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DETECTION AND NUCLEOTIDE SEQUENCE ANALYSIS OF NEW AICHI VIRUS IN WASTEWATER SAMPLES

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Aichi virus (AiV) is a species of the genus Kobuvirus in family Picornaviridae, consists of a single serotype, and is divided into three genotypes A, B, and C with approximately 90% sequence homology in VP1 region. Primers for RT-PCR were designed based on the sequences of the AiV and Bovine kobuvirus genomes. Primers corresponding to the nucleotide sequence of Kobuvirus 3D polymerase gene were used in RT-PCR for amplification of the viral genome from 207 of wastewater samples. Out of 207 samples, 137 samples (67.8%) were positive for amplification of AiV nucleotide. AiV A sequences were detected in all 137 samples. AiV B sequence was detected in only one sample. New sequences of AiV were detected from 9 samples, had 83% similarity to AiV A in this region, and distinct from AiV A and B by phylogenetic analysis. Out of the 9 samples containing with new type of AiV, one (Y12/04) was used for sequence analysis of the other regions. A total of 6 overlapping cDNA clones spanning from VP3 to 3'UTR regions genome of Y12/04 wastewater sample were obtained and their nucleotide sequences determined. The genome consist of 5,125 nt, which encode a potential polyprotein precursor of 1,737 aa, and the 3' end by 244 nt. No. of nucleotide in each regions were as same as that of AiV except 3A and 3'UTR regions. The nucleotide (amino acid) identity of complete VP1 region was 69.1 % (64.9%) between Y12/04 and AiV. The phylogenetic analyses based on the nucleotide and the deduced amino acid sequences of VP1, 2C, 3C and 3D showed that Y12/04 was independent from all other genotypes of AiV, and the evolutionally distance was equivalent to that among the different serotypes of enteroviruses.