

Short communication

Correlation between monkeypox viral load and infectious virus in clinical specimens

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ABSTRACT

Background: In the 2022 mpox outbreak, several studies have explored longitudinal DNA shedding of mpox virus (MPXV) using PCR. However, there are fewer studies assessing infectivity in cell culture, and, by inference, MPXV transmissibility. Such information could help inform infection control and public health guidelines.

Aims and Methods: The aim of this study was to correlate cell culture infectivity of clinical samples with viral loads in clinical samples. Between May to October 2022, clinical samples from different body sites sent to the Victorian Infectious Diseases Reference Laboratory in Melbourne, Australia for MPXV PCR detection were cultured in Vero cells as a surrogate for infectivity.

Results: In the study period, 144 samples from 70 patients were tested by MPXV PCR. Viral loads in skin lesions were significantly higher than those in throat or nasopharyngeal samples (median Ct 22.0 vs 29.0, $p = 0.0013$ and median Ct 22.0 vs 36.5, $p = 0.0001$, respectively). Similarly, viral loads were significantly higher in anal samples compared to throat or nasopharyngeal samples (median Ct 20.0 vs. 29.0, $p < 0.0001$ and median Ct 20.0 vs. 36.5, $p < 0.0001$, respectively). Viral culture was successfully performed in 80/94 samples. Using logistic regression analysis, 50% of the samples were positive in viral culture at Ct 34.1 (95% confidence intervals 32.1–37.4).

Conclusions: Our data further validate recent findings showing that samples with a higher MPXV viral load are more likely to demonstrate infectivity in cell culture. Although the presence of infectious virus in cell culture may not directly translate with clinical transmission risk, our data may be used as an adjunct help inform guidelines on testing and isolation policies in individuals with mpox.

1. Introduction

Since early May 2022, over 85,000 cases of monkeypox (mpox) have been reported from multiple countries not previously regarded as endemic for mpox infection[1,2]. Several studies have described the longitudinal detection of mpox virus (MPXV) DNA in clinical specimens [3–5]. These studies have demonstrated high viral loads in skin lesions, with viral DNA detected from a range of other samples, including anal swabs, nasopharyngeal and throat swabs, saliva, semen and urine[6]. Although quantitative detection of viral DNA provides useful

information on viral detection and shedding dynamics[6], additional work assessing viral infectivity is required to further understand the possible transmissibility of MPXV.

Growth in viral cell culture systems is often used as a proxy for the presence of infectious, replication-competent virus, although in the case of MPXV, viral cell culture should only be performed in laboratories with appropriate biosafety facilities[6]. Given the specialised nature of these facilities, there are relatively few studies assessing mpox viral infectivity in clinical samples. Such information could be used to better inform infection control and public health guidelines for individuals with mpox.

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Here, we describe the comparative MPXV viral loads in patient specimens and correlate these findings with infectivity in cell culture.

2. Methods

2.1. Clinical samples and PCR testing

A laboratory evaluation study was conducted at the Victorian Infectious Diseases Reference Laboratory (VIDRL) at The Peter Doherty Institute for Infection and Immunity (Melbourne, Australia). VIDRL is the public health virology laboratory for the state of Victoria, serving a population of approximately 6.24 million people. Clinical swabs for MPXV PCR testing were collected in viral transport medium (VTM) and were stored at 4 °C for up to 48 h before PCR testing. PCR testing was performed using a previously described assay [7].

2.2. MPXV viral culture

Clinical samples that tested MPXV PCR-positive were cultured for 7 days in a 24-well cell culture plate containing a Vero cell monolayer in an incubator at 37°C with 5% CO₂. Prior to use for isolation and to improve virus to cell contact, Earle's minimum essential medium (EMEM) containing 5% heat inactivated fetal bovine serum (FBS) was completely removed from each well and 100 µL of specimen fluid inoculum was overlaid to cover all surface of the cell monolayer. The plate was returned to the 37°C incubator to allow the virus to adsorb for 30 min before addition of 500 µL virus culture media (EMEM 2% FBS). The viral inoculum was allowed to remain to avoid additional manipulation in the high containment laboratory. Wells were monitored daily for virus-induced cytopathic effect (CPE) and CPE readings were recorded by two independent readers for each sample. In the majority of samples with viable virus, extensive CPE was observed between 2 and 4 days. Blind passage of CPE-negative cell culture supernatant beyond 7 days in general did not yield viable virus. All viral culture was performed in a physical containment 3 (PC3) laboratory at VIDRL by trained personnel.

2.3. Statistical analysis

Analyses and data visualization were performed using Prism 9 (version 9.4.1), and except where otherwise specified, p-values < 0.05 were considered statistically significant. A logistic regression model was used to determine the Ct value at which 50% of samples grew in viral culture, with corresponding 95% confidence intervals (CI), using culture positivity as the dependent variable and Ct value as the independent variable. Kruskal-Wallis or chi-squared tests were used to compare Ct

values between clinical samples.

3. Results

3.1. MPXV PCR positivity in clinical samples

Between 19th May 2022 and 19th October 2022, 2057 samples from 1040 patients were sent to VIDRL for MPXV PCR, using a previously described assay [7]. Of these, 199 samples from 70 patients tested positive for MPXV (Table 1). When only considering samples collected on the day of diagnosis from individual patients (144 samples from 70 patients), viral loads in skin lesions were significantly higher than those in throat or nasopharyngeal samples (median Ct 22.0 vs 29.0, $p = 0.0013$ and median Ct 22.0 vs 36.5, $p = 0.0001$, respectively) (Fig. 1A). Similarly, viral loads were significantly higher in anal samples compared to throat or nasopharyngeal samples (median Ct 20.0 vs. 29.0, $p < 0.0001$ and median Ct 20.0 vs. 36.5, $p < 0.0001$, respectively). (Fig. 1A). Paired anal samples from the day of diagnosis and day seven post-diagnosis were available from seven individuals; the median Ct increased significantly from 17.0 to 29.0; $p = 0.03$ (Fig. 1C), suggesting a decrease in viral load.

3.2. Correlation of PCR positivity with growth in viral culture

In total, 94 MPXV PCR-positive samples underwent viral culture, with Ct values ranging from 16 to 40. Of these, 80/94 samples (85.1%) grew in viral culture (Fig. 1D). There was a significant difference between the median Ct value of samples that grew in viral culture (Ct 22; range 16–36) compared to samples that did not grow (Ct 33; range 26–40) ($p < 0.001$). Logistic regression analysis demonstrated that the Ct value at which 50% of the samples were positive in viral culture was 34.1 (95% confidence intervals 32.1–37.4) (Fig. 1B). There was no significant difference in viral culture positivity between different sites (skin 30/35, 85.7%; anus 37/42, 88.0%; throat, 13/15, 86.7%) ($p = 0.95$, chi-squared test).

4. Discussion

Here, we assess the comparative MPXV viral loads in clinical specimens, and correlate these with growth in cell culture. Our data are in keeping with other recent studies demonstrating viral loads are higher in skin lesions and anal samples compared to oral samples [3,8,9]. The relatively high load and culture positivity from anal samples is consistent with anal infection being a key site of infectiousness. Moreover, a recent study has also demonstrated infectious MPXV (assessed by cell culture positivity) in the saliva of infected individuals [10]. Collectively,

Table 1

Anatomical sites and test results of clinical samples received for monkeypox PCR testing at the Victorian Infectious Diseases Reference Laboratory (VIDRL) between 19th May 2022 and 19th October 2022.

Specimen site	Samples (n = 2057)	%	All positive samples (n = 199)	%	Median Ct (range)	Positive samples collected on day of diagnosis (n = 144)	%	Median Ct (range)
Skin	1097	53.3	87	43.7	23.0 (16.0–38.0)	76	52.8	20.0 (15.0–37.0)
Anus	558	27.1	71	35.6	22.0 (15.0–40.0)	46	31.9	20.0 (15.0–37.0)
Oral	213	10.4	27	13.6	32.0 (20.0–40.0)	12	8.3	29.0 (20.0–40.0)
Nasopharyngeal	24	1.2	7	3.5	36.5 (23.0–37.0)	6	4.1	36.5 (23.0–37.0)
Blood	44	2.1	2	1.0	NA*	2	1.4	NA*
Urine	118	5.7	5	2.5	27.0 (21.0–40.0)	2	1.4	NA [#]
Unknown	3	0.1	0	0	–	0	0	–

Abbreviations: Ct, cycle threshold; NA, not applicable.

* Ct values of two samples from blood were 34.0 and 41.0.

[#] Ct values of two samples from urine were 21.0 and 27.0.

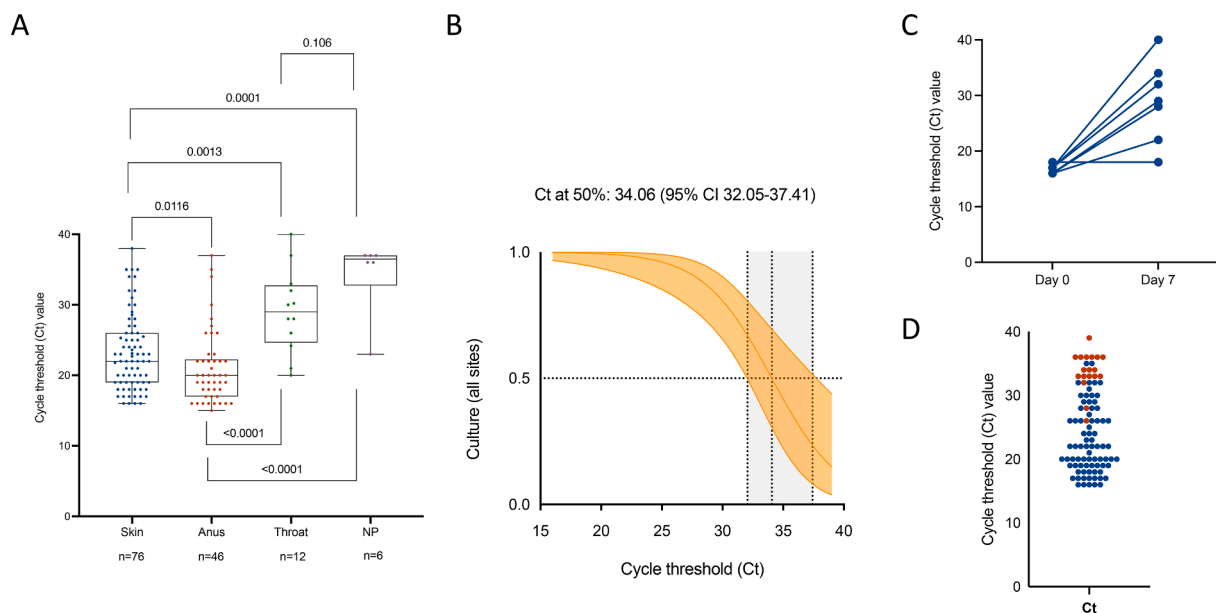


Fig. 1. (A) Monkeypox virus (MPXV) viral loads given as cycle threshold (Ct) values, according to clinical sites, and including samples collected on the day of diagnosis from individual patients. Results are presented as box and whisker plots, with median and interquartile ranges represented in boxes, and range presented as whiskers. (B) Graphical representation of binary logistic regression analysis demonstrating the Ct value at which 50% of samples grew in cell culture (represented in grey with 95% confidence intervals). Shown in yellow is the 95% CI for the regression curve. (C) Change in monkeypox PCR cycle threshold (Ct) values from paired anal samples from day 0 and day 7. (D) Monkeypox virus (MPXV) cycle threshold (Ct) values coloured by culture positivity (denoted by red dots). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

these findings further highlight the multi-site, systemic nature of the recent mpox outbreak.

Our data further validate recent findings showing that samples with a higher MPXV viral load are more likely to demonstrate infectivity in cell culture [8,10,11]. For example, a recent study from Israel observed that clinical samples with a Ct value of ≥ 35 rarely grew in viral culture [8]. Similarly, Hernaez et al., showed that growth in viral culture was more likely in samples with Ct values < 26 [10]. It is possible that slight differences in cell culture positivity between studies reflect factors such as sample storage and processing, PCR assays, and cell culture methods. Although the presence of infectious virus in cell culture may not directly translate with clinical transmission risk, our data provide additional information that may be used as an adjunct with clinical and epidemiological information to help inform clinical and public health guidelines on testing and isolation policies in individuals with mpox infection.

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CRediT authorship contribution statement

Chuan Kok Lim: Conceptualization, Supervision, Data curation, Writing – original draft, Writing – review & editing. **Charlene McKenzie:** Methodology, Writing – review & editing. **Joshua Deerain:** Methodology, Writing – review & editing. **Eric P.F. Chow:** Conceptualization, Writing – review & editing. **Janet Towns:** Conceptualization, Writing – review & editing. **Marcus Y Chen:** Conceptualization, Writing – review & editing. **Christopher K Fairley:** Conceptualization, Writing – review & editing. **Thomas Tran:** Methodology, Writing – review & editing. **Deborah A Williamson:** Conceptualization, Supervision, Data curation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

We declare no competing interests. DAW, EPFC, and CKF are

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