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Investigations for pestiviruses in camels and deer from the UAE

Dear Dr Mohamed,

With reference to your request to Dr TW Drew in May 2003 regarding diagnostic verification and typing of pestiviruses from various ruminants, I'd like to report on our findings based on the submitted samples.

The request for biosecurity reasons to submit samples suspended in Trizol reagent only enabled us to analyse RNA extracted from the samples for known pestiviruses sequences. All 12 samples were subjected to individual RNA extraction as specified by the reagent manufacturer, and reverse transcribed (RT) to generate cDNA. Subsequently, cDNA aliquotes were amplified by polymerase chain reaction (PCR) using pestivirus-specific primers targeting the 5'-UTR, which is the most conserved genetic region of pestiviruses. The primers used were the 324/326 (originally described by Vilcek et al., 1944), and A11/A14 pairs, which have succeeded in amplifying DNA from a wide range of diverse pestiviruses. These PCR gave only rise to faint bands from some of the samples. For selected samples, repeated PCRs were set up to generate sufficient DNA to allow purification and sequencing of the PCR product, but the results of these repeated PCRs were not always consistent with the initial results. An example of gel electrophoresis bands obtained with this primer pair is shown in Fig. 1, where specific faint bands can be seen for samples 1, 3, 9, 10, 11 and 12.

For samples no 7, 9 and 12 sufficient DNA was generated to set up sequencing reactions. Only for one of these (no 12) a pestivirus-like sequence longer than 100 nucleotides was obtained. When aligned with corresponding sequences from a variety of pestiviruses, the no 12 sequence (113 nucleotides, compared to ~245 for the others) showed greatest similarity to CSFV. However, a suboptimal quality of the no 12 sequence and also the shorter length makes this classification only approximate. It also has to be mentioned that CSFV diagnostic work, including handling of a CSFV type 1.1 isolate as positive control is carried out in our laboratory as well. In Fig. 2 the genetic relatedness of various pestiviruses, including the sequence obtained from sample no 12 is shown.

For a few of the submitted samples, there was sufficient cDNA to also set up PCRs with experimental primers we have used for characterisation of atypical pestiviruses. Some additional PCR products were obtained, but since these PCR products were of different sizes compared to the expected ones, this did not add useful information beyond what was obtained with the 5'-UTR PCR primers.

The conclusion from the above investigations should be drawn with care. What is certain is that it is highly unlikely that the submitted samples contained a BVDV-1 or -2, or border disease virus similar to those found in domestic cattle and sheep, which all amplify very efficiently with the 5'-UTR primer pair combinations used.

On the other hand, several "atypical" pestiviruses have been isolated from non-bovine ruminants over the years, and it cannot be excluded that an exotic pestivirus may have been involved in the described cases. Any such may have only partial genetic homology to the primers used, and thus amplify inefficiently, resulting in faint PCR products. Contamination with previously amplified DNA might have been picked up as a consequence of the high sensitivity of the employed PCR, but we have rarely seen several faint PCR products only obtained from only the sample material under testing.

Since the initial test results leading to a suspicion of infection with BVD were by antigen ELISA, I'd like to mention that we have heard of cases with both false negative and false positive test results with such diagnostic kits. Generally, commercially available diagnostic kits are reliable, but when applied on sample material they previously not have been evaluated for, aberrant test results are more likely to be seen. We have on one occasion got submitted a cattle serum sample that had tested positively with the IDEXX Herdcheck "serum" antigen ELISA; but when tested by RT-PCR and virus isolation in cell cultures we found it negative with both tests. To minimise the probability of false pestivirus diagnoses when using diagnostic kits, is useful to combine testing for both BVDV antibodies and viral antigens in the same group of animals. In groups of cattle where BVDV is circulating, the majority will eventually become antibody positive, and only persistently infected individuals will remain viremic or antigen positive. However, the epidemiology of pestivirus infections in other species than cattle and sheep is not well known. Consequently, the performance of antigen detection tests can be difficult to predict, since all commercially available BVDV antigen tests are based on the fact that viremic/antigenemic animals are immunotolerant and persistently infected. When it comes to serology in camels and deer, only blocking BVDV antibody ELISA kits are suitable, since the specificity of the anti-bovine conjugated secondary antibodies in indirect ELISAs may not cross react sufficiently with e.g. camelid immunoglobulins. Alternatively, virus neutralisation tests are among the most specific for detection of pestivirus antibodies, but they are obviously dependent on suitable cell cultures and reference viruses. Independent of choice of assay, it is important to keep aliquots of original sample material for future reference. The OIE Manual of Standards for diagnostic tests and vaccines describes protocols suitable for BVD laboratory diagnosis.

I would recommend that you continue surveilling for ruminant pestiviruses in your country. Initially I would suggest doing an antibody prevalence surveillance of dairy herds – even if there are specific pestiviruses among camelids, it is useful to know to what extent the dairy cattle are infected with BVDV. In northern Europe wild ruminants such as deer are occasionally found to be BVDV antibody positive, and this is most often due to transmission of BVDV from domestic cattle. If the dairy cattle in the UAE are BVDV positive, they are likely to serve as sources of infection for all other susceptible animals, including camels, if contact through animal movements should occur.

It may be difficult and resource demanding to maintain cell culture testing facilities suitable for pestivirus diagnosis, but given the situation for countries where other OIE list A viruses are present in ruminants, it will otherwise be very difficult to transfer sample material for confirmative testing – this will have to be isolated viruses. If you see this as an option for future investigations into the existence of pestiviruses in camels or other ruminant species in the UAE, we can supply you with specific antisera, monoclonal antibodies and reference virus strains useful for such tests.

Although not confirmative with regard to the testing of your samples, I hope this have added some useful information to aid with further investigations.

Yours sincerely,

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Fig. 1. Agarose gel electrophoresis of PCR products obtained with cDNA from UAE samples. Identity 1 – 12 as for submitted samples. The specific band for sample 1 (290 nucleotides) is framed. Molecular weight markers: 100 bp ladder.

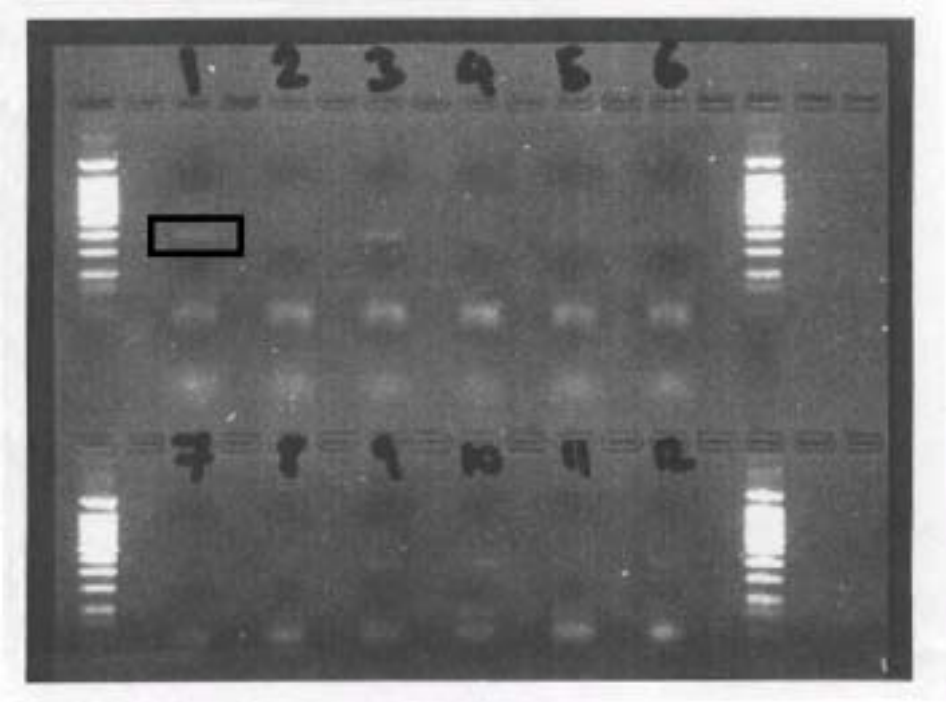

Fig. 2. Phylogram of selected BVDV, BDV, CSFV plus other pestivirus 5'-UTR sequences.