Veterinary Microbiology 155 (2012) 191–197

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Co-circulation of bluetongue and epizootic haemorrhagic disease viruses in cattle in Reunion Island

Corinne Sailleau [a,*], Gina Zanella [a], Emmanuel Breard [a], Cyril Viarouge [a], Alexandra Desprat [a], Damien Vitour [a], Micheline Adam [a], Laurent Lasne [b], Arnaud Martrenchar [c], Labib Bakkali-Kassimi [a], Laura Costes [b], Stéphan Zientara [a]

[a] French Agency for Food, Environmental and Occupational Health and Safety (ANSES), 23 Avenue du Général de Gaulle 94706 Maisons-Alfort Cedex, France
[b] Directorate for Food, Agriculture and Forestry – Parc de la Providence 97488 Saint Denis Cedex, France
[c] General Delegation of French Overseas Regions, 27, rue Oudinot, 75007 Paris, France

ARTICLE INFO

Article history:
Received 3 May 2011
Received in revised form 29 August 2011
Accepted 5 September 2011

Keywords:
Orbivirus
Epizootic haemorrhagic disease in deer
Bluetongue
Molecular diagnosis

ABSTRACT

Bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV) in deer have already been isolated in Reunion Island and have caused more or less severe clinical signs in cattle (EHDV) or in sheep (BTV), as observed in 2003. In January 2009, cattle in Reunion Island showed clinical signs suggesting infection by one or the other of these arboviral diseases. A study was set up to determine the etiology of the disease. Analysis by reverse transcriptase-polymerase chain reaction (RT-PCR) performed on blood samples from 116 cattle from different districts of the island detected the presence of the EHDV genome in 106 samples and, in 5 of them, the simultaneous occurrence of BTV and EHDV. One strain of EHDV (7 isolates) and one of BTV were isolated in embryonated eggs and a BHK-21 cell culture. Group and subgroup primer-pairs were designed on the segment 2 sequences available in GenBank to identify and type the EHDV strains.

Phylogenetic analysis of the genomic segment 2 (encoding the VP2 serotype-specific protein) of the isolates confirmed the serotypes of these two orbiviruses as BTV-2 and EHDV-6 and allowed them to be compared with previously isolated strains.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV) belong to the family *Reoviridae* and the genus *Orbivirus* (Verwoerd and Erasmus, 2004; Maclachlan and Osburn, 2004). These two viruses have structural, antigenic and molecular similarities. Both viruses are transmitted to their host range (ruminants) by the bites of *Culicoides* midges. Serological and molecular techniques for the laboratory diagnosis of these two diseases are very similar.

Both viruses have seven different structural proteins (VP1 to VP7) divided into two capsids (Roy, 2005). The outer capsid consists of VP2 and VP5. The VP2 protein, the major constituent of the outer capsid, is exposed on the surface of the virus particle and determines the serotype-specific antigen. Twenty-four BTV serotypes and 7 EHDV serotypes have been identified (Anthony et al., 2009). The specific antigens of each serotype induce the production of serotype-specific neutralising antibodies. Segment 2, which encodes VP2, is favoured for conducting studies of genetic variability between different serotypes.

EHDV primarily infects wild ruminants such as deer, which develop the most severe clinical signs. Several strains – including strains of EHDV serotype 2 (Ibaraki strain), 6 and 7 – can also cause a severe disease in cattle; clinical signs observed are similar to those caused by the European strain of BTV-8. EHD is endemic in the United States and Canada, where it causes clinical signs exclusively in certain species of deer. In Australia, Southeast

* Corresponding author. Tel.: +33 149772707; fax: +33 143689762.
E-mail address: corinne.sailleau@anses.fr (C. Sailleau).

0378-1135/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2011.09.006

Asia, sub-Saharan Africa and, since 2006, in the Maghreb countries (serotype 6) and the Arabian Peninsula (serotype 7), it occurs in outbreaks and causes seasonal clinical signs in cattle.

In January 2009, cattle in Reunion Island showed clinical signs of hyperthermia, anorexia, congestion and petechia of the oral mucosa, prostration and nasal discharge. Some animals had a severe dyspnea, facial oedema and lameness. The morbidity rate was 0.8% and the case fatality rate was estimated at 11%. This clinical pattern could be attributed to BTV or EHDV.

In this article, we present a study, which was set up in February 2009 to determine the etiology of this disease and to quantify the clinical signs displayed by cattle in 2009 in Reunion Island. The laboratory diagnostic techniques developed and used to identify viruses responsible for the observed pathology are described.

2. Materials and methods

2.1. Clinical studies

Private veterinarians participating in the study were asked to take blood samples from at least 100 cattle showing EHD clinical signs. They also had to fill out a detailed questionnaire on clinical signs and lesions observed.

2.2. Blood samples

EDTA blood samples were collected from cattle displaying clinical signs, for viral isolation and RT-PCR assays.

2.3. Nucleic acid sample preparation, RT-PCR reactions and sequencing

Total RNA was extracted from 100 μl of blood samples using the QIAcube robot (QIAGEN) and the QIAamp Viral kit (QIAGEN; reference: 52906) according to the manufacturer's instructions. Finally, the RNAs were eluted with 50 μl of ultrapure water and used in specific EHDV and BTV RT-PCR.

2.3.1. BTV group-specific RT-PCR

Reverse transcription (RT) and amplification were performed using a commercial real-time RT-PCR kit (ADI-352, AES) according to the manufacturer's instructions. This kit allows all 24 serotypes to be detected (by amplifying BTV segment 10 which encodes NS3).

2.3.2. EHDV group-specific RT-PCR

Group-specific primer-pairs (EHDVF617 cgc aca Rga Kac Rccaca; EHDVR1070 ccc tca cat/c ata ggc gtt tt) derived from the nucleotide sequence data of genome segment 7 of EHDV available in GenBank were used in conventional RT-PCR using the one-step RT-PCR Kit (Qiagen, Courtaboeuf, France). Briefly, 2.5 μl of denatured RNA were added to a mixture containing 15.2 μl of RNase-free water, 5 μl of 5X QIAGEN one-step RT-PCR buffer, 1 μl of dNTP mix (400 μM of each dNTP), 0.6 μM of each primer, 1 μl of QIAGEN one-step RT-PCR Enzyme. The amplification was carried out according to the following cycling parameters: 50 °C for 30 min, 95 °C for 15 min, followed by 40 cycles of 1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C. A quota of ten microlitres of each RT-PCR product was analysed by electrophoresis on a 2% agarose gel.

2.3.3. EHDV subgroup-specific primers

The alignment (Megalign) of the segment 2 nucleotide sequences available in GenBank shows 4 clusters (Fig. 1). Sequence data were used to design one primer-pair for each cluster (Table 1) allowing the amplification of each serotype for each group. These primers were selected in a conserved region of each group. These four primer-pairs were used in RT-PCR assays using the same protocol as described above. In order to determine the serotype, each amplification product was sequenced.

2.3.4. Sequencing of amplified products

2.3.4.1. EHDV Sequencing. Amplified RT-PCR products were sequenced directly, in both directions, using the primer-pairs (Table 1) (Cogenics). Sequences were assembled by SeqMan (DNAstar programs, Lasergene) and compared (Blastn 2.2.23) to the homologous sequences available in

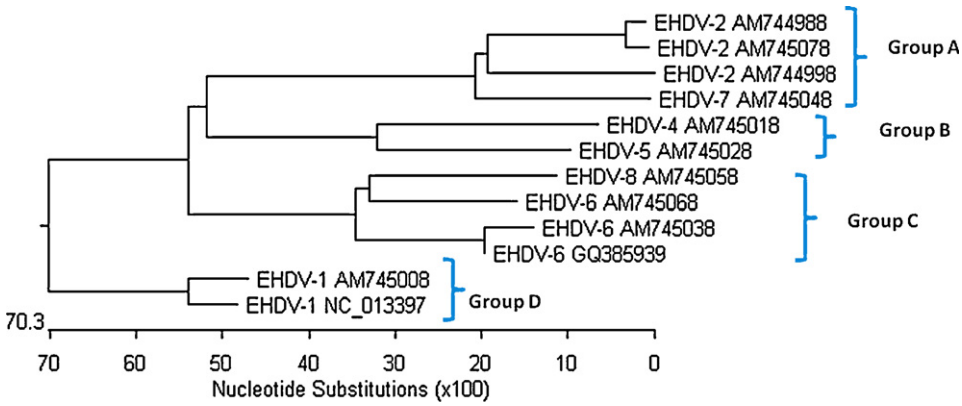

Fig. 1. Phylogenetic tree of full-length segment 2 sequences showing relationships between EHDV serotypes (GenBank). The serotypes of EHDV are associated in 4 Clusters (A, B, C and D).

Download English Version:

https://daneshyari.com/en/article/2467438

Download Persian Version:

https://daneshyari.com/article/2467438

Daneshyari.com