The emergence of new lineages of the Monkeypox virus could affect the 2022 outbreak.

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Abstract

Human monkeypox is a contagious zoonotic viral disease caused by *Monkeypox virus* and is causing a current outbreak in various regions of the world, being already considered an epidemic and a global public health problem. From the sequenced monkeypox genomes of clades B.1, A.1.1 and A.2 available, we performed analyzes of 9 proteins considered important in the pathogenesis of the disease (A9L, A36R, A50L, B9R, B16L, C3L, C7L, C12L (SPI-1) and H5R) and 4 important proteins for the host's immune response (A27L, A33R, B5R and L1R). We identified four synonymous mutations and six amino acid changes, of which four are in conserved domains, such changes can alter the function of proteins. Furthermore, we did not find the C3L protein in monkeypox genomes from the 2022 outbreak, an important protein for disease pathogenicity. Our analyses suggest that lineage/clade A.2 may be suffering the different effects of various selective pressures than lineage/clade B.1. In conclusion, the mutations identified in the present study have not yet been associated with genetic alterations, significant changes in the transmission route, mean age, signs/symptoms at the clinical presentation, and their evolution could be detected. Therefore, further research in the field is needed since our findings need to be confirmed by new studies.

Introduction

In May 2022, numerous cases of monkeypox started to be identified in several non-endemic countries. In about a month, more than 3.500 confirmed cases of monkeypox have been reported in, at least, 50 non-African countries until past week. (Kraemer et al., 2022; World Health Organization, 2022). These features are totally new for this disease in humans, since *Monkeypox virus* was endemic in West and Central Africa, and only occasionally caused short outbreaks elsewhere in the world, which were quickly contained or peter out by themselves (Huhn et al., 2005; Reed et al., 2004). In endemic African countries, published mortality rates vary from 1% to 10%. Despite the data restriction, the lineage/clade responsible for outbreaks in the Congo Basin appears to be associated with higher virulence (Likos et al., 2005).

Monkeypox virus is a double-stranded DNA virus with about 200-kb genome, being a member of the *Orthopoxvirus* genus from the *Poxviridae* family. Recently, two lineages of the Monkeypox virus were identified in the current outbreak in non-endemic countries (Gigante et al., 2022). The most sequenced lineage/clade, to date, is related with a 2021 travel-associated case from Nigeria to Maryland in the USA (USA_2021_MD) that displays high similarity to the predominant 2022 *Monkeypox virus* outbreak sequences. The second lineage/clade is related to *Monkeypox virus* from a 2021 traveler from Nigeria to Texas in the USA (USA_2021_TX) (Figure 01). In 2005, Likos and collaborators compared clinical, laboratory and epidemiological features of confirmed human monkeypox case-patients. They suggested that human disease pathogenicity was associated with the viral lineage/clade (West African and Congo Basin (Central African)). A comparison of proteins between *Monkeypox virus* clades permitted the prediction of viral proteins that could cause the observed differences in human pathogenicity (Likos et al., 2005).

The re-emergence and dissemination of the *Monkeypox virus* have resulted in infections across the globe. Something has changed. Before the 2022 outbreak, cases outside Africa have previously been limited to a handful that was associated with travel to Africa or with the importation of infected animals. Moreover, the ongoing cases differ from previous outbreaks in terms of age (thirties), sex/gender (most cases being males), and transmission route, being sexual transmission being highly likely. The clinical presentation is atypical and unusual, being characterized by anogenital lesions and rashes that relatively spare the face and extremities (Bragazzi et al., 2022).

Methods

Complete genome sequences of deposited Monkeypox virus were retrieved from the GenBank® (www.ncbi.nlm.nih.gov). Multiple sequence alignment as well as the comparison of nucleotide sequences were performed with MAFFT version 7.4 employing the E-INS-I algorithm (https://mafft.cbrc.jp/alignment/software/). The phylogenetic relations of the complete genome were estimated using Maximum Likelihood Method implemented in RAxML (https://cme.h-its.org/exelixis/web/software/raxml/) under the HKY+G+I model of sequence evolution. Statistical support of the clades was measured by a heuristic search with 1,000 bootstrap replicates in RAxML. The best-fit evolutionary model was determined using the Bayesian Information Criterion.

Results and Discussion

We analyzed nine proteins (A9L, A36R, A50L, B9R, B16L, C3L, C7L, C12L (SPI-1 and H5R) identified by Likos and collaborators in the two lineages/clades (B.1 and A.2) identified in the current outbreak and one lineage/clade (A1.1) identified in 2021 (https://nextstrain.org/monkeypox/hmpxv1). Five proteins are involved with either immune evasion or host range and the remaining four proteins are involved with various aspects of the viral life cycle in other poxviruses (Black et al., 1998; Legrand et al., 2004;

Moon et al., 1999). We observed nucleotide substitutions in six of the nine genes analyzed. The nucleotide changes result in five missense mutations and four synonymous mutations. Some of these changes are within protein domains (table 01). Herein, we found in lineage/clade 2 the development of a signature amino acid sequence in position 442 (aspartic acid > asparagine) in A50L gene. Interestingly, when we evaluated the genomes of the current outbreak, we did not find the C3L gene. The vaccinia virus complements control protein is a 35-kDa protein that is encoded by the C3L gene and secreted by cells infected with the *Vaccinia virus*. Members of this family can block complement-mediated induction of the inflammatory response, and engulfment, killing and lysis of bacteria and viruses (Chen et al., 2005; Isaacs et al., 2003; Kotwal and Moss, 1988).

Orthopoxviruses produces two antigenically distinct infectious virions, intracellular mature virus (IMV) and extracellular enveloped virus (EEV). Structurally, EEV consists of an IMV with an additional outer membrane containing proteins that are absent from IMV. Due to their stability in the environment, IMVs play a predominant role in host-to-host transmission, whereas EEVs play an important role in dissemination within the host (Vanderplasschen et al., 1998). Additionally, we analyzed 4 genes used successfully in vaccine studies and important to the host's immune response in the two antigenically distinct infectious virions. Hooper and collaborators reported that a gene-based vaccine comprised of the A27L and L1R proteins associated with IMV and, A33R and B5R proteins associated with EEV may be a useful candidates to protect against other orthopoxviruses, including those that cause smallpox and *Monkeypox virus* (Hooper et al., 2003). Again, we found lineage/clade A.1 signature amino acid in position 221 (proline > serine) for B5R protein (table 01). However, the remaining genes had no nucleotide changes. These data demonstrate that such proteins are attractive targets for future studies in vaccine production since only B5L had an amino acid substitution.

Additionally, it has already been described that the combination of the four VACV genes (A27L + A33R + L1R + B5R) can provide an alternative vaccine for poxvirus, without the known side effects and serious adverse reactions.

The genomic surveillance has been vital to the early detection of mutations, monitoring of virus evolution and evaluating the degree of similarities between circulating. Molecular clock analyses assumed an evolutionary rate of 5×10^{-6} (Firth et al., 2010). These mutations arise as a natural by-product of viral replication. Our analyses suggest that lineage/clade A.2 may be suffering the different effects of various selective pressures than lineage/clade B.1. Some studies analyzing single genes or whole genomes have suggested a relation between lineage/clade with differences in the human monkeypox disease pathology. Combined, these observations propose that the effect of changes among a moderately small number of genes could account for the modifications in viral clearance and pathogenesis of human infections. (Esposito and Knight, 1985; Likos et al., 2005; Reed et al., 2004).

Therefore, with the emergence of new lineages/clades the evaluation of novel *Monkeypox* variants should include an assessment of the following questions: What effect do these mutations have on transmissibility and spread, antigenicity, aspects of pathogenesis, or virulence? Although it is not yet associated with genetic alterations, significant changes in the transmission route, mean age, signs/symptoms at the clinical presentation, and their evolution could be detected (Bragazzi et al., 2022; Patrocinio-Jesus and Peruzzu, 2022).

Regardless of why the mutations were selected, it is reasonable to expect that many mutations in these genes affect viral fitness. Sometimes a mutation that enhances one viral property, can reduce another property. Although most cases in current outbreaks have presented with mild disease symptoms, *Monkeypox virus* may cause severe disease

in certain population groups as immunosuppressed persons, young children and pregnant women (Di Giulio and Eckburg, 2004). Even if there are few data linking pregnant women and the effects of human *Monkeypox virus* infection, there is evidence that viruses of the *Orthopoxvirus* genus are associated with an increased risk of maternal and perinatal morbidity and mortality (Dashraath et al., 2022; Khalil et al., 2022; Mbala et al., 2017). Understanding how virulence evolves after a virus jumps or adapts to a new host species is critical to the effective prevention and treatment of viral infections. Finally, it is possible that an increased understanding of virulence evolution drawn from a relevant data set (phylogenetics, epidemiology, and experimental studies of virus virulence and fitness) may contribute to new strategies for human monkeypox control and eradication.

Figure 01. Phylogenetic analysis of human monkeypox virus based on 171 genomes complete sequences using the Maximum Likelihood Method using RAxML. The Hasegawa-Kishino-Yano model with gamma-distributed heterogeneity (HKY + G) was selected as the best-fit evolutionary model. Bootstrap: 1000.

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References

Black, E.P., Moussatche, N., Condit, R.C., 1998. Characterization of the Interactions among Vaccinia Virus Transcription Factors G2R, A18R, and H5R. Virology 245, 313–322. https://doi.org/10.1006/viro.1998.9166

Bragazzi, N.L., Kong, J.D., Mahroum, N., Tsigalou, C., Khamisy-Farah, R., Converti, M., Wu, J., 2022. Epidemiological trends and clinical features of the ongoing monkeypox epidemic: A preliminary pooled data analysis and literature review. J.

Med. Virol. https://doi.org/10.1002/jmv.27931

Chen, N., Li, G., Liszewski, M.K., Atkinson, J.P., Jahrling, P.B., Feng, Z., Schriewer, J., Buck, C., Wang, C., Lefkowitz, E.J., Esposito, J.J., Harms, T., Damon, I.K., Roper, R.L., Upton, C., Buller, R.M.L., 2005. Virulence differences between monkeypox virus isolates from West Africa and the Congo basin. Virology 340, 46–63. https://doi.org/10.1016/j.virol.2005.05.030

Dashraath, P., Nielsen-Saines, K., Mattar, C., Musso, D., Tambyah, P., Baud, D., 2022. Guidelines for pregnant individuals with monkeypox virus exposure. Lancet. https://doi.org/10.1016/S0140-6736(22)01063-7

Di Giulio, D.B., Eckburg, P.B., 2004. Human monkeypox: an emerging zoonosis. Lancet Infect. Dis. 4, 15–25. https://doi.org/10.1016/S1473-3099(03)00856-9

Esposito, J.J., Knight, J.C., 1985. Orthopoxvirus DNA: A comparison of restriction profiles and maps. Virology 143, 230–251. https://doi.org/10.1016/0042-6822(85)90111-4

Firth, C., Kitchen, A., Shapiro, B., Suchard, M.A., Holmes, E.C., Rambaut, A., 2010. Using Time-Structured Data to Estimate Evolutionary Rates of Double-Stranded DNA Viruses. Mol. Biol. Evol. 27, 2038–2051. https://doi.org/10.1093/molbev/msq088

Gigante, C.M., Korber, B., Seabolt, M.H., Wilkins, K., Davidson, W., Rao, A.K., Zhao, H., Hughes, C.M., Minhaj, F., Waltenburg, M.A., Theiler, J., Smole, S., Gallagher, G.R., Blythe, D., Myers, R., Schulte, J., Stringer, J., Lee, P., Mendoza, R.M., Griffin-Thomas, L.A., Crain, J., Murray, J., Atkinson, A., Gonzalez, A.H., Nash, J., Batra, D., Damon, I., McQuiston, J., Hutson, C.L., McCollum, A.M., Li, Y., 2022. Multiple lineages of Monkeypox virus detected in the United States, 2021- 2022.

bioRxiv 1–15. https://doi.org/10.1101/2022.06.10.495526

Hooper, J.., Custer, D.., Thompson, E., 2003. Four-gene-combination DNA vaccine protects mice against a lethal vaccinia virus challenge and elicits appropriate antibody responses in nonhuman primates. Virology 306, 181–195. https://doi.org/10.1016/S0042-6822(02)00038-7

Huhn, G.D., Bauer, A.M., Yorita, K., Graham, M.B., Sejvar, J., Likos, A., Damon, I.K., Reynolds, M.G., Kuehnert, M.J., 2005. Clinical Characteristics of Human Monkeypox, and Risk Factors for Severe Disease. Clin. Infect. Dis. 41, 1742–1751. https://doi.org/10.1086/498115

Isaacs, S.N., Argyropoulos, E., Sfyroera, G., Mohammad, S., Lambris, J.D., 2003. Restoration of Complement-Enhanced Neutralization of Vaccinia Virus Virions by Novel Monoclonal Antibodies Raised against the Vaccinia Virus Complement Control Protein. J. Virol. 77, 8256–8262. https://doi.org/10.1128/JVI.77.15.8256-8262.2003

Khalil, A., Samara, A., O'Brien, P., Morris, E., Draycott, T., Lees, C., Ladhani, S., 2022. Monkeypox and pregnancy: what do obstetricians need to know? Ultrasound Obstet. Gynecol. https://doi.org/10.1002/uog.24968

Kotwal, G.J., Moss, B., 1988. Vaccinia virus encodes a secretory polypeptide structurally related to complement control proteins. Nature 335, 176–178. https://doi.org/10.1038/335176a0

Kraemer, M.U.G., Tegally, H., Pigott, D.M., Dasgupta, A., Sheldon, J., Wilkinson, E., Schultheiss, M., Han, A., Oglia, M., Marks, S., Kanner, J., O'Brien, K., Dandamudi, S., Rader, B., Sewalk, K., Bento, A.I., Scarpino, S. V, de Oliveira, T., Bogoch, I.I., Katz, R., Brownstein, J.S., 2022. Tracking the 2022 monkeypox outbreak with

epidemiological data in real-time. Lancet Infect. Dis. 22, 941–942. https://doi.org/10.1016/S1473-3099(22)00359-0

Legrand, F.A., Verardi, P.H., Jones, L.A., Chan, K.S., Peng, Y., Yilma, T.D., 2004. Induction of Potent Humoral and Cell-Mediated Immune Responses by Attenuated Vaccinia Virus Vectors with Deleted Serpin Genes. J. Virol. 78, 2770–2779. https://doi.org/10.1128/JVI.78.6.2770-2779.2004

Likos, A.M., Sammons, S.A., Olson, V.A., Frace, A.M., Li, Y., Olsen-Rasmussen, M., Davidson, W., Galloway, R., Khristova, M.L., Reynolds, M.G., Zhao, H., Carroll, D.S., Curns, A., Formenty, P., Esposito, J.J., Regnery, R.L., Damon, I.K., 2005. A tale of two clades: monkeypox viruses. J. Gen. Virol. 86, 2661–2672. https://doi.org/10.1099/vir.0.81215-0

Mbala, P.K., Huggins, J.W., Riu-Rovira, T., Ahuka, S.M., Mulembakani, P., Rimoin, A.W., Martin, J.W., Muyembe, J.-J.T., 2017. Maternal and Fetal Outcomes Among Pregnant Women With Human Monkeypox Infection in the Democratic Republic of Congo. J. Infect. Dis. 216, 824–828. https://doi.org/10.1093/infdis/jix260

Moon, K.B., Turner, P.C., Moyer, R.W., 1999. SPI-1-Dependent Host Range of Rabbitpox Virus and Complex Formation with Cathepsin G Is Associated with Serpin Motifs. J. Virol. 73, 8999–9010. https://doi.org/10.1128/JVI.73.11.8999-9010.1999

Patrocinio-Jesus, R., Peruzzu, F., 2022. Monkeypox Genital Lesions. N. Engl. J. Med. https://doi.org/10.1056/NEJMicm2206893

Reed, K.D., Melski, J.W., Graham, M.B., Regnery, R.L., Sotir, M.J., Wegner, M. V., Kazmierczak, J.J., Stratman, E.J., Li, Y., Fairley, J.A., Swain, G.R., Olson, V.A., Sargent, E.K., Kehl, S.C., Frace, M.A., Kline, R., Foldy, S.L., Davis, J.P., Damon,

I.K., 2004. The Detection of Monkeypox in Humans in the Western Hemisphere. N. Engl. J. Med. 350, 342–350. https://doi.org/10.1056/NEJMoa032299

Vanderplasschen, A., Hollinshead, M., Smith, G.L., 1998. Intracellular and extracellular vaccinia virions enter cells by different mechanisms. J. Gen. Virol. 79, 877–887. https://doi.org/10.1099/0022-1317-79-4-877

World Health Organization, W., 2022. Monkeypox - United Kingdom of Great Britain and Northern Ireland [WWW Document]. URL https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON383

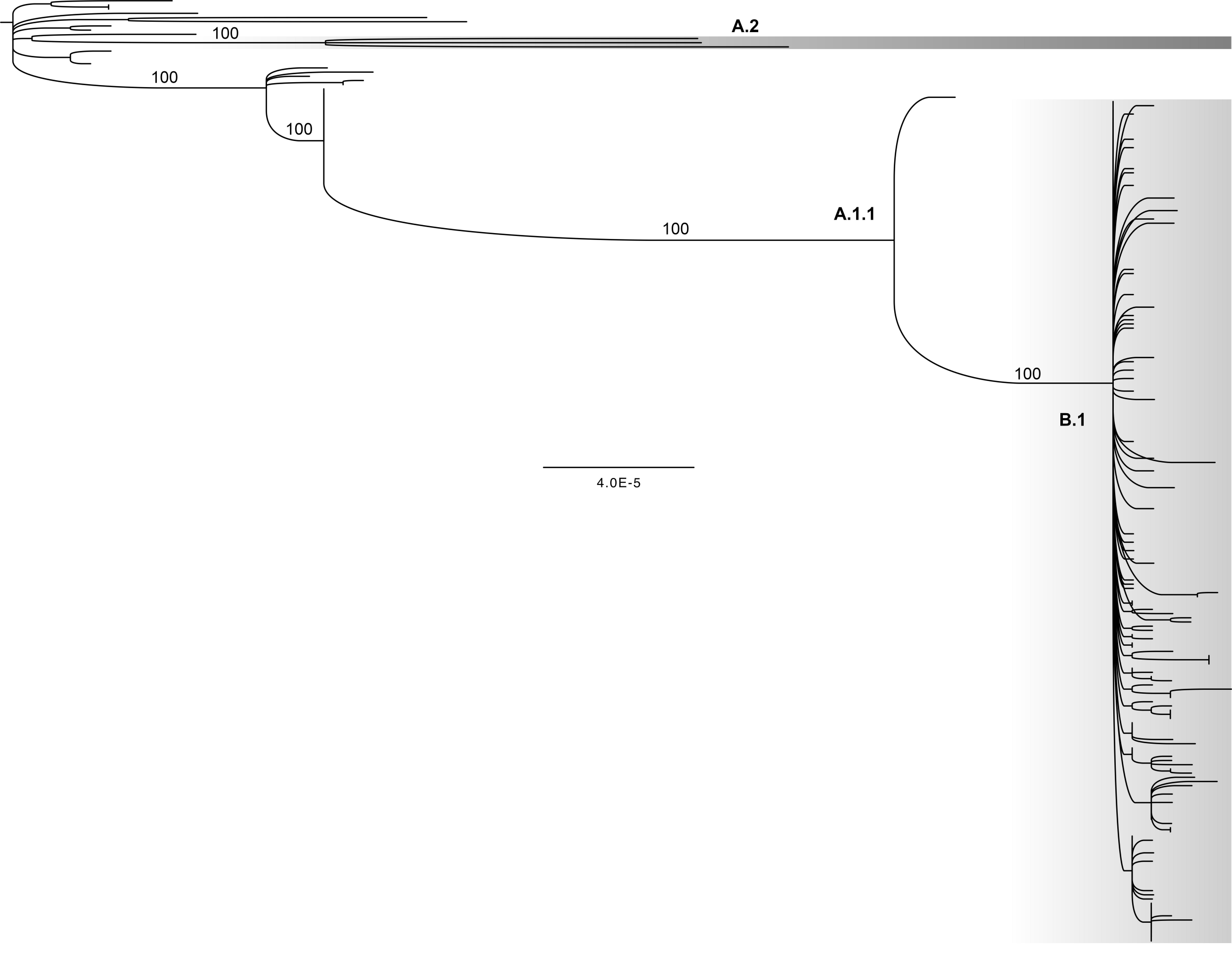
A.2
100
100
100
A.1.1
100
100
B.1
4.0E-5

Table 1: Nucleotide changes between clades/lineages A.2 and B.1. was used 155 completes genomes 2022 outbreak. Gene homologs in *Vaccinia virus* strain Copenhagen (AY315828) are given for each gene as well as notes on proposed functions taken from annotation.

Gene (VACV-Cop)	Note	NT Change	AA Change	Domains (Vaccinia Reference)	Accession	Clade	Importance
A36R	IEV transmembrane (Viral life cycle)	141 C > T	Synonymous	-	ON675438	A.2	Pathogenies
A50R	DNA ligase	413 C > T	Cys138Phe	DNA ligase N terminus	ON803435	B.1	Pathogenies
		1324 G > A	Asp442Asn	ATP dependent DNA ligase C terminal region	ON674051	A.2	Pathogenies
					ON675438		
					ON676707		
B5R	EEV type-I membrane	661 C > T	Pro221Ser	Sushi	ON674051	A.2	Immune response
					ON675438		
					ON676707		
B9R	Expressed late during infection	303 C > T	Synonymous	-	ON675438	A.2	Pathogenies
C7L	Host-range factor for vaccinia virus life cycle in mammalian cells	151 G > A	Asp051Asn	-	ON675438	A.2	Pathogenies
C12L (SPI-1)	Serpin (serine protease inhibitor)	272 C > T	Ser091Leu	-	ON674051	A.2	Pathogenies
		363 A > G	Synonymous	-	ON675438	A.2	Pathogenies
		588 C > T	Synonymous	-	ON675438	A.2	Pathogenies
H5R	Multifunctional protein*	275 C > T	Ser092Phe	Disordered	ON602722	B.1	Pathogenies

* Protein involved in viral DNA replication, postreplicative gene transcription, and virion morphogenesis.