT-PCR with a mean cycle threshold (Ct) value of 36.20 (Table 2S **of the supplementary file**), indicating a low viral load. The monkeypox RT-PCR differentiation assay also revealed that the same samples belong to monkeypox Clade II (previously known as the Western African clade) with mean Ct value of 35.62 (Table 2S **of the supplementary file**). No viral copies of the Congo Basin clade were detected via RT-PCR among all the samples.

The PCR-confirmed samples from the patient were endorsed to the Molecular Biology Laboratory for genetic characterization by shotgun metagenomic sequencing (mNGS) using the Illumina DNA Prep kit and the Illumina MiSeq instrument. Analysis of the recovered sequences from shotgun mNGS showed too few reads mapped to the Monkeypox virus reference sequence. Moreover, further analysis showed recovery of a relatively large number of sequencing reads (n = 280,805) aligning to *Human alphaherpesvirus 3* or commonly known as *Varicella zoster virus* (VZV) (Table 1).

Table 1
Metagenomic sequencing reads mapped to *Human alphaherpesvirus 3*

Sample ID	Human alphaherpesvirus 3 (mapped sequencing reads)	Lineage
MPOX22-00061DSA Lesion surface dry swab	40,662	Viruses > Herpesvirales> Herpesviridae>
MPOX22-00061RA Lesion roof	240,143	Alphaherpesvirinae> Varicellovirus
MPOX22-000061 Total Number of reads	280,805	

Figure 4 shows the identified microbial and viral taxa from the metagenomic sequences of MPOX22-00061DSA (Fig. 4a) and MPOX-00061RA (Fig. 4b) samples as depicted by Sankey diagrams, which show the counts of paired-end reads assigned to a particular taxon as indicated by the number on the upper left corner of the taxon. The diagrams show that the majority of the viral sequences recovered from the lesion specimens aligned to the *Human alphaherpesvirus 3*, supporting the high viral infection detected from the patient serum through RT-PCR testing. Additional reads for the *Pseudomonas aeruginosa* group in the MPOX22-0061DSA sample and for *Spirometra erinaceieuropaei* and *Ralstonia solanacearum* in the MPOX-00061RA sample were also obtained. Since shotgun metagenomic sequencing was used, reads from all organisms present in the sample were obtained. The detected bacterial organisms and other taxa with very low read counts may be considered misassigned taxa, contaminants, or otherwise members of the normal host tissue microflora. Regardless, additional analysis and filtering are needed to draw a definite conclusion.

The patient was fully advised regarding her disease conditions, and she was started on Acyclovir 800 mg/capsule 1 capsule five times a day for five days accompanied by Acyclovir + Zinc oxide ointment 50 mg/100 mg twice a day to treat the active VZV infection. Mupirocin ointment was also applied to eroded areas. On the second hospital day, the patient had a low grade fever of 37.9°C with the appearance of new pustules, papules, and vesicles on the face, chest, back, and palms. She was discharged on the fourth hospital day in stable condition, with no recurrence of fever for 24 hours. She was advised on

continued isolation at home until all crusts and scabs have completely disappeared. Home isolation and daily monitoring of symptoms and rash progression or resolution were done via teleconsultations (Fig. 5). The patient's total isolation lasted 30 days from the appearance of her skin lesions. There was no serious complication during the course of her illness (Fig. 6).

Discussion

This report describes the first documented case of Monkeypox (MPXV)-Varicella zoster virus (VZV) co-infection in the Philippines in a female patient with significant travel history to Switzerland, who has no prior history of VZV infection. She presented with vesiculo-pustular lesions and tested positive for both Monkeypox and Varicella viral infections using a quantitative real-time polymerase chain reaction (qPCR) test. There are a few studies that describe and explain the occurrence of MPXV-VZV co-infection in humans, and many of them are surveillance studies carried out in African nations where Monkeypox is endemic. A previous study conducted in the Democratic Republic of Congo showed that MPXV-VZV co-infection occurred in 13% of the study population and in 19.3% of those who had laboratory-confirmed MPXV infection (8). Mechanisms explaining the occurrence of this phenomenon remain unknown but previous studies suggested that prior infection with either MPXV or VZV may make the host susceptible to acquiring a secondary infection (8). A break on the skin also becomes an ideal point of entry for MPXV via direct contact with infected animals or humans. Moreover, the presence of both viruses in the same host prompted theories from previous studies that acute MPXV infection somehow activates latent VZV infection leading to shingles (12, 13). Whether or not the co-occurrence of the two viruses in a single host is a coincidence or not, further evidence is still required to prove their association.

Overlapping clinical features of MPXV and VZV infections were appreciated in this case, which has not been reported in the local setting. A few surveillance studies in the Democratic Republic of Congo previously investigated cases of MPXV-VZV co-infection and results showed a higher burden of skin lesions found in patients with MPXV-VZV co-infection than VZV infection alone and a lower burden of skin lesions than MPXV infection alone, which suggested the possibility of the two viruses modulating the severity of the infection (8). It is also important to be familiar with the classic presentation of both MPXV and VZV infections for proper diagnosis, especially in countries where both viruses are found to be naturally occurring. The centrally umbilicated pustular lesions with accompanying bilateral lymphadenopathies observed in the case are consistent with the classic monkeypox infection as described in previous studies (5). The typical MPXV infection usually has a centrifugal pattern of lesion distribution, with most of the lesions observed at the face, and upper and lower extremities which were also observed in our patient. The recent 2022 Monkeypox outbreak also reported anogenital rashes among patients in non-endemic countries (14). On the other hand, the patient was also observed to have lesions that were at different stages as well as lesions on the trunk which are more commonly seen with VZV infection (5, 15). Interestingly, the patient's fever was seen to have occurred after rash onset which was not commonly observed in patients with MPXV nor VZV infection (5). With the recent 2022 monkeypox outbreak in multiple non-endemic countries, the need for updated diagnostic pathways arises

to differentiate MPXV infection from VZV infection and to determine the presence of possible co-infections.

Tzanck smear was performed in this case since Varicella infection was considered. The result was consistent with a viral etiology showing neutrophils with rare, atypical round cells exhibiting viral cytopathic effects (Fig. 2). However, a Tzanck smear alone does not distinguish a monkeypox infection from other herpetic infections (16). The gold standard in diagnosing both MPXV and VZV infection involves qPCR tests which were used to diagnose the patient presented in this case (6, 17, 18). For qPCR of monkeypox samples, the recommended types of specimens are swabs of skin lesions with or without exudates, roofs, or crusts from more than one lesion (17). On the other hand, fluid or scabs from vesicular lesions are collected for VZV PCR (6). Plasma was used for Varicella PCR in this case. Although plasma and serum specimens are not usually used for VZV PCR tests, previous studies showed their role in the diagnosis and management of VZV infection (19–21).

Sequencing can be performed for further genetic characterization of PCR-positive samples. Metagenomic sequencing (mNGS) is a preferable tool for detecting multiple pathogens present in a sample. Shotgun metagenomic sequencing is a hypothesis-free or untargeted (no pathogen target) sequencing method that allows for the sequencing of all microbial genomes. This sequencing method has been widely used to detect the monkeypox virus, other unknown pathogens, and pathogen co-infections. However, mNGS requires high viral concentrations to be able to recover pathogen sequences. In this case, the patient sample demonstrated a high Ct value in the MPXV PCR assay denoting a low viral load of the monkeypox virus. While the VZV PCR assay detected a high viral load (5,350 copies/mL) of Varicella zoster virus. Therefore, relative to the viral load of the patient, the sequencing results corroborate the PCR results wherein too few reads were recovered for MPXV while a large number of reads were recovered for VZV. Although mNGS was not able to successfully detect MPXV based on the sequences that were recovered, this does not necessarily negate the results of the PCR test. Low genome sequence recovery may be due to the low viral load of MPXV present in the patient sample.

Strict isolation and supportive management of symptoms remain central to the management of both MPXV and VZV infections (17). There are currently no available antiviral medications for MPXV infection; however, Acyclovir remains one of the approved drugs for the treatment of early VZV infection (22). Local guidelines recommend that confirmed monkeypox patients undergo isolation until symptoms have resolved and until all scabs are gone (17). On the other hand, patients with confirmed Varicella infection are advised isolation until all lesions have crusted (23). The patient reported in this case underwent isolation for 30 days from the occurrence of her rashes until all scabs had disappeared. No serious complication was observed during her home isolation. She was treated for VZV infection with oral and topical Acyclovir for five days. No other systemic antibiotics or antivirals were used by the patient.

Conclusion

Strategies have been formed by the country's healthcare facilities to properly identify monkeypox infection and differentiate it from other infectious diseases. However, monkeypox co-infection with other viral diseases, specifically Varicella zoster infection, clinically presented a challenge in the proper diagnosis of our patient. It is unusual for different infectious agents to cause comparable diseases and circulate in the same population. This prompted a high index of suspicion and the usage of suitable diagnostic tests. Tzanck smear could not differentiate between monkeypox and Varicella zoster viruses but real-time quantitative polymerase chain reaction (qPCR) tests confirmed the presence of both monkeypox and Varicella zoster virus infections in the patient. Shotgun metagenomic sequencing (mNGS) successfully recovered sequences from Varicella zoster virus, while there were too few reads mapped to the Monkeypox virus reference sequence. With proper clinical evaluation and utilization of appropriate diagnostic tests, we were able to diagnose the first Filipino patient with monkeypox and varicella zoster virus co-infection. Future studies on the possible mechanisms responsible for the presence of both monkeypox and varicella zoster virus are vital in the further understanding and surveillance of the disease.

Abbreviations

MPXV: monkeypox virus; VZV: varicella zoster virus; qPCR: real-time quantitative polymerase chain reaction; CT value: cycle threshold value; DNA: deoxyribonucleic acid; mNGS: shotgun metagenomic sequencing

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethics approval for this case report was waived as it was not considered part of a research study. Written and duly signed informed consent was given by the patient.

CONSENT FOR PUBLICATION

The patient provided written and duly signed informed consent for the preparation and publication of this case report.

CONFIDENTIALITY

Patient names and other information that can be used to identify the patient were kept private and confidential. The patient will not be personally identified in published results or in presentations.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

COMPETING INTERESTS

All the authors have declared that they have no competing interests.

FUNDING

Not applicable.

AUTHORS' CONTRIBUTION

A.B. and N.R. contributed to the study conceptualization, investigation, methodology, and visualization. P.G. contributed to study conceptualization, investigation, project administration, and visualization. M.O. and J.G. contributed to conceptualization, investigation and supervision. M.C., A.N., and F.P. contributed to study data curation, formal analysis, investigation, methodology, resources, software programming, validation and visualization. All authors contributed to the manuscript writing. All authors reviewed the manuscript for intellectual content, approved the final version of the manuscript for submission, and agreed to be accountable for all aspects of the work.

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Figures

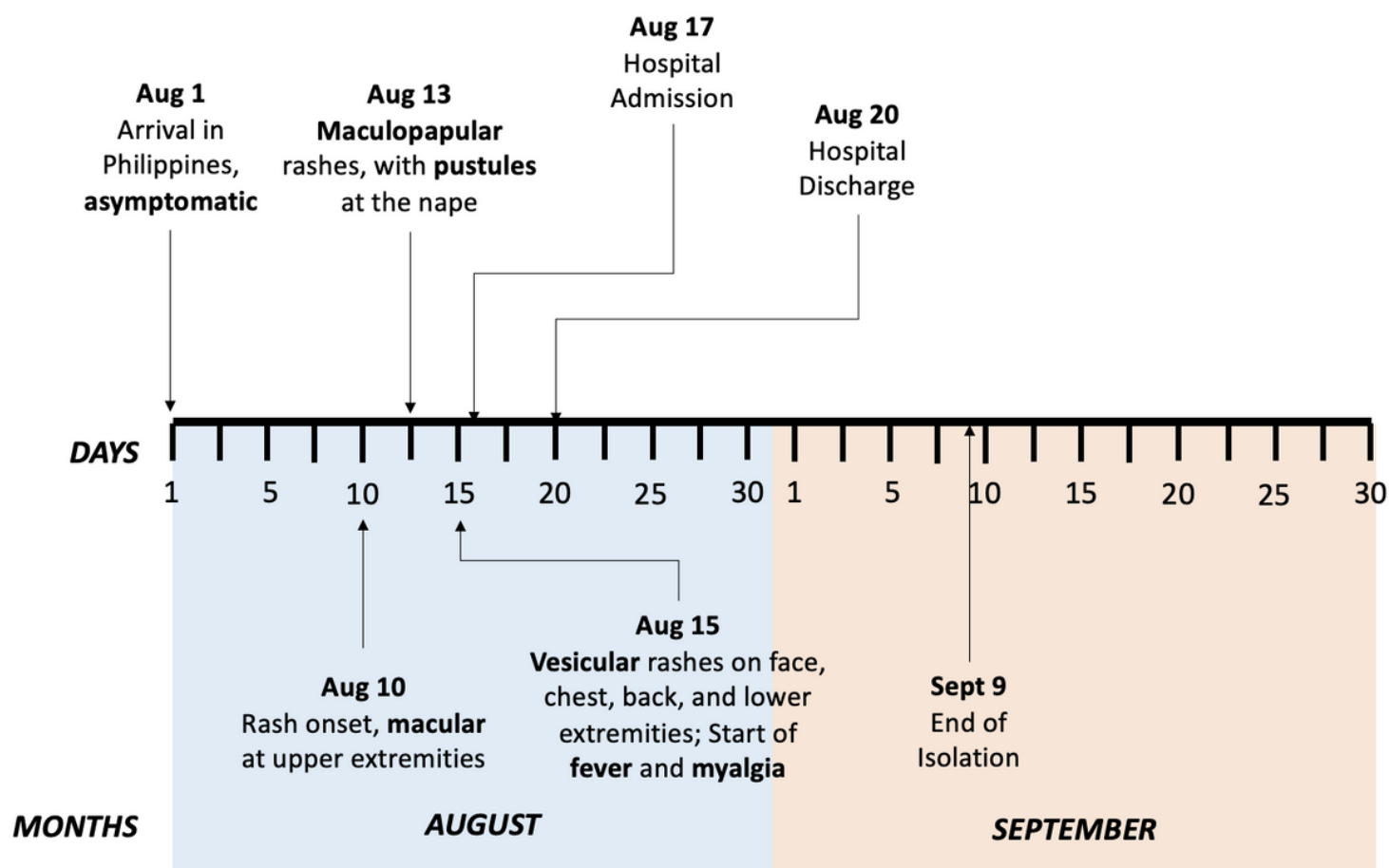


Figure 1

Timeline of patient's symptom progression to end of isolation, August to September 2022



Figure 2

Skin lesions presenting at different stages upon admission. Locations: a) chest, b) neck, c) right cheek, d) left arm, e) nape

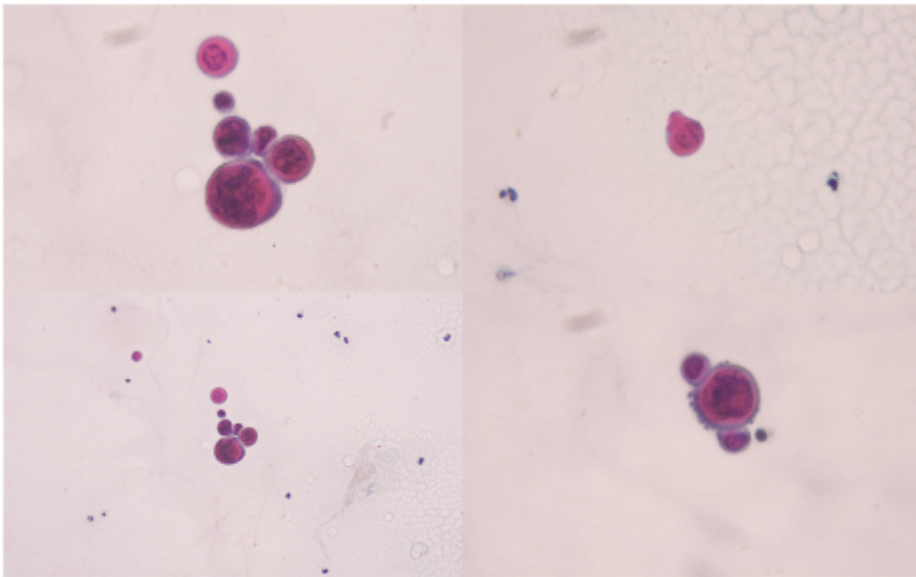
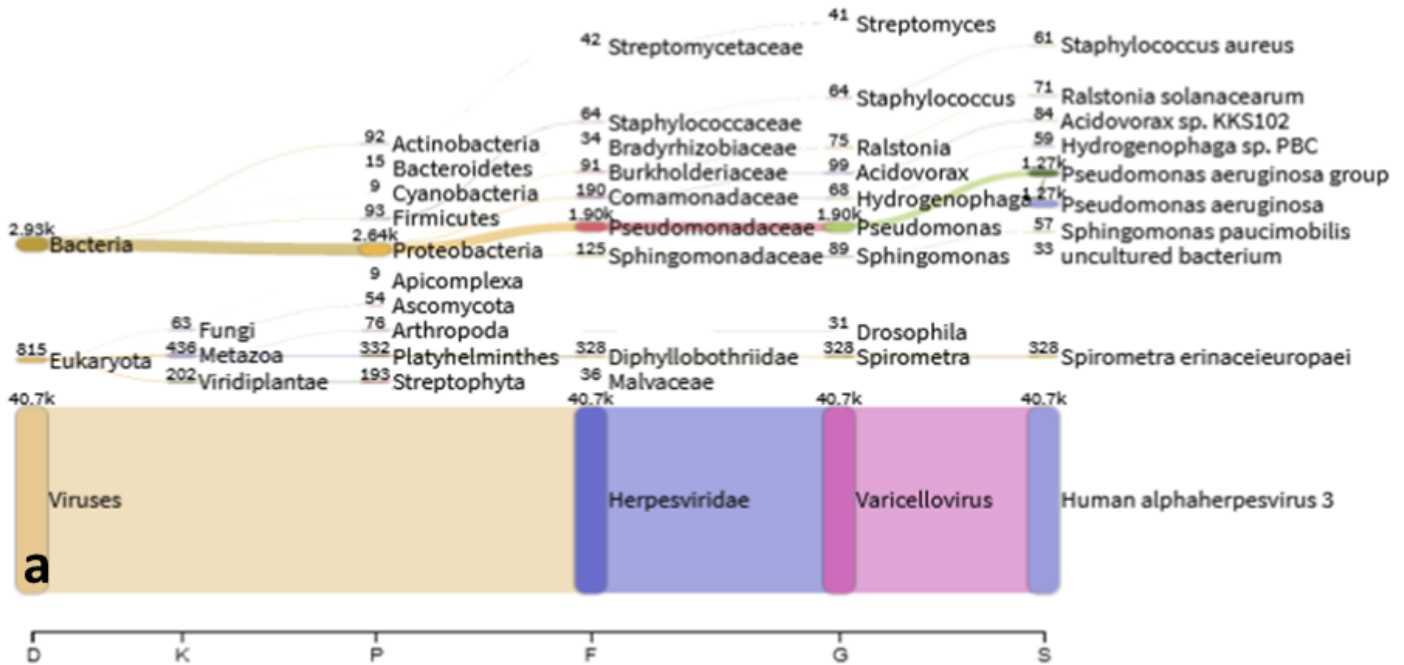


Figure 3

Tzanck smear of the vesicular skin lesions. Tzanck smear of the patient's vesicular skin lesion shows neutrophils with atypical round cells suspected to exhibit viral cytopathic effects.

6 Archaea



20 Archaea

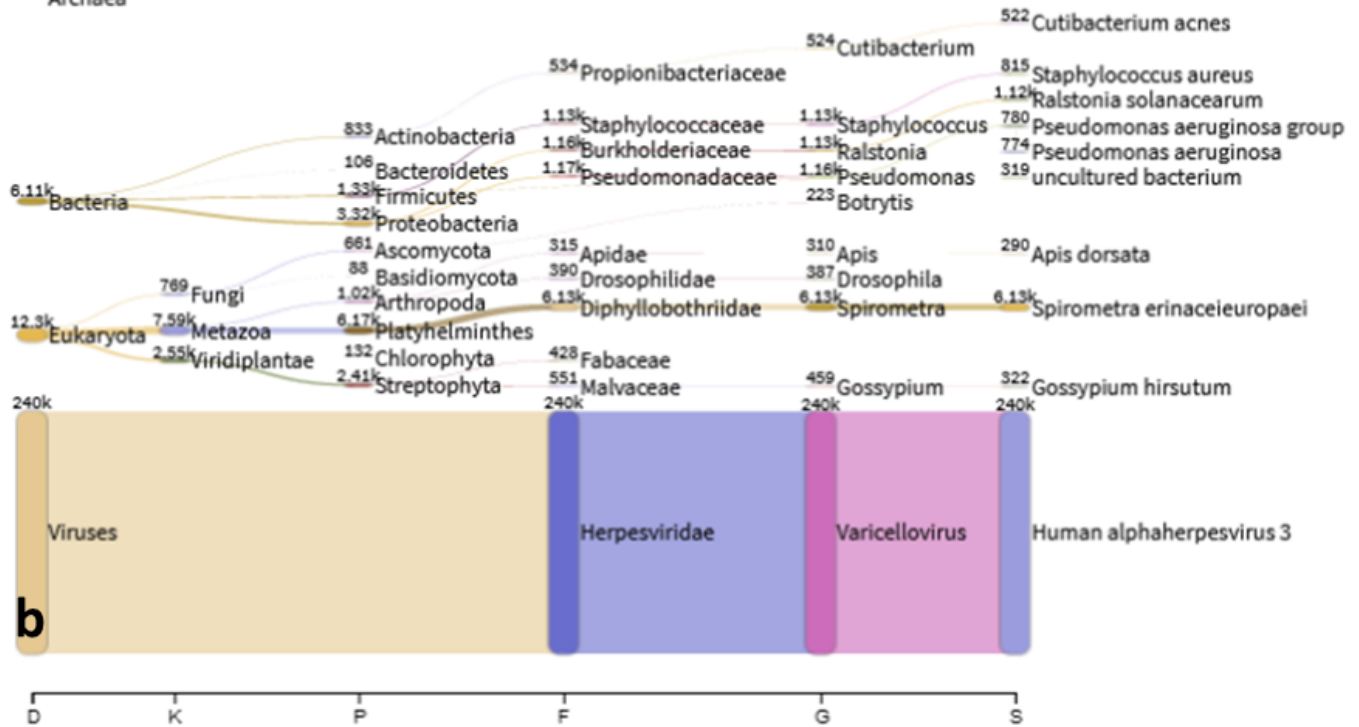


Figure 4

Sankey plot. Classified viral and microbial taxa from MPOX22-0061DSA (a) and MPOX22-0061RA (b) metagenomic samples

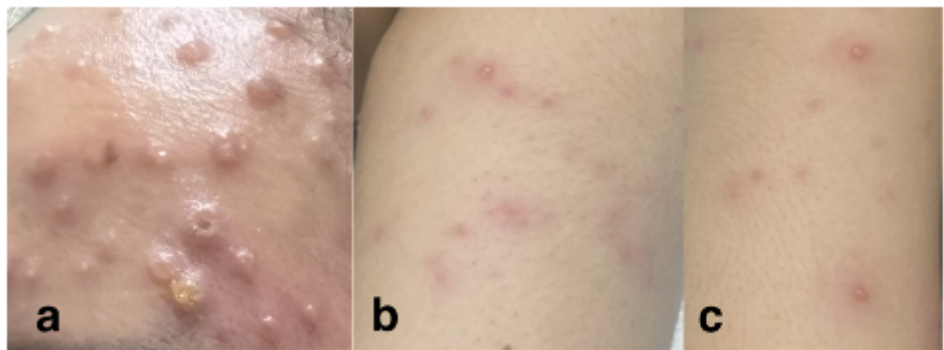


Figure 5

Skin lesions on the 7th day. Locations: a) forehead, b) left arm, c) right leg.

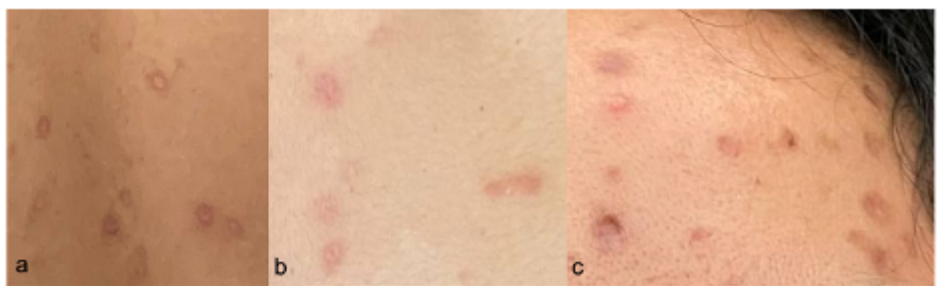


Figure 6

Skin lesions on the 30th day. Locations: a) back, b) chest, c) forehead.

Supplementary Files

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