Available online at www.sciencedirect.com
ScienceDirect
The Veterinary Journal 174 (2007) 316–324
The Veterinary Journal
www.elsevier.com/locate/tvjl

Genetic and antigenic typing of border disease virus isolates in sheep from the Iberian Peninsula

B. Valdazo-González [a], M. Álvarez-Martínez [a], T. Sandvik [b,*]

[a] Departamento de Patología Animal, Sanidad Animal, Facultad de Veterinaria, Universidad de León, 24071 León, Spain
[b] Virology Department, Veterinary Laboratories Agency, Weybridge, New Haw, Addlestone, Surrey KT15 3NB, UK

Accepted 2 October 2006

Abstract

A selection of 10 pestiviruses isolated from sheep from the Iberian Peninsula from 2001 to 2004 was characterised at the molecular level. The 5′ untranslated region (5′-UTR) and N^{pro}-coding gene were amplified by the reverse transcription-polymerase chain reaction (RT-PCR) and sequenced directly from purified products. All isolates were also typed antigenically with a panel of monoclonal antibodies (mAbs) raised against representative isolates of the four recognised pestivirus species. The genetic typing placed all the isolates in a new tentative type 4 of border disease virus (BDV), which was closely related to a pestivirus recently found in Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*). Overall, the genotyping indicated a relatively wide diversity of the BDV type 4, which was best defined on the basis of N^{pro} sequences. Antigenically, the isolates were recognised by two pan-pestivirus specific anti-NS3 mAbs, but only by some of the anti-glycoprotein specific mAbs raised against BDV, indicating partial antigenic overlap with other BDV isolates.

Keywords: Pestivirus; Border disease virus; Sheep; Genetic typing; Monoclonal antibodies

1. Introduction

Pestiviruses cause economically important diseases in domestic ruminants and pigs worldwide: bovine viral diarrhoea (BVD) in cattle, border disease (BD) in sheep and goats and classical swine fever (CSF) in pigs (Moennig and Plagemann, 1992; Nettleton and Entrican, 1995). Currently, four virus species are recognised within the genus *Pestivirus* in the *Flaviviridae* family: BVD virus (BVDV)-1 and -2, BD virus (BDV) and CSF virus (CSFV) (Thiel et al., 2005). Pestiviruses can infect animal species other than their natural hosts but, historically, they were named according to the animal from which they were isolated. Analysis of virus isolates by cross neutralisation, monoclonal antibody (mAb) typing and sequence analysis has shown that classification based on virus characteristics is more logical (Becher et al., 1995; Paton et al., 1995). Thus, although BDV is a mainly an ovine pathogen, it can infect pigs (Roehe et al., 1992; Vilcek and Belak, 1996) and has been isolated from cattle, reindeer (*Rangifer tarandus*) and bison (*Bison bonasus*) (Becher et al., 1999), as well as Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) (Arnal et al., 2004). Similarly, BVDV-1 and BVDV-2 are the predominant pestiviruses of cattle, but also occur in sheep (Hofmann et al., 1994; Becher et al., 1995), goats (Becher et al., 1997), pigs (Becher et al., 1999; Vilcek et al., 1999) and different wild ruminants (Becher et al., 1999; Pizarro-Lucero et al., 2005).

Pestiviruses have single-stranded positive polarity RNA genomes of approximately 12.3 kb. A single open reading frame that encodes 10 major viral proteins is flanked by two non-coding regions (5′-UTR and 3′-UTR), each of less than 400 nucleotides. Of these, the non-structural amino terminal autoprotease N^{pro} and the major envelope glycoprotein E2 have most commonly been used for genetic classification of new virus isolates (Becher et al., 2003).

* Corresponding author. Tel.: +44 1932 357677; fax: +44 1932 357239.
E-mail address: t.sandvik@vla.defra.gsi.gov.uk (T. Sandvik).

1090-0233/$ - see front matter
doi:10.1016/j.tvjl.2006.10.002

However, since the 5′-UTR region of pestiviruses is easier to detect by the reverse transcription-polymerase chain reaction (RT-PCR), more partial 5′-UTR sequences of all pestivirus species are available in sequence databases.

On the basis of N^{pro} plus partial 5′-UTR and C gene sequences, ovine BDV isolates from the UK and New Zealand were divided into types A and B (Vilcek et al., 1997). A new subtype C was recently proposed by Hurtado et al. (2003) based on analysis of the 5′-UTR region of pestivirus isolates from sheep in northern Spain. Concurrent studies of entire N^{pro} and E2 gene sequences and cross-neutralisation for a different set of German ovine pestiviruses, has led to the recognition of genotypes BDV-1, BDV-2 and BDV-3, where the "classical" BDV isolates were designated as BDV-1 (Becher et al., 2003). Finally, three other sets of small ruminant isolates have recently been suggested as additional BDV subtypes (Arnal et al., 2004; De Mia et al., 2005; Thabti et al., 2005). To allow a more definitive classification of the various types of BDV, further genetic studies are required, including comparative data sets of all recently described isolates.

Within the European Union, Spain is a major sheep and goat meat producing country, ranking second only after the UK (Anonymous, 2005). Despite the importance of this meat producing sector, pestivirus infections in sheep and goats have largely been overlooked. Regional serological studies have shown individual and flock prevalences of 17–18 and 39–50%, respectively (Álvarez et al., 1989; Mainar-Jaime and Vazquez-Boland, 1999). A recent study on the aetiology of sheep abortions in northern Spain showed that BD was the most common diagnosed cause (Barandika et al., 2002). Knowledge of the predominant pestiviruses among sheep and goats in a specific area is necessary to ensure optimal performance of diagnostic tests, to determine the immunological protection likely to be provided by available vaccines and to develop effective measures for control of ruminant pestiviruses. Therefore, the aim of this study was to characterise genetically and antigenically a selection of ovine pestivirus isolates obtained from within Spain, as well as from neighbouring and epidemiologically linked geographical regions.

2. Materials and methods

2.1. BDV isolates

A total of 10 noncytopathogenic (ncp) BDV isolates were included in this study (Table 1). These isolates were selected on the basis of a preliminary analysis of 5′-UTR sequences of a larger group of pestiviruses obtained from small-ruminant flocks with BD-like clinical signs, in slaughterhouses and from a fattening unit from different regions of the Iberian Peninsula from 2001 to 2004 (Fig. 1). The flocks of origin comprised mainly indigenous (Spanish and Portuguese Merino and Aragonese), but also foreign (Assaf and Lacaune) sheep breeds.

The 10 viruses were isolated from serum samples using pestivirus-free sheep choroid plexus (SCP) cells, cultured in minimum essential medium containing 10% fetal bovine serum proven free from pestiviruses and neutralising antibodies to pestiviruses. For subsequent characterisation, culture supernatants up to second or third passages were used.

2.2. Genetic typing

Viral RNA was extracted from cell culture supernatants using either the TRIZOL method (Gibco) or a spin column system (QIAamp Viral RNA Mini Kit, Qiagen) according to the manufacturer's instructions. RT-PCRs were carried out in two-tube reactions, as previously described (McGoldrick et al., 1999), but with triplicate 50 μL volume PCR tubes and with the annealing step at 55 °C instead of 60 °C. Various combinations of the primers listed in Table 2 were used to amplify partial 5′-UTR and full N^{pro} sequences. Amplified PCR products were separated by electrophoresis in 2% agarose gels in tris-acetate EDTA

Table 1
Details of border disease (BD) virus isolates characterised in the present study

Name	Origin[a]	Year of isolation	Animal status	GenBank accession no.	
				N^{pro}	5′-UTR
LE31C2	León, L	2001	Clinically healthy lamb	DQ273161	
M3	León, S	2001	Clinically healthy lamb	DQ273163	DQ275626
M96	León, S	2001	Clinically healthy lamb	DQ273162	
BU1-CRA22	Burgos, L	2002	PI ewe lamb, BD in flock	DQ273155	DQ275622
ZA1-CRA1	Zamora, L	2002	PI ewe lamb, BD in flock	DQ273164	
C27	Teruel, F	2004	Lamb with diarrhoea	DQ273156	DQ275623
C41	Teruel, F	2004	Lamb with diarrhoea	DQ273157	
C52	Teruel, F	2004	Lamb with diarrhoea	DQ273158	
C121	Teruel, F	2004	Clinically healthy lamb	DQ273159	DQ275625
C290	Teruel, F	2004	Lamb with diarrhoea	DQ273160	DQ275624

[a] L = local farm, S = slaughterhouse, F = fattening unit, PI = persistently infected.