THE EFFECTS OF BVDV ON THE CYTOSKELETON OF BOVINE MACROPHAGES AND EPITHELIAL CELLS: TRACKING ACTIN CHANGES USING IMMUNOFUORESCENCE

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ABSTRACT

One of the most threatening endemic diseases facing the cattle industry today is bovine viral diarrhea virus (BVDV). Economic losses caused by high-virulent strains are as high as $57 million per million calvings (Houe, H. 1999. Epidemiological features and economical importance of bovine virus diarrhoea (BVDV) infections. Vet. Microbiol. 64(2-3): 89-107). Within the host, the virus affects many different cell types, the most significant of which are immune cells. Our lab has shown previously that different strains of the virus inhibit the phagocytic capabilities of bovine macrophages. We believe that this is due to the ability of the virus to interfere with the restructuring of actin filaments within the cell. Monocyte derived macrophages were infected with different strains of BVDV. At 48 hours post-infection, the cells were fixed and stained using rhodamine phalloidin. The strains were found to vary in the extent to which they affected actin structure within the cell. Our current efforts are focused on obtaining time-lapse images of actin reorganization in living cells. To accomplish this, we are transfecting a plasmid that induces the expression of mCitrine-labeled actin into macrophages. Because macrophages are difficult to transfect and to maintain in culture, we have been working on optimizing procedures in Madin Darby Bovine Kidney Cells (MDBKs), as epithelial cells are also primary targets of BVDV. So far, the observable difference in actin rearrangement in MDBKs has been comparable to that seen in macrophages. To overcome the difficulty of transfection, we are constructing a VSV-G pseudotyped lentiviral vector for the plasmid to express mCitrine-labeled actin.