

# Liver tolerance as a mechanism for the development of BVDV persistent infection

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## Introduction

Bovine viral diarrhea virus (BVDV) is a member of the pestivirus group of the Flavivirus family that also includes hepatitis C virus. Infection of a pregnant cow with BVDV from 40 to 120 days of gestation may result in persistent infection (PI) of the fetus with BVDV. In pregnant cows, BVDV infects the cells of the placenta and crosses into fetal circulation. Persistently infected calves have been recognized as the major reservoir for BVDV; therefore, identification and removal of PI calves are the focus of BVDV eradication efforts. The objective of this study was to evaluate the association of the fetal liver and liver tolerance with the development of PI with BVDV.

## Materials and Methods

This study was approved by the Colorado State University Institutional Animal Care and Use Committee. Eight heifers were artificially inseminated and became pregnant. On day 75 of gestation, four of the eight heifers were inoculated with a noncytopathic type 2 strain of BVDV (96B2222). For each heifer, a cesarean section was performed to retrieve the fetus on day 89 of gestation. During fetal necropsies, liver specimens were obtained, fixed in formalin followed by ethanol, and then embedded in paraffin. Tissue specimens were cut into 5- $\mu$ m thick sections, and each section was mounted onto lysine-charged slides for immunohistochemical and immunofluorescent staining. Treatment of slides with heat in citrate buffer provided antigen retrieval. Antibodies against 15C5 for BVDV Erns (IDEX, USA) and MAC 387 (AbCam, USA) for Kupffer cells were diluted 1:200 and used to identify BVDV-infected Kupffer Cells. Dual immunofluorescent staining was also used to co-localize the BVDV antigen with Kupffer cells in paraffin-embedded sections.

## Results

Immunohistochemical staining identified Kupffer cells in all fetal liver specimens. Kupffer cells were identified histomorphologic characteristics and location and were distributed in the sinusoidal regions of the liver. No histologic lesions were observed in BVDV-infected or control liver specimens.

Within liver specimens obtained from the fetuses of BVDV-infected dams, BVDV antigen was detected in cells located primarily in the liver sinusoids and near central veins. Hepatocytes and hematopoietic precursor cells did not appear to be infected with BVDV.

Confocal microscopy positively identified the population of cells infected with BVDV in fetal livers as exclusively Kupffer cells. Kupffer cells were not uniformly infected with BVDV at this stage of infection as uninfected Kupffer cells were also observed in all livers evaluated. Other hepatic cell populations, such as hepatocytes, liver sinusoidal endothelial cells, hematopoietic precursors, and lymphocytes, did not test positive for BVDV antigen.

## Significance

In fetal liver specimens, only Kupffer Cells tested positive for BVDV antigen 14 days after maternal infection. Kupffer cells are responsible for antigen presentation to lymphocytes which are also present at this critical stage of gestation. In the context of the specific microenvironment of the fetal liver at this stage of gestation, antigen presentation likely results in systemic tolerance rather than immune activation. Thus, BVDV infection of Kupffer cells is important for the development of persistent infection.