Note: Snapshot PDF is the proof copy of corrections marked in EditGenie, the layout would be different from typeset PDF and EditGenie editing view.

Author Queries & Comments:

AQ1 : Please check the spelling and accuracy of all author names and affiliations, particularly for any of your co-authors. Please also check that author surnames are correctly identified with an orange background. This is to ensure that forenames and surnames are tagged correctly for online indexing. Incorrect names and affiliations may lead to an author not being credited for their work by funders, institutions, or other third parties.

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AQ4 : Please check and confirm the same labels for Figures 3 and 4 part labels (a), (b), etc. and its inner parts (a), (b), etc.

Response: - In Figure 3 (b) the inner part could be marked with an arrow instead of (a) to avoid misunderstanding. - In Figure 4 labels (a)(b) and (c) can be changed for A, B, C or I, II, III. The inner part inside the second figure can remain as (a), (b), (c), (d).

CM1 (A) : Figure 1. Scattered skin lesions at different stages on the penis shaft (a) and glans penis (b)

CM2 (A) : (Figure 1a,b)

CM3 (A) : I have reviewed the spelling and accuracy of all authors names and affiliations.

A Memorable Patient

Clinical, histopathological and ultrastructural features of human monkeypox infection in the 2022 outbreak: report of a case with immunohistochemistry for vaccinia virus

Verso running head : A memorable patient

Recto running head : A memorable patient

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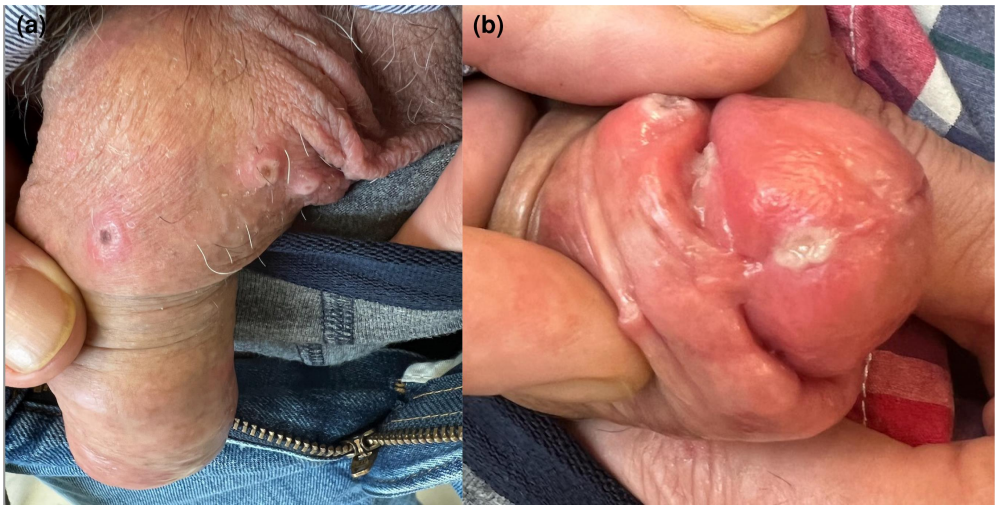
Human monkeypox (HMPX) is an emerging viral zoonosis that has now become the most prevalent Orthopoxvirus worldwide.[1] The Poxviridae family is formed by large, double-stranded DNA viruses that replicate in vertebrate cells. Currently, there is a substantial outbreak of HMPX, with more than 64 200 cases reported to date.[2]

Reports on the histology and ultrastructure of the current HMPX are currently scarce.[3] We describe the clinical, histological, immunohistochemical and ultrastructural features of the current HMPX virus, emphasizing the applicability of both transmission electron microscopy (TEM) and immunohistochemistry (IHC) for a fast visualization and identification of viruses.

A 52-year-old patient sought medical advice because of scattered, mildly painful, genital skin lesions (Figure 1[2]). He was HIV-positive, with the infection well controlled under antiretroviral treatment, and reported recent unprotected sex with other men. A skin snip was performed, and diagnosis of HMPX was confirmed by real-time PCR.

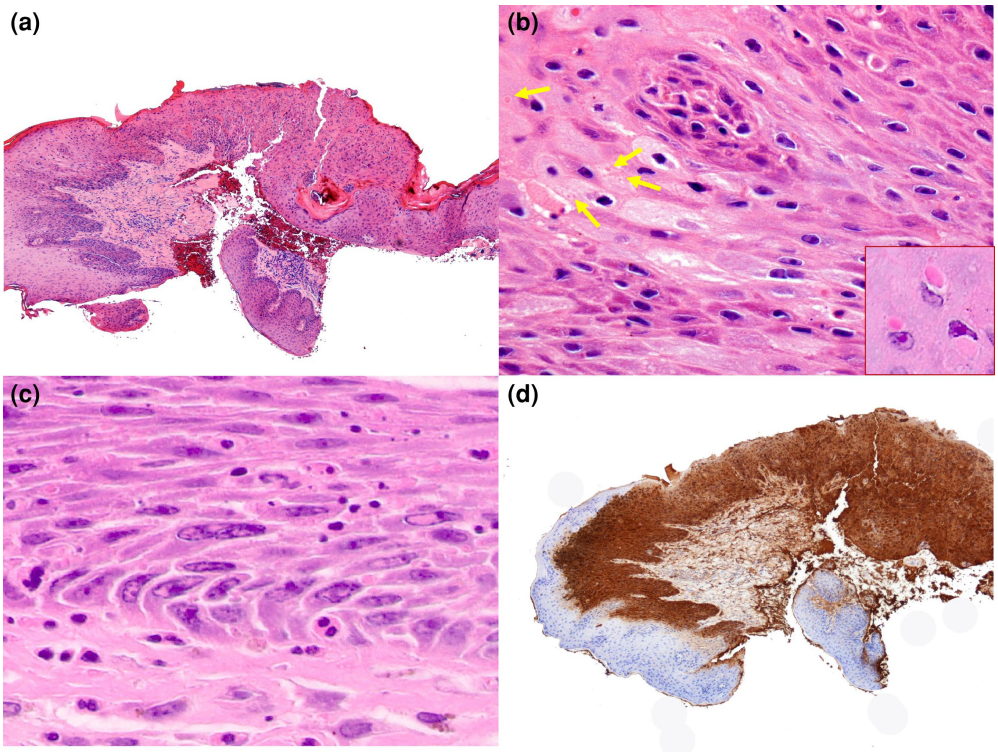
Figure 1[1] Scattered skin lesions at different stages on the penis shaft and glans penis.



Histological sections showed a hyperplastic epidermis with mild hypergranulosis and a central area of keratinocyte necrosis with moderate neutrophilic infiltration. Keratinocytes of the viable epidermis showed varying degrees of ballooning, with abundant pale cytoplasm that occasionally harboured round eosinophilic inclusions. A few multinucleated keratinocytes were seen, and some nuclei showed margination of their chromatin although no clear-cut intranuclear inclusions were identified (Figure 2a-c 1a–c).

IHC for anti-vaccinia virus antibody revealed strong and diffuse cytoplasmic staining in the ballooned and necrotic keratinocytes, as well as in some dendritic dermal cells. No apparent highlighting of the aforementioned inclusions was noted (Figure 2d).

Figure 2 (a) **[AQ4]**Biopsy from the skin of the penis shaft, showing acanthosis and a central area of keratinocyte necrosis; (b) ballooned, viable keratinocytes, some of them harbouring cytoplasmic eosinophilic inclusions (arrows and inset); (c) epidermal basal and parabasal cells with cytoplasmic inclusions and focal chromatin margination. Haematoxylin and eosin, original magnification (a) × 40; (b, c) × 400. (d) Immunohistochemistry for anti-vaccinia virus antibody showing intense positivity in the epidermis and focal staining of dermal cells (original magnification × 40).



Under TEM, mature viruses appeared extracellularly scattered among keratinocytes, while the assembling virions and mature intracellular viruses were clustered within cytoplasmic vesicles in keratinocytes of the stratum granulosum and the Malpighian layer of the epidermis (Figure 3). Their cytoplasm contained numerous vesicles with viral particles in different maturation stages, ranging from 186 to 498 nm in length and 130·5 to 273·1 nm in width. When observed in a full section, some viruses revealed an hourglass-shaped central core, lateral bodies and external membranes with superficial tubules, whereas others appeared as brick-shaped particles. Immature virions appeared as round particles with no visible core (Figure 4).

Figure 3 (a, b) Transmission electron microscopy showed (a) monkeypox viruses inside intracytoplasmic vacuoles and (b) extracellular location of viruses among keratin fibre. Arrowhead indicates outer membrane. Original magnification (a) × 1000; (b) × 4000.

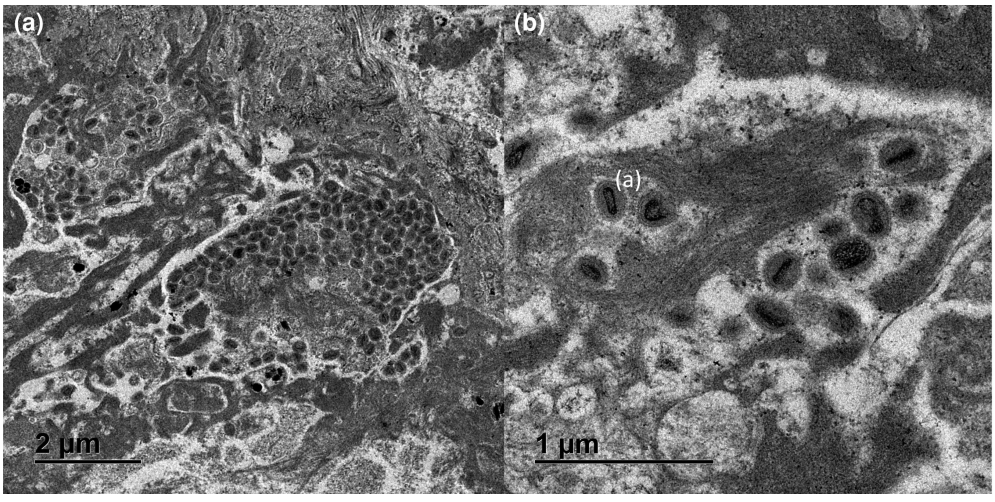
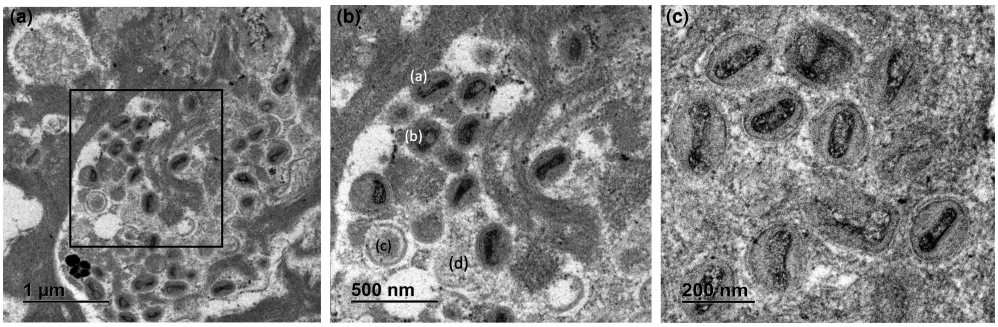


Figure 4 (a) Different maturation stages of monkeypox viruses inside of an intracytoplasmic vacuole in a keratinocyte; (b) detail of viruses assembling in an intracellular vesicle. (a) Both the core and the lateral bodies around it are observed. (b) The typical brick-shape of Orthopoxvirus is noticeable in some of them. (c) Immature form of virus. (d) Groups of scattered small protein particles. (c) Detail of extracellular viruses with a visible outer membrane. Original magnification (a) × 3000; (b) × 8000; (c) × 12000.



This paper illustrates the morphological characteristics of the HMPX virus, as well as its tropism within host cells. Previous descriptions confirm that orthopoxviruses are large, enveloped viruses that replicate in the cytoplasm of the host cell,[4] in contrast to herpes viruses, which replicate in the nucleus. Our histological findings differ from those of other viral infections because of the absence of intranuclear inclusions. TEM allows the distinction of herpesviruses from the various poxviruses and papilloma viruses both on morphological grounds and by the site where replication occurs.

In this study, the typical brick-shaped particles were seen, despite negative staining not being performed. To our knowledge, no previous descriptions of the typical brick-shape particles of HMPX have been observed under TEM in the absence of negative staining.

Unquestionably, the role of molecular biology is essential for an accurate and rapid diagnosis of novel viral diseases. However, modern techniques are far from a diagnostic panacea, as they are capable only of detecting genomic sequences that are already known, and in addition, they can also be inhibited by contaminants and require commercially available reagents, which is not possible for new diseases.

In agreement with Molina-Ruiz*et al*,[5] we consider that IHC can be used to confirm the diagnosis of some viral infections that may be difficult to diagnose under light microscopy alone. IHC for anti-vaccinia antibody produced a strong and diffuse signal in the cytoplasm of the keratinocytes, and did not highlight the cytoplasmic Guarineri's bodies, contrary to a previous report.

Therefore, EM still represents a useful tool in virus diagnosis, with its rapidity and nonselective nature[6] being among its advantages.

Learning points

- The current human monkeypox outbreak has aroused interest in the various aspects of this viral zoonosis, neglected in the developed countries since its first description in Zaire in 1970.
- The present paper is the first detailed report on the ultrastructure of the virus in the current human monkeypox virus outbreak, and provides a full description of the clinical, histopathological and immunohistochemical features (anti-vaccinia antibody) of a recent case of human monkeypox virus infection.
- Anti-vaccinia virus antibody produced distinct and strong immunohistochemical staining for ballooned and necrotic keratinocytes.
- TEM and IHC allow rapid visualization of viruses and their site of replication in any diagnostic sample.
- They both represent useful tools in the diagnosis of viral cutaneous infections that may facilitate clinical decisions in patient care.

References

1 **Kmiec D, Kirchhoff F**. Monkeypox: A New Threat? *Int J Mol Sci. 2022 Jul 17;23(14):7866* .**[AQ2]**

2 Centers for Disease Control. 2022 Monkeypox Outbreak Global Map. Available at: https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html (accessed 29 September 2022).

3 **Maronese CA, Beretta A**, Avallone G et al. Clinical, dermoscopic and histopathological findings in localized human monkeypox: a case from northern Italy. *Br J Dermatol* 2022; doi: https://doi.org/10.1111/bjd.21773.

4 **Moss B**. Poxviridae: the viruses and their replication. In: *Fields Virology*, 5th edn (**Knipe DM, Howley PM**, eds) Lippincot Williams & Wilkins; 2007; 2905–45.

5 **Molina-Ruiz AM, Santonja C, Rütten A et al.** Immunohistochemistry in the diagnosis of cutaneous viral infections. Part I.

Cutaneous viral infections by herpesviruses and papillomaviruses, *Am J Dermatopathol* 2015 37: 1–14; quiz 12–14.

6 **Gelderblom H, Madeley D**. Rapid viral diagnosis of orthopoxviruses by electron microscopy: optional or a must? *Viruses* 2018; 10:142.**[AQ3]**

CPD questions

Learning objective

To demonstrate up-to-date knowledge in the management of human monkeypox infection.

Question 1

What is the diagnostic test procedure of choice for confirmation of human monkeypox infection?

a Cell culture.
b Immunohistochemistry.
c Polymerase chain reaction.
d Clinical suspicion.
e Electron microscopy.

Question 2

What treatment would you prescribe to a patient with confirmed human monkeypox (HMPX) infection with mild symptoms?

a Aciclovir.
b Tecovirimat.
c Oseltamivir.
d Supportive care.
e Brincidofovir.