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**Characterization, including VP1 and full-length sequencing of type A and O strains collected from foot-and-mouth disease outbreaks since 1996 in Turkey**

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**Abstract:**

The present report adds years to the monitoring since 1964 of FMDV strains causing disease outbreaks in Turkey. Full-length VP1 sequencing confirms a significant discrepancy in epidemiological dynamics of serotype A versus serotype O strains where distinct A genotypes are repeatedly introduced from exogenous sources while serotype O strains re-emerge from islands of persistent circulation. The introduction in November 2005 of a novel genotype A05 to Turkey is revealed. In addition, the data reveal a significant temporal clustering of isolates suggesting that a limited number of strains, emerging, show a strong tendency to quickly become widely distributed in Turkey. Preliminary analyses of full-length sequences of serotype A genotypes does not reveal significant information on evolutionary origin.

**Introduction:**

FMD is the most serious disease in the cattle industry in Turkey and the introduction of FMDV strains from the east and the presence of FMDV in Turkey represents a continuous threat to the FMD-free European countries. The epidemiology of FMD in Turkey has been subject to several studies during the past decade (Aktas, 1998; Parlak, 2005; Gilbert, 2005). Since 1952, serotypes A, C, O, Asia1 and SAT 1 among the 7 serotypes of FMDV, circulating globally, have been reported from outbreaks of FMD in Turkey and Asia1, A and O are currently reported in spite of considerable efforts to control the disease including vaccination which now involves biannual mass vaccination covering more than 50% of the cattle. The epidemiological situation apparently is very complex and is believed to involve a variety of epidemiological dynamics.

**Materials and Methods:**

Virus isolates included tissue samples from FMD cases submitted over the years for laboratory diagnosis according to the national surveillance practice, as well as multiple tissue and probang samples collected from outbreaks of FMD during October 2005 in the Kirsehir and Nevsehir districts for high-resolution strain identification. The origins of isolates collected during 1998 to July 2004 were widely distributed as shown elsewhere (Parlak et al., 2006) and the origins of isolates collected from August 2004 to December 2005 are shown in Fig. 1. Serotype Asia1 strains are infrequently isolated and these isolates were not included in the present study.



**Figure 1. Localisation of FMD outbreaks in Turkey during August 2004 to December 2005**

Isolates were characterised by full-length VP1 sequencing using two sets of primers, one specific for O strains and one specific for A strains identified in Turkey as shown in Table 1. Representatives of the various genotypes were characterised by full-length sequencing of a set of overlapping PCR products to be described elsewhere. Sequence information is to be deposited in Genbank.

**Table 1 Primers used for VP1 full-length sequencing**

Primer name	Sequence in 5'-3' orientation	Specificity	Reference
A1	ACCAATGTACAGGGATGGGT	Serotype O	Klein et al., 2006
A1	TTGCACCGTAGTTAAAGGAA		
3135-F(A)	ACAAACGTACAGGGGTGGGT	Serotype A	The present study
3926-R(A)	ACGTCTCCCGCCAACTTGAG		

Multiple alignments and phylograms were done with ClustalX (EMBL, Heidelberg, Germany, May 1994) using the default parameters and 1000 bootstrap replications. The aligned sequences were presented with GeneDoc (Nicholas et al., 1997) and dendrograms were visualized with MEGA3 version 3.1 (Kumar et al., 2004).

## Results:

Dendrograms of the type A and O isolates are shown in Fig. 2 and 3, respectively. The A96, A99 and A05 clusters are very distinct ones with distances > 0.2 between them and distances >1.4 to any of the A genotypes previously identified by Aktas (1998) (Parlak et al., 2006). Antigenic characterisation based on  $r_1$  estimations indicated that vaccines based on an A96 isolate protected against A99 strains (Parlak et al., 2006) while no firm conclusion has yet been reached for A05 strains. The A/Afyon/03

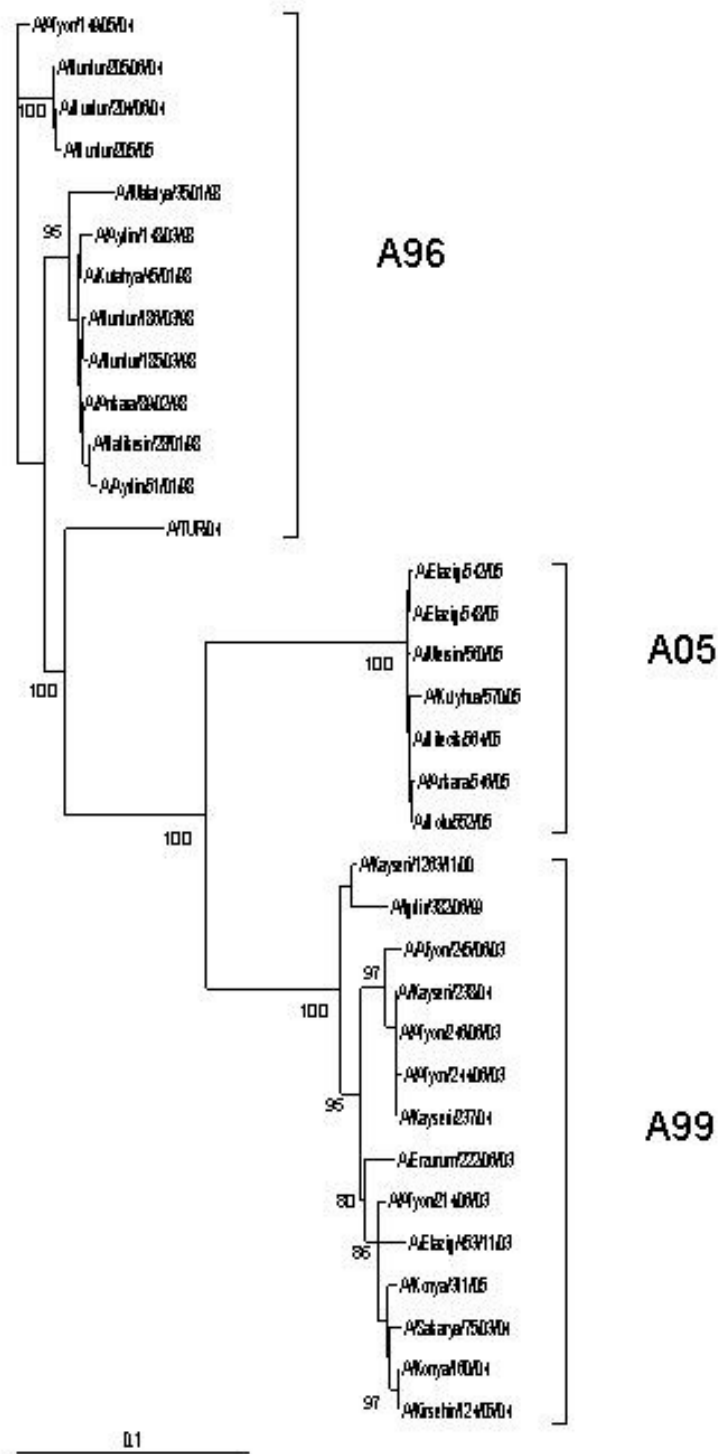


Figure 2. Dendrogram based on VP1 sequences of A isolates from outbreaks in Turkey during 1998 to 2005. Bootstrap values >80% are indicated.

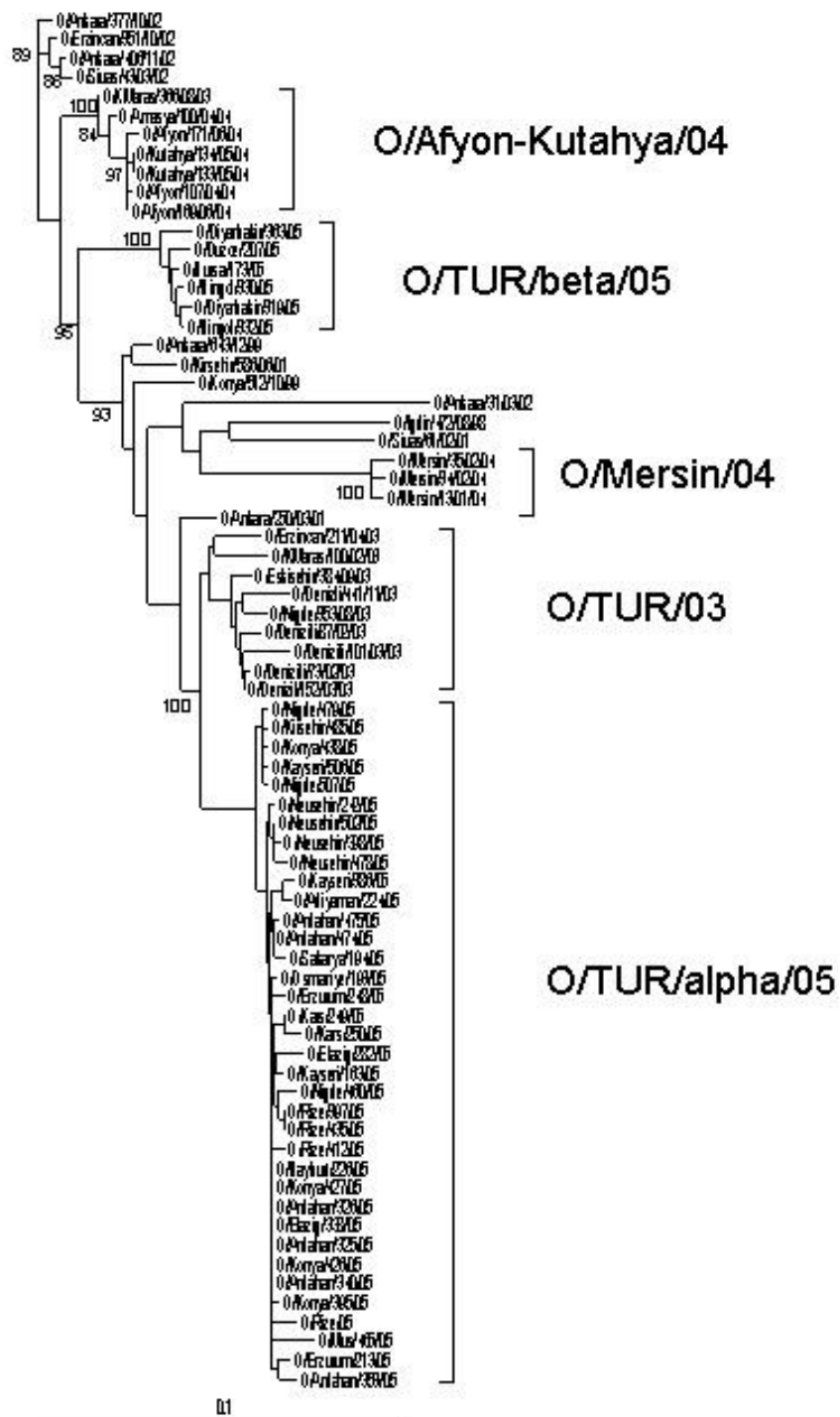


Figure 3. Dendrogram based on VP1 sequences of O isolates from outbreaks in Turkey during 1998 to 2005. Bootstrap values >80% are indicated.

and A/Kayseri/04 isolates differed by perfect linkage of 9 nucleotide markers in VP1 none of which were found in any of the other A99 isolates (data not shown) suggesting this to be a distinct strain within the A99 genotype, in turn suggesting at least two introductions of A99 strains to Turkey. The isolates belonging to genotype A05 represent a highly homogenous cluster with only few singleton deviations. Full-length sequencing of representatives of the 3 genotypes was concordant with the genetic distances based on VP1 and comparison with other serotype A genotypes revealed no additional evidence of evolutionary origin (data not shown).

Clusters are also observed among O isolates but differences between closest related clusters are  $< 0.05$ . The clusters prior to 2005 in some cases reflected that multiple isolates were collected from the same areas such as isolates of the O/TUR/03 cluster collected in Denizli in 2003 and isolates of the O/Mersin/04 cluster, while the cluster of other isolates collected in 2004 such as the O/Afyon-Kutahya/04 cluster originated from different areas of Turkey. The situation in 2005 differed significantly in revealing two distinct O clusters, O/TUR/alpha/05 and O/TUR/beta/05, both representing isolates collected in widely separated parts of Turkey. The O/TUR/alpha/05 cluster is highly homogenous. Within each of the two clusters the same deviating markers are found in isolates from the various outbreak regions (data not shown) strongly suggesting that the two clusters represent two strains emerging and spreading rapidly.

### **Discussion:**

Multiplicity of strains being introduced to a population or reactivated in a population versus tendency of spread in the population, once emerged, are significant parameters in terms of understanding the initial dynamics of emerging epidemics and might be of great importance in the design of evidence-based control measures. The present study is addressing these questions, in particular by exploiting the quasi-species nature of FMDV strains cf. Christensen et al. (2005) by analysing multiple samples from outbreaks.

It was reported by Aktas (1998) and Parlak et al. (2006) that serotype A and O strains differ significantly in epidemiological dynamics, serotype A genotypes being introduced from exogenous sources and circulating only for a limited span of years in Turkey while serotype O strains re-emerge from islands of persistent circulation. The study of isolates collected in 2005 confirms this picture revealing an introduction of a new genotype A05 to Turkey from an exogenous source and re-emergence in epidemics of two serotype O strains apparently representing the evolutionary continuum of serotype O strains previously shown to circulate in Turkey. Since 1999 and unlike previous years, both A96 and A99 genotype strains were found to be continuously circulating in Turkey in spite of increased vaccination efforts.

As the span of years of monitoring strains emerging in FMD outbreaks in Turkey increases and in particular as the number of isolates increases, a temporal clustering of isolates becomes the more evident. The genotype A05 strain introduced in 2005 and strain O/TUR/alpha/05 identified in outbreaks from many different parts of Turkey during 2005 are highly homogenous strains, strongly indicating that they represent a single introduction and a single re-emergence, respectively, with a subsequent aggressive spread from two epicentres. Gilbert et al. (2005) used a meta-population approach to conclude that long distance transmission have become more significant in FMD epidemiology in Turkey. Our data comply perfectly with this conclusion. Yet, sporadic cases and limited spread of A96 and A99 strains was also seen in 2005.

### **Conclusions:**

- A significant temporal clustering of isolates from outbreaks of FMD is becoming increasingly evident.
- The wide geographical dissemination of strains suggest animal movements to be the most significant risk parameter.
- Sporadic cases with limited spread of strains re-emerging still play a role.

### **Recommendations:**

- Strains emerging in epidemics should be monitored continuously.
- Appearance of strains, identified, should be linked to transmission modes.

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