Letters to the Editor

False-Positive Results Obtained when Bluetongue Virus Serotype 1 Algeria 2006 Was Analyzed with a Reverse Transcription-PCR Protocol for Detection of Epizootic Hemorrhagic Disease Virus[∇]

During the analysis of the bluetongue virus serotype 1 (BTV-1) strain Algeria 2006 grown in Vero cells at our laboratory (Laboratorio Central de Veterinaria [LCV]), false-positive results for epizootic hemorrhagic disease virus (EHDV) were detected when the reverse transcription-PCR (RT-PCR) technique previously described by Aradaib et al. was used (1). Bluetongue outbreaks caused by BTV-1 in Algeria and Morocco at the end of 2006 represented a high risk of introduction of this serotype in Spain. To assure the capabilities of LCV routine diagnostic procedures for detection of this BTV-1 strain, the BTV-1 Algeria 2006 isolate was obtained from the European Reference Laboratory. The virus was grown in Vero cells and analyzed with the BTV-specific RT-PCR assay previously described by Toussaint et al. (2), confirming the suitability of the method for sensitive detection of this viral strain. It was also tested to discard the concomitant presence of EHDV and other pathogens. EHDV detection was carried out with Aradaib's multiplex BTV-EHDV RT-PCR but using only EHDV-specific primers. Surprisingly, an amplification fragment of the specific size was obtained (Fig. 1) when assaying BTV-1 Algeria 2006 in EHDV-specific RT-PCR with the external primers E1 and E4 (targeted at nucleotides [nt] 175 to 194 and 543 to 562, respectively, of the EHDV NS1 gene). However, no amplification was produced using this PCR product in nested PCR with the internal primers E2 and E3 (targeted at nt 216 to 235 and 421 to 440, respectively). The DNA fragment size, established by sequence, was 384 bp and, consequently, could not be distinguished from the EHDV NS1 gene expected specific band (388 bp) on agarose gels. The sequence of this DNA fragment was compared with that in the GenBank database using BLASTN (NCBI), and high homology (94.8%) was found within a region of the VP2 gene (nt 2080 to 2464) belonging to the BTV-1 South Africa reference isolate (GenBank accession number AJ585122). In order to explain the BTV-1 Algeria 2006 unspecific amplification with primers specific for E1 and E4 EHDV, the nucleotide sequence around this VP2 region was determined in the BTV-1 Algeria 2006 isolate. A comparative study showed that the 3'

FIG. 1. Agarose gel showing DNA products obtained in the EHDV-specific RT-PCR with external primers E1 and E4 (1st RT-PCR) and in the nested PCR with internal primers E2 and E3 (Nested PCR). C+, EHDV used as a positive control in the reaction; BT-1, BTV-1 Algeria 2006 grown in Vero cells; C+(L), EHDV used as a sensitivity detection limit positive control in the reaction.

end of E1 and E4 oligonucleotides shared 9 and 5 nt, respectively, with the sequence of the BTV-1 Algeria 2006 VP2 gene (Fig. 2). This finding could justify the unexpected amplification, as it is known that correct annealing of the 3' terminus of the primer with a target template is very important for efficient polymerase extension. Even though the same homology is observed in the 3' end of the E1 and VP2 sequences of other BTV-1 isolates (GenBank accession numbers AJ585122 and X55800), only 2 nt of the E4 3' end are shared, making false-positive results with these BTV-1 strains unlikely. No significant homology between the primers and reference strains of other BTV serotypes has been found. EHDV and BTV infect wild and domestic ruminants and share transmission vectors (*Culicoides* spp.), making it essential to have a differential diagnosis of these viruses in areas

FIG. 2. Comparison of the BTV-1 Algeria 2006 VP2 gene, the partial sequence, and oligonucleotides E1 and E4. Coincident nucleotides are indicated with an asterisk. E4c, E4 oligonucleotide complementary sequence. The BTV-1 sequence is numbered according to AJ585122.

where both viruses are present. Furthermore, the absence of EHDV and other pathogens must be proven in virus seeds employed in the development of BTV vaccines. Therefore, it is necessary to perform an EHDV-specific detection test on several occasions for samples containing BTV-1. Only a few RT-PCR methods for EHDV detection have been reported, and Aradaib's method of detection is probably the most widely used. This has encouraged us to communicate these results showing the requirement of confirmatory tests, as nested PCR or sequencing, when an EHDV-positive result is obtained using Aradaib's RT-PCR method.

REFERENCES

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