



Complete Genome Sequence of a Novel Pestivirus from Sheep

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We report here the complete genome sequence of pestivirus strain Aydin/04-TR, which is the prototype of a group of similar viruses currently present in sheep and goats in Turkey. Sequence data from this virus showed that it clusters separately from the established and previously proposed tentative pestivirus species.

Destiviruses are enveloped viruses with a single-stranded positive-sense RNA genome of about 12.3 kb. Due to similarities in genome organization and strategy of gene expression, pestiviruses and the genera Hepacivirus and Flavivirus form the family Flaviviridae (5). The genus Pestivirus comprises the established species Bovine viral diarrhea virus 1 (BVDV-1), BVDV-2, Classical swine fever virus (CSFV), and Border disease virus (BDV) as well as a growing number of additional tentative pestivirus species isolated from domestic and wild ruminants and from pigs (1, 5). Complete genomic sequences are available for the four approved pestivirus species and three tentative species represented by a group of atypical pestiviruses (HoBi-like) isolated from cattle and buffalo, porcine Bungowannah virus, and giraffe pestivirus. The established pestivirus species can be further subdivided into several genotypes. Based on comparative sequence analysis of partial 5' nontranslated region (NTR) and N^{pro} coding sequences, it has been proposed that the ovine pestivirus strain Aydin/04-TR and other viruses isolated from sheep and goats in Turkey form a novel genotype of the BDV species (4).

Here we report the complete genome sequence of pestivirus strain Aydin/04-TR. Total cellular RNA was extracted from sheep fetal thymus (SFTR) cells 3 days after infection with Aydin/04-TR. The complete virus genome sequence was obtained using overlapping amplicons produced by one-step reverse transcription-PCR (RT-PCR) with Superscript III reverse transcriptase (Invitrogen) and Platinum Taq DNA polymerase (high fidelity; Invitrogen). PCR products were purified and subjected to double-stranded Sanger sequencing (Qiagen, Hilden, Germany). For determination of the 5' and 3' terminal sequences of pestivirus strain Aydin/ 04-TR, a previously described RNA ligation method was used (2, 3). The genome of Aydin/04-TR comprises 12,292 nucleotides (nt) and contains one large open reading frame (ORF). The 5' and 3' NTRs are 377 nt and 227 nt long, respectively. The ORF is 11,688 nt long and encodes a polyprotein encompassing 3,895 amino acids. The highest genomic nucleotide identities were obtained with CSFV strains Alfort-T, Paderborn, Brescia, C, and 39 (72.3 to 72.7%), whereas the identities with BDV (BDV-1 X818, BDV-2 Reindeer-1, BDV-3 Gifhorn, BDV-4 H2121) were below 72.1% and those with the other pestivirus species below 68%. Taken together, comparisons of full-genome sequences revealed that Aydin/04-TR is more closely related to CSFV than to BDV

and other pestivirus species. This argues against the previously proposed classification of Aydin/04-TR as a member of a new BDV subgroup. Instead, comparison of genomic sequences provides evidence that Aydin/04-TR represents a novel pestivirus species.

In addition to nucleotide and deduced amino acid sequence relatedness, antigenic relatedness represents an important criterion for pestivirus species demarcation. To provide a solid basis for classification of ovine pestivirus strain Aydin/04-TR, a detailed phylogenetic analysis together with an analysis of antigenic relatedness is currently under study.

Nucleotide sequence accession number. The genomic sequence of pestivirus strain Aydin/04 has been submitted to GenBank (accession number JX428945).

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REFERENCES

- 1. Becher P, Thiel H-J. 2011. Genus Pestivirus (*Flaviviridae*), p 483–488. *In* Tidona CA and Darai G (ed), Springer index of viruses, second ed. Springer Verlag, Heidelberg, Germany.
- Becher P, Orlich M, Thiel H-J. 1998. Complete genomic sequence of border disease virus, a pestivirus from sheep. J. Virol. 72:5165–5173.
- Becher P, Orlich M, Thiel H-J. 2000. Mutations in the 5' nontranslated region of bovine viral diarrhea virus result in altered growth characteristics. J. Virol. 74:7884–7894.
- Oguzoglu TC, et al. 2009. Border disease virus (BDV) infections of small ruminants in Turkey: a new BDV subgroup? Vet. Microbiol. 135:374–379.
- Simmonds P, et al. 2012. Family *Flaviviridae*, p 1003–1020. *In* King A, Lefkowitz E, Adams MJ, Carstens EB (ed), Virus taxonomy. Ninth report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, CA.

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