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Malignant Catarrhal Fever in Brazilian cattle presenting with neurological syndrome

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ABSTRACT

Malignant Catarrhal Fever (MCF) was investigated in the central nervous system of cattle with neurological syndrome. Two-hundred-ninety samples were analyzed by histology, and molecular methods to detect ovine herpesvirus type 2 (OvHV-2) were optimized and validated. The qualitative polymerase chain reaction (qualitative PCR) analytical sensitivity was 10^1 DNA copies/μL and found 4.8% (14/290) positive for OvHV-2. The quantitative polymerase chain reaction (qPCR) analytical sensitivity was 10^0 DNA copy/μL and 5.9% (17/290) positivity, with 47.1% (8/17) of the positive samples presenting histological evidence of non-purulent meningo-encephalitis. The qualitative PCR products (422 bp of the ORF75 region) were sequenced and submitted to phylogenetic analysis. Identity matrices showed 100% similarity in OvHV-2 samples obtained in this study and those recovered from GenBank, corroborating other studies.

Introduction

Encephalitis and encephalopathies have serious impact on public health, produce economic losses, and are a barrier to international trade.[1] The differential diagnosis of bovine neurological syndrome is essential, and is challenging due to the difficulty in identifying potential agents and obtaining conclusive results. Diagnosis begins with investigations for the most probable agents, and, in the case of negative results, proceeding to include other pathogens.[1]

Malignant Catarrhal Fever (MCF) is a widespread disease that affects multiple systems and is often fatal to many artiodactyl species. It is caused by *Herpesvirus* of the family Gammaherpesvirinae and *Macavirus*, a group of antigenic and genetically related viruses, known as MCF viruses,[2] which cause clinical or subclinical infection in susceptible animals.[3–5] To date, ten MCF viruses have been identified, with the most frequent being ovine herpesvirus type 2 (OvHV-2), which causes MCF in sheep (SA-MCF).[6]

Diagnosis of SA-MCF is generally made from clinical history and signs, non-specific lesions observed during necropsy,

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histology, and epidemiologic data.[7] Molecular methods are recommended, as they allow viral DNA detection both ante- and post-mortem, to identify the disease and assess its epidemiology.

Lack of awareness of MCF as a cause of neurological syndrome in cattle and the importance of the differential diagnosis of neurological diseases reinforces the need for optimization and validation of specific and sensitive diagnostic methods for the molecular identification of the OvHV-2.

This research aimed to evaluate suspected cases of MCF in cattle by clinical and anatomopathological evaluation and by molecular characterization in Brazil.

Materials and methods

Survey design

Retrospective molecular and histological analyses were made of 290 central nervous system (CNS) samples from cattle with neurological symptoms referred to the *Centro de Pesquisa e Desenvolvimento de Sanidade Animal do Instituto Biológico* for differential diagnosis of neurological syndrome. The samples came from several regions of Brazil, with 285 collected from January 2012 to March 2014, one in 2007, and four in 2009. All samples were negative for rabies virus, bovine *herpesvirus* types 1 and 5, bovine viral diarrhea, and *Neospora caninum*.

Standard virus selection

A sample of bovine CNS from Assis County, São Paulo State was used as virus standard. The sample was found MCF-positive on histological examination. The viral DNA was extracted using TRizol according to the manufacturer's instructions. Amplification of the ORF75 segment that encodes the phosphoribosylformylglycinamidine synthase (FGARAT) enzyme, participating in purine metabolism and production of viral tegument proteins, was conducted by PCR, using the primers MCF 556 and MCF 755, to amplify a 422 base pair (bp) segment. The positive PCR products were purified, sequenced, and identified as ovine herpesvirus-type 2 (OvHV-2). It was quantified using the QuantiFluor dsDNA System following the manufacturer's instructions. The purified DNA was used as standard to optimize and validate molecular methods.

To determine the sensitivity of the molecular methods, ten-fold serial dilutions of a positive sample (10^0–10^7 DNA copies/μL) were performed in nuclease free water and in CNS negative for OvHV-2.

Extraction of nucleic acid

The DNA samples were extracted using TRizol, according to the manufacturer's instructions. The viral load was determined by comparing to the standard curve, expressed as DNA copies per gram tissue.

Optimizing and validating molecular methods

Primers and an hydrolysis probe targeting the ORF75 regions of the OvHV-2 genome were used. For qualitative PCR, the primers used were MCF 556 (5′ GTC TGG GGT ATA TGA ATC CAG ATG GCT CTC 3′), MCF 755 (5′ AAG ATA AGC ACC AGT TAT GCA TCT GAT AAA 3′), and MCF 555 (5′ TTC TGG GGT AGT GGC GAG CGA AGG CTT C 3′).[8] For qPCR, the primers used were forward oF-OvHV-2 (5′ TGG TAG GAG CAG GCT ACC GT 3′), reverse oR-OvHV-2 (5′ ATC ATG CTG ACC CCT TGC AG 3′), and the hydrolysis probe FAM/TAMRA oP-OvHV-2 (5′ TCC ACG CCG TCC GCA CTG TAA GA 3′).[9]

The temperature gradient was tested in the qualitative PCR with MCF 556 vs. MCF 755, MCF 556 vs. MCF 555, and MCF 755 vs. MCF 555 with various reagent concentrations and cycling conditions (data not shown).

Reaction conditions in both amplifications were adjusted to 12.5 μL PCR Master Mix (Promega). The concentration of external and internal primers was 0.25 μM (556 and 755 in the first amplified 422 bp, 556 and 555 in the second 238 bp). The volume of DNA in the first and second amplification was 2.5 μL and 2 μL, respectively, plus nuclease free water to a total volume of 25 μL.

Quantitative PCR used SYBRGreen and TaqMan systems at primer concentrations of 200–1000 nM. The hydrolysis probe was at 60–100 nM concentration (data not shown). All reactions were carried out in a LightCycler 480 (Roche).

Table 1 – Cycling and temperature conditions of the first and second amplification for qualitative PCR and both qPCR systems (SYBR Green and TaqMan Systems) for OvHV-2.

Method	System	Primers	Initial denaturing	30 cycles			Final extension	Curve of melting
				DNA denaturing	Primer hybridization	Polymerization		
Qualitative PCR		First amplification: MCF 556/755	95 °C/3 min	95 °C/30 s	60 °C/30 s	72 °C/30 s	72 °C/3 min	
		Second amplification: MCF 556/555	95 °C/3 min	95 °C/30 s	67 °C/30 s	72 °C/30 s	72 °C/3 min	
Quantitative PCR	SYBR Green	oF-OvHV-2	95 °C/5 min	40 cycles				72 °C 5 s/65 °C 1 min/97 °C continuous
				95 °C/10 s	60 °C/20 s	72 °C/6 s		
	TaqMan	oR-OvHV-2	95 °C/5 min	95 °C/10 s	60 °C/20 s	72 °C/6 s		

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