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INFECTIOUS DISEASE

Comparative Neuropathology of Major Indian Bluetongue Virus Serotypes in a Neonatal BALB/c Mouse Model

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Summary

Bluetongue virus (BTV) is neurotropic in nature, especially in ruminant fetuses and in-utero infection results in abortion and congenital brain malformations. The aim of the present study was to compare the neuropathogenicity of major Indian BTV serotypes 1, 2, 10, 16 and 23 by gross and histopathological lesions and virus distribution in experimentally infected neonatal BALB/c mice. Each BTV serotype (20 µl of inoculum containing 1×10^5 tissue culture infectious dose $[TCID]_{50}$/ml of virus) was inoculated intracerebrally into 3-day-old mice, while a control group was inoculated with mock-infected cell culture medium. Infection with BTV serotypes 1, 2 and 23 led to 65–70% mortality at 7–9 days post infection (dpi) and caused severe necrotizing encephalitis with neurodegenerative changes in neurons, swelling and proliferation of vascular endothelial cells in the cerebral cortex, cerebellum, midbrain and brainstem. In contrast, infection with BTV serotypes 10 and 16 led to 25–30% mortality at 9–11 dpi and caused mild neuropathological lesions. BTV antigen was detected by immunohistochemistry, direct fluorescence antibody technique and confocal microscopy in the cytoplasm of neuronal cells of the hippocampus, grey matter of the cerebral cortex and vascular endothelial cells in the midbrain and brainstem of BTV-1, -2, -10, -16 and -23 infected groups from 3 to 20 dpi. BTV nucleic acid was detected in the infected brain tissues from as early as 24 h up to 20 dpi by *VP7* gene segment-based one-step reverse transcriptase polymerase chain reaction. This study of the relative neurovirulence of BTV serotypes is likely to help design suitable vaccination and control strategies for the disease.

Keywords: BALB/c mice; bluetongue virus; histopathology; neuropathogenicity

Introduction

Bluetongue (BT), a non-contagious, re-emerging arthropod-borne disease of sheep, cattle and other wild ruminants transmitted by biting midges of genus *Culicoides* (Mellor, 1990; Maclachlan and Guthrie, 2010), is caused by bluetongue virus (BTV), which belongs to the genus *Orbivirus* in the family Reoviridae (Pringle, 1999; Saminathan *et al.*, 2016). The double-stranded RNA (dsRNA) genome of BTV is formed by 10 segments encoding seven structural (VP1 to VP7) and four non-structural (NS1 to

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NS4) proteins (Ranjan *et al.*, 2015; Rao *et al.*, 2016). The BTV virion is an icosahedral particle assembled in a triple layered protein capsid. The outer capsid layer is formed by VP2 and VP5 proteins responsible for cell attachment and entry. The internal core is composed of VP3 (subcore) and VP7 (core surface layer) proteins (Ranjan *et al.*, 2015; Rao *et al.*, 2016). The core also contains three minor enzymatic proteins: VP1 (RNA-dependent RNA polymerase), VP4 (capping enzyme and transmethylase) and VP6 (RNA-dependent ATPase and helicase). The largest non-structural protein, NS1, forms tubules in the cytoplasm of BTV-infected cells. NS2 is a major component of the viral inclusion bodies, where morphogenesis and RNA replication take place. Two different isoforms, NS3 and NS3a, are glycoproteins involved in excretion of virus from infected cells against the innate immune response of the host cells. The recently discovered smallest BTV protein, NS4, has a role in replication of the virus in host cells (Ranjan *et al.*, 2015; Rao *et al.*, 2016).

At present, at least 27 distinct BTV serotypes have been recognized worldwide, which include the recently characterized putative BTV-27 serotype in goats from Corsica and France (Jenckel *et al.*, 2015). The serotype is predominantly determined by the VP2 segment, which is the most variable of BTV proteins and a main target of neutralizing antibodies (Huismans and Erasmus, 1981). BT is considerably variable in its clinical manifestations, which can range from asymptomatic infection to lethal haemorrhagic fever (MacLachlan *et al.*, 2009). The clinical outcome of BTV infection depends on the strain/topotype/serotype of BTV, passage history of the virus, virus dose, route of inoculation, age, species, breed, individual susceptibility and immune status of the infected host, nutritional status, stress and environmental factors such as solar irradiation and high temperature (MacLachlan *et al.*, 2009; Maclachlan and Guthrie, 2010). Great genetic and phenotypic variations called BTV topotypes have been observed, even within the same serotype, related to geographical locations (Gould and Pritchard, 1990). Variability in virulence exists among BTV strains due to frequent genome segment re-assortments adapting to new ecological zones (Shaw *et al.*, 2013).

Caporale *et al.* (2011) studied the molecular basis of BTV virulence in murine models. Genome segments encoding VP1, VP2 and NS2 revealed consistent differences between the virulent and attenuated strains of BTV. Multiple genome segments determined the virulence of BTV serotypes (Janowicz *et al.*, 2015).

The disease is enzootic in many tropical and subtropical regions and certain temperate regions. Since the first outbreak of BT in India was recorded during the 1960s, the country has been endemic for BT and there is evidence for circulation of at least 23 BTV serotypes (except for 19, 22, 25 and 26) evidenced by serology and/or virus isolation (Prasad *et al.*, 2009; Ranjan *et al.*, 2015; Ayanur et al., 2016; Rao *et al.*, 2016). Until now, 15 serotypes (BTV-1—4, -5, -6, -9, -10, -12, -16—18, -21, -23 and -24) have been isolated from animals in India (Ranjan *et al.*, 2015; Rao *et al.*, 2016). BTV-1, -2, -10, -16 and -23 are common and recently, an inactivated pentavalent vaccine comprised of these serotypes has been developed for use (Prasad *et al.*, 2009; Reddy *et al.*, 2010).

The BTV serotypes or strains are neuropathogenic in nature, occasionally causing fetal death and congenital cerebral malformations in newborn calves and lambs (Enright and Osburn, 1980; MacLachlan and Osburn, 1983; MacLachlan *et al.*, 1985; van der Sluijs *et al.*, 2013). Experimental in-utero infection of BTV in sheep and cattle induced fetal death and/or teratogenic effects, including severe encephalomalacia, hydranencephaly, porencephaly and retinal dysplasia varying with the gestational age of the fetus (Osburn *et al.*, 1971; Barnard and Pienaar, 1976; Luedke, 1985; Richardson *et al.*, 1985). Recently, in India, the *VP2* gene of the BTV-1 strain was isolated from the spleen of an aborted goat fetus by genome sequencing, indicating transplacental transmission of this naturally occurring strain. There has been no exposure to vaccine strains in this region (Chauhan *et al.*, 2014). Two strains of BTV serotype 11 (UC-2 and UC-8) were used to determine the difference in neuroinvasiveness in bovine fetuses and neonatal mice. It was found that UC-8 strains induced more severe necrotizing encephalitis in the cerebrum and cerebellum than the UC-2 strain (Waldvogel *et al.*, 1987, 1992a; Brewer and Osburn, 1998). It was also noticed that gene segment 5 of UC-8 was associated with neurovirulence in newborn mice (Carr *et al.*, 1995). Variations in virulence or pathogenesis among BTV serotypes have been reported experimentally in natural host sheep (Groocock *et al.*, 1982; Hamblin *et al.*, 1998; van der Sluijs *et al.*, 2013; Sánchez-Cordón *et al.*, 2013, 2015) and murine models of disease (Caporale *et al.*, 2011). The aim of the present study was to compare the neruropathogenicity of common Indian strains of BTV in BALB/c mice.

Materials and Methods

Virus

BTV serotype 1 was isolated from a clinically affected cross-breed sheep during 1994 in Avikanagar in Rajasthan State, India (Prasad *et al.*, 1994). BTV-2 and