with BVDV type 1 (study 1) or type 2 (study 2) at 75-80 days gestation. Heifers were assessed for clinical signs of BVDV infection including viremia and leukopenia for 14-24 days following challenge. Fetuses were recovered at 152-156 days gestation, and fetal tissues were analyzed for BVDV by virus isolation.

Results

In study 1, no fetuses from vaccinated heifers and all control fetuses were positive for BVDV type 1. In study 2, one fetus from vaccinated heifers and all control fetuses were positive for BVDV type 2.

Significance

Examination of the ability of a BVDV vaccine to prevent disease has historically centered on the magnitude of the antibody response induced by the vaccine. The current study provided evidence that a single dose of a bivalent BVDV vaccine diluted to contain minimum protective levels of type 1 and type 2 BVDV can stimulate a high level of protective immunity to prevent BVDV fetal infections.

A commercial MLV combination vaccine containing type 1 and type 2 BVDV protected 100% of fetuses from BVDV type 1 infection and 95% of fetuses from type 2 infection. The use of a bivalent BVDV MLV vaccine with a comprehensive BVDV control program should result in decreased incidence of BVDV persistent infections and, therefore, minimize the risk of BVDV infections in the herd.

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Rapid Detection of Bovine Viral Diarrhea Virus using a Conductometric Biosensor

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Introduction

Bovine viral diarrhea virus (BVDV) is one of the most important viral pathogens of cattle worldwide. A key to controlling BVDV is identifying and eliminating carriers of the virus, better known as cattle persistently infected (PI) with BVDV. PI's shed large amounts of virus and serve as the major source of virus transmission within and between farms. Several laboratory methods are currently available to detect PI's including virus isolation, antigen capture ELISA's, skin immunohistochemistry/fluorescent antibody and PCR. The development of a rapid and cost effective field based system to detect PI's would be beneficial for more practical implementation of BVDV control strategies. In this study, a conductometric biosensor previously developed for use in detecting foodborne pathogens was adapted to detect BVDV in cell culture media and blood. The architecture of this biosensor allows for rapid field based applications.

Materials and Methods

A biosensor was developed using an architecture previously designed by one of the investigators (Alocilja). The biosensor uses antibodies as the biological sensing element and polyaniline as the transducer and molecular switch. The principle of detection can be described briefly as follows: A liquid sample containing the antigen (Ag) is dropped on the sample application membrane. The sample containing the antigen flows to the conjugate membrane by capillary action and binds to the primary antibody (Ab) that is conjugated to the conductive polyaniline molecules (Pan). The bound Ag forms a soluble Pan-Ab-Ag complex that is transported by capillary flow to the electrical capture membrane that is

coated with the secondary antibody and with silver electrodes fabricated on both sides. The Pan-Ab-Ag binds to the secondary antibody, forming a Pan-Ab-Ag-Ab sandwich, and which creates a molecular bridge between the two electrodes. This molecular bridge reduces the resistance between the electrodes, and resistance measurement is taken using a multimeter. In phase I of the study the biosensor was constructed and initially tested using both type 1 and 2 BVDV grown in cell culture and diluted to different concentrations. Concentrations of the various capture antibodies and polyaniline was optimized, and the detection limit of the sensor determined. In phase 2 of the study, serum known to be free of BVDV virus and BVDV neutralizing antibodies was inoculated with known concentration of BVDV virus and then assayed using the optimized biosensor.

Results

A conductometric biosensor was successfully constructed that was capable of detecting both type 1 and type 2 BVDV viruses grown in cell culture. The minimum virus concentration in cell culture that was detectible using the current architecture was 10(-3) cell culture infective doses per milliliter (CCID/ml). When applied to BVDV spiked serum, the biosensor was capable of detecting virus at the same concentration of 10 (-3) CCID/ml. The amount of time between sample application in the biosensor to stabilization of the electrical current averaged two minutes.

Significance

Results of this preliminary study provide proof of concept that a rapid field-based biosensor can be developed that is capable of detecting BVDV in both cell culture media and blood at a concentration that is biologically relevant for identifying PI's. This biosensor architecture has the capability of being miniaturized and automated to facilitate large scale field-testing if necessary. With mass production, the cost of the biosensor has the capability of being very low. Extension of this biosensor architecture to other disease agents is of great interest, including agents of bioterrorism and foreign animal diseases.

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Penetration of Ceftiofur into Sterile versus *Mannheimia Haemolytica*-Infected Tissue Chambers in Beef Calves after Subcutaneous Administration of Ceftiofur Crystalline Free Acid Sterile Suspension in the Ear Pinna

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Introduction

Ceftiofur is a third-generation cephalosporin approved in the US for the treatment of bovine respiratory disease in beef and dairy cattle. Ceftiofur crystalline free acid sterile suspension (CCFA-SS) is a single administration product developed as an extension to the ceftiofur product line. The efficacy of a single dose of CCFA-SS administered subcutaneously in the neck was established in several clinical field studies. However, this route of administration was not acceptable for registration because of extended residues at the injection site. Consequently, the middle third of the posterior aspect of the ear pinna was selected as an alternative site of administration, and pilot studies were conducted to investigate the duration of efficacy resulting from a