DEVELOPMENT OF LINEAGE-SPECIFIC REAL-TIME PCR ASSAYS FOR DETECTION OF THE FMDV LINEAGE O/SEA/MYA-98

[1]Saduakassova M.A., [1]Sultanov A.A., [2]Bankowska K.B.

[1]"KazSRVI" LLP, Kazakhstan
[2]The Pirbright Institute, United Kingdom
E-mail: sadumeru_87@mail.ru

FMD is endemic across much of Asia and West Eurasia where field outbreaks due to co-circulating serotypes O, A and Asia 1 are regularly reported in domesticated livestock. Of these, serotypes O (ME-SA/PanAsia and ME-SA/PanAsia-2 lineages) and A (ASIA/Iran-05 and ASIA/Sea-97 lineages) have been previously detected in Kazakhstan. However, there are still gaps in the understanding of virus movements. The FMD viruses currently circulating in countries in the neighbourhood of Kazakhstan are known for their rapid and unpredictable expansion into new geographical areas. Therefore, in addition to the FMDV strains previously reported in Kazakhstan the O/SEA/Mya-98 additional viral lineage should be considered as a threat to Kazakhstan's agricultural industry. To facilitate identification of Mya-98 lineage a real-time RT-PCR assay detecting a specific FMDV lineage of serotype O was designed and validated.

The causative agent of foot and mouth disease belongs to the genus Aphthovirus, family Picornaviridae in the taxonomy of viruses. It has 7 typical variants: A, O, Asia-1, C, SAT-1, SAT-2, SAT-3. Animals, infected by one serotype of foot-and-mouth disease virus, can be affected again with another type variant of the virus. Therefore, each serotype of foot-and-mouth disease must be considered as a separate noso unit and take appropriate preventive and combat measures. In addition, for each serotype of foot and mouth disease virus, during the circulation in nature (reproduction in the body of various species of animals and animals with different specific and nonspecific immune status), lineage appears that differ significantly and insignificantly in antigenic and genetic relationship.

VP1 sequence data, including historical and current strains of FMDV, were aligned, analysed and conserved regions were identified as targets for individual lineage-specific assay. Multiple primers and probes for each of the assays were designed according to TaqMan specification and tested for diagnostic sensitivity and performance. Best assay candidates were selected and validated with a panel of field samples at the Pirbright Institute, UK and the Kazakh Scientific-Research Institute, Kazakhstan.

The Mya-98 lineage-specific RT-PCR assay for detection of FMDV strains circulating in Asia were designed; to detect FMDV the O/SEA/Mya-98 lineage. This assay was shown to correctly identify FMDV lineage in a panel of field and tissue cultured samples originated from Asia. Mixed serotype samples could also be identified using the system. In addition, FMDV positive samples which could not be propagated in tissue culture were investigated and, in many cases, the serotype of the samples could be determined. The assay developed have been shown to be of a similar efficiency to the "gold standard" 3D assay (Callahan at al., 2002).

Real-time RT-PCR assay was developed able to determine the serotype O/SEA/Mya-98 of FMD viruses circulating in Asia. This assay can aid molecular epidemiology of FMDV and help to inform FMDV control policy in the region. The system could be also adapted to field diagnostic platforms aiding transfer of technology and use in the countries concerned.

Accurate and timely diagnosis of FMD using laboratory tests provides vital support to surveillance and disease control programmes. Molecular assays such as real-time RT-PCR are now established as front-line tests, and are widely used for routine diagnosis in most Reference Laboratories.