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Differentiation of five strains of infectious bursal disease virus: Development of a strain-specific multiplex PCR

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Abstract

Infectious bursal disease virus (IBDV) is a major cause of disease problems in the poultry industry and vaccination has therefore been applied intensively to control the infection. The classical methods of detection and characterization of IBDV are by the use of immunodiffusion test and histopathology. Since these methods are laborious and have low specificity alternatives are needed. In the present study, we report the development of a strain-specific multiplex RT-PCR technique, which can detect and differentiate between field strains of IBDV and vaccine virus strains including a so-called hot vaccine strain widely used in the European poultry industry. The method, which is highly specific, fast and inexpensive, can be applied in all laboratories with basal PCR capabilities and equipment.

Keywords: vvIBDV; Multiplex PCR; RT-PCR; Virus differentiation

1. Introduction

Infectious bursal disease (IBD) is considered endemic in poultry producing regions worldwide. IBD is causing significant economic losses in the production of young birds as a consequence of increased mortality and immuno-suppression of infected animals.

Within recent years the Danish broiler industry has experienced several outbreaks of IBD caused by very virulent IBD virus (vvIBDV) strains similar to the strains detected in several EU countries in the past decade. Vaccination, although not common practice in Denmark, has shown in some cases to prevent continuous outbreaks of IBD when implemented for up to three subsequent productions of broilers at IBD affected premises (Flensburg, 2001). However, since vaccination of flocks does not always prevent outbreaks of IBD, a fast and sensitive method for differentiation between vaccine strains from field strains in diagnostic material is highly desirable.

IBDV belongs to the *Birnaviridae* family characterized by a bi-segmented double stranded RNA genome, designated segment A and B. The segment A encodes the viral structural proteins VP2 and VP3, the

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protease VP4 and the non-structural VP5. The smaller segment B encodes the polymerase VP1. For distinguishing between IBDV strains, a region located in the VP2 gene between nucleotide position 618 and 1050, called the hyper variable region (HVR) of VP2, is particularly useful (van den Berg, 2001). This region is highly variable and is used as an indicator for different IBDV strains in some of the presently developed diagnostic protocols such as immunohistochemistry and genomic sequencing. Until now, the diagnosis of IBD has been based mainly on clinical signs, pathology, histopathology, virus isolation and serological tests. These techniques are all laborious, time consuming and in some cases, expensive. However, recent years' progress within the field of molecular biology has provided new methods for fast and reliable diagnostic procedures (Muller et al., 2003). Thus, reverse transcriptase polymerase chain reaction (RT-PCR) protocols for detection of IBDV have been under development and sequencing of the RT-PCR product delivers a precise and robust analysis of the viral genome (Lin et al., 1994; Eterradossi et al., 1998). Restriction fragment length polymorphism (RFLP) technique applied on PCR products has also been used for characterization of viral strains (Jackwood et al., 2001; Zierenberg et al., 2000; Lin et al., 1994).

In this study, a multiplex RT-PCR-based assay was developed to enable fast and precise typing of various IBDV strains, focusing on the Danish vvIBDV strain DK01; the classical virulent IBDV reference strain F52/70; the vaccine strains used in the Danish poultry industry, D78 and Bursine-2; and a hot vaccine strain 228E. The method proved successful in discriminating between the base compositions of the HVR in the VP2 gene from these five virus strains and the test can be performed within 8 h. Hence, the developed technique shows potential as a standard diagnostic protocol to be used in all laboratories with access to basal PCR equipment.

2. Materials and methods

2.1. IBDV virus strains

Five IBDV strains were used in this study. Field virus DK01 was a Danish field virus isolate collected in 2001 during IBDV outbreaks in Denmark (1997–2001) (see Section 2.2). The reference field virus strain F52/70 is the classical virulent field virus strain, Faragher 52/70. Preparation of these strains was made by inoculation in specific pathogen free (SPF) chickens (Lohmann Tierzucht, Cuxhaven, Germany) and harvest of tissue from bursa of Fabricius. The vaccine strains Nobilis Gumboro D78 Vet. and Nobilis Gumboro 228E (Intervet International B.V. Boxmeer, the Netherlands) and Bursine-2 (Fort Dodge Animal Health, Fort Dodge, Iowa) are commercially available. D78 and Bursine-2 are commonly used vaccine strains in Denmark, and 228E is an intermediary plus vaccine strain used in many European countries. The vaccine virus strains were obtained as lyophilized cells and RNA was purified from suspensions hereof.

2.2. Typing of Danish field virus isolates

Twenty-eight Danish field virus isolates from the 2001 outbreak were characterized by sequencing part of the HVR in the VP2 gene. The resulting sequences were aligned and a consensus sequence referred to as DK01, representing this family, was deduced and submitted to Genbank with accession number AY850693. The DK01 sequence was aligned with the very virulent IBDV reference strain UK661 (accession number X92760) and the sequences of the four strains mentioned above (accession numbers: F52/70: D00869, Bursine-2: AF498631, D78: Y14962 and 228E: AF457104, Fig. 1). The DK01 nucleotide sequence was homologous to the UK661 sequence and the translated amino acid residue sequence of DK01 shows full identity to UK661 within amino acid residue 210–343 of the HVR in VP2 (not shown). Therefore, DK01 is considered a very virulent IBDV strain, which has been confirmed at the OIE reference laboratory for IBDV using an antigen capture ELISA assay (Eterradossi et al., 1997). The sequence data were analyzed with the programs ClustalX (Thompson et al., 1997) and Genedoc (Nicholas et al., 1997).

2.3. Isolation of RNA

Total RNA, used as template for the first strand cDNA preparation (reverse transcription), was extracted from tissue or cells by use of the RNeasy