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Title: Draft Genome of a Bovine Enterovirus recovered from Sewage in Nigeria

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ABSTRACT

We describe the draft genome of a Bovine enterovirus (EV) recovered from sewage in Nigeria. The virus replicates on both RD and L20B cell lines, but is negative for all EV screens in use by the GPEI. It contains 7,368nt, with 50.2% G+C content and an ORF with 6,525nt (2,174aa).

Keywords: Bovine enterovirus, EV-E, Nigeria, Sewage, Complete Genome

Enteroviruses are members of the genus Enterovirus (EV), family Picornaviridae, order Picornavirales. Poliovirus (PV) is the type member of the genus and courtesy the Global Poliovirus Eradication Initiative (GPEI), is isolated in about 150 specialized laboratories globally. The laboratories use RD (of human origin) and L20B (Engineered Mouse cells expressing the poliovirus receptor) cell lines for PV isolation (1,2). All isolates that grow on both cell lines are assumed to be polioviruses and subsequently subjected to molecular identification (3). Here, we describe the genome of an isolates that grew on both cell lines but is not poliovirus.

The isolate was recovered from a sewage contaminated water sample collected in Borno State, Nigeria in 2017 and inoculated into RD and L20B cell lines. The isolate replicated on both cell lines but was not poliovirus. The genome of the isolate was extracted using the Total RNA extraction kit (Jena Bioscience, Jena, Germany) and used for cDNA synthesis as previously described (4). The single-stranded cDNA was shipped to a commercial facility (MR, Texas, USA) where library preparation, genome sequencing and assembly were done. Library preparation was done using the TruSeq™ RNA LT Sample Preparation Kit (Illumina) following the manufacturer's recommendations. Sequencing was done paired end for 300 cycles using the MiSeq system (Illumina).

Post assembly of the 3.9 million reads, annotation of the EV-E genome was done by aligning it with previously characterised and annotated EV-E genomes. The draft genome is 7,368nt in

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length, with G+C content of 50.2% and was assembled from 1,963 reads. The 5′-untranslated region (UTR), Open Reading Frame (ORF) and a 3′-UTR contains 800nt, 6,525nt (2,174aa) and 43nt respectively. The ORF encodes one polyprotein that can be cleaved into three (P1 [2,517nt; 839Aa], P2 [1,737nt; 579aa] and P3 [2,271nt; 756aa]) and subsequently into the eleven proteins encoded in EV genomes. A BLAST of the draft genome showed it to be most closely related to LC150009 (an enterovirus recovered from a Cow in Japan in 1990). A BLAST of the VP1 gene however showed it to be most closely related to DQ092778 (an enterovirus recovered from a Cow in Germany in 1999). In the VP1 gene, EV_NGR_2017 and LC150009 are 71.6% and 93.7% similar in nucleotides and amino acid, respectively. On the other hand, EV_NGR_2017 and DQ092778 are 71.7% and 91.4% similar in nucleotides and amino acid, respectively.

Classically Bovine enteroviruses are cultured in Mardin-Darby Bovine kidney (MDBK) cells (5,6). Therefore, the biological basis of EV_NGR_2017's replication in both Human (RD) and Mouse (L20B) cell lines and the implications for zoonosis need to be investigated. Kaundal et al., (7) recently described unidentifiable isolates from sewage in India, that replicated in both RD and L20B cell lines but were not polioviruses. These isolates might be Bovine enteroviruses too.

Accession number

The draft genome assembly described here and the raw reads have been deposited in the GenBank and SRA under the accession numbers MH719217 and SUB4559641, respectively. The BioProject ID is PRJNA493004.

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