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Development of a real-time RT-PCR assay for improved detection of Borna disease virus

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Abstract

Borna disease virus (BDV) is a non-segmented, negative-stranded RNA virus, which infects cells of the central nervous system (CNS) in many different species. BDV is the causative agent of the neurological disorders in horses and sheep termed classical Borna disease (BD), as well as staggering disease in cats. At present, the diagnosis staggering disease or feline BD is made by histopathology or immunohistochemistry of the CNS. In order to obtain a better clinical diagnostic tool, a duplex real-time RT-PCR assay (rRT-PCR) was developed. TaqMan® probes and primers specific for the BDV P and BDV L genes were designed by aligning the sequences of known BDV strains. After optimisation, the sensitivity and specificity of the rRT-PCR were established. The detection limit was set to 10–100 viral genomic copies per reaction and the assay detects the BDV strains V and He/80, as well as the most divergent BDV strain known so far, No/98. Furthermore, the system detected feline BDV variants in five naturally infected cats and a feline isolate used in experimental infection of cats. This rRT-PCR assay will be a powerful tool in further studies of BDV, including epidemiological screening and diagnosis.

Keywords: Borna disease virus; rRT-PCR; TaqMan®; Diagnostics; Cat; Staggering disease

1. Introduction

Borna disease virus (BDV) is a neurotropic, enveloped, non-segmented, negative-stranded RNA virus that belongs to the family *Bornaviridae*, genus *Bornavirus* within the order *Mononegavirales* (Briese et al., 1994; Cubitt et al., 1994). Borna disease, which was first described in horses in Germany in 1767, was considered as an endemic among horses and sheep in certain parts of central Europe (Ludwig and Bode, 2000). Today, however, natural infection has been confirmed in a broad range of hosts worldwide, among them cats. A fatal neurological disorder in cats, known as staggering disease, has been described in Sweden since the early 1970s (Kronevi et al., 1974). This disorder has been characterised as a non-suppurative meningoencephalomyelitis, thereby indicating a viral disease (Lundgren, 1992). However, it was not until the1990s that the disease was linked to BDV infection (Lundgren et al., 1995, 1997).

The size of the BDV genome is approximately 8900 nucleotides and it consists of six major open reading frames (Briese et al., 1994; Cubitt et al., 1994). These encodes for six polypeptides named after their molecular weight in kilodaltons (kDa). The polypeptide p40 corresponds to the viral nucleoprotein (N), p24 to the phosphoprotein (P), p16/gp18 to the matrix protein (M) and p56/gp94 to the surface glycoprotein (G), whereas p190 is thought to correspond to a RNA-dependent RNA polymerase (L). The function of the p10 polypeptide, sometimes referred to as the X protein, is largely unknown, though it has been reported to be involved in the regulation of the viral polymerase as a co-factor to the P protein (Poenisch et al., 2004).

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The molecular epidemiology of BDV implicates that the viral genome is well conserved. Most BDV strains are genetically similar, and in a study where 40 field viruses, vaccine and laboratory strains were analysed, the different isolates showed a nucleotide sequence identity of 97–99% in the p24 region (Kolodziejek et al., 2005). So far, only one published BDV strain is divergent compared to the others, around 16% genetic divergence. This strain was recovered from an Austrian horse in 1998, and is called No/98 (Nowotny et al., 2000; Pleschka et al., 2001).

At present, there are no consensuses on the diagnosis of BDV. Several different serological methods, as well as molecular biological methods have been used (reviewed in Ludwig and Bode, 2000). Previously, conventional nested RT-PCR assays have been used for the detection of BDV RNA (for example, Sauder and de la Torre, 1998; Zimmermann et al., 1994). Conventional PCR, however, is difficult to use for quantitation, and it is also laborious and time-consuming. Most recently, a real-time RT-PCR (rRT-PCR) assay for the detection of BDV in horse and sheep brain has been described (Schindler et al., 2007). However, it is not established whether this assay can detect more divergent strains, like No/98.

Staggering disease or feline Borna disease is a fatal neurological disorder in cats characterised by a staggering movement (hind-leg ataxia), behavioural changes, lumbosacral pain and an inability to retract the claws (Lundgren, 1992). The disease is suspected clinically by excluding other reasons for clinical signs. At present, the diagnosis is often made by histopathology or immunohistochemistry of the central nervous system (CNS). However, serological methods have been developed and used both for epidemiological screening and to diagnose cats with neurological symptoms (reviewed in Kamhieh and Flower, 2006). The seroprevalence of BDV-antibodies in cats showing symptoms of unspecified neurological disease is ranging from 0 to 66.6%, depending on method used and number of animals studied (Helps et al., 2001; Horii et al., 2001; Ouchi et al., 2001; Reeves et al., 1998). The seroprevalence in the normal cat population has not yet been fully characterised, but studies performed indicates highly variable results (2–41.6%) (Helps et al., 2001; Horii et al., 2001; Nakamura et al., 1996; Nishino et al., 1999; Ouchi et al., 2001; Reeves et al., 1998). In staggering disease, however, the humoral response seem to be weak or not present in naturally infected cats (Johansson et al., 2002; Lundgren and Ludwig, 1993). Experimentally infected cats on the other hand show high titres (Johansson et al., 2002).

The variation of seroprevalence in different studies suggest the presence of false-positive results, as a study using three different serological methods including specificity tests showed a significantly lower seroprevalence of BDV in humans compared to other studies (Fukuda et al., 2001). In the same study, no correlation between immunological and BDV RNA findings using nested RT-PCR of PBMCs were seen.

The lack of a good diagnostic tool for the living animal is a problem for the clinician and the owner. Apart from the clinical situation, the study of the epidemiology of BDV, as well as therapy evaluation is difficult without a clinical diagnostic tool. The current study presents a new, rapid, sensitive and specific duplex, one-step rRT-PCR for the simultaneous detection and quantification of the p24 and the L polymerase genes of BDV. This novel tool could be useful for future diagnostic methods and for epidemiological screening of different populations.

2. Materials and methods

2.1. Cells

C6 (rat glioma) cells infected with BDV He/80 (Cubitt et al., 1994) were used during the optimisation of the rRT-PCR assays. In the specificity test, Vero monkey cells infected with BDV No/98 (Nowotny et al., 2000) were also used.

2.2. Animals

In the specificity test, brain tissue samples from cats experimentally infected with BDV strain V and a feline isolate (Lundgren et al., 1997) were used. Brain tissue samples of five naturally infected cats, showing symptoms of staggering disease, as well as asymptomatic cats were also investigated. These cats have been autopsied and sampled at the Department of Pathology, Swedish University of Agricultural Sciences (SLU) from 1993 to 2005 (Table 1).

Table 1
Naturally infected cats positive by the BDV duplex rRT-PCR assay

Cat no. (year of sampling)	Breed/sex/age (years)	Duration of signs	Signs	Serology	Histopathology
A (1993)	DSH/NM/9	3 weeks	Depression, hindleg incoordination, fever	FIV, FeLV, Borrelia, Ehrlichia (all negative)	Non-suppurative meningoencephalomyelitis
B (1994)	DSH/F/3	1.5 years	Incoordination, loss of appetite	Not done	Non-suppurative encephalitis
C (1996)	DSH/NF/2	6 weeks	Incoordination, vocalising, inability to retract the claws, staring gaze	No data	Non-suppurative meningoencephalomyelitis
D (1996)	DSH/NM/3	4 weeks	Incoordination	No data	Non-suppurative meningoencephalomyelitis
E (2005)	DSH/NF/8	6 months	Incoordination, loss of weight, increased vocalisation	No data	Non-suppurative meningoencephalomyelitis

DSH: domestic shorthair; NM: neutered male; NF: neutered female; F: female; FIV: feline immunodeficiency virus; FeLV: feline leukemia virus.