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Serial passage of the etiologic agent of epizootic bovine abortion in immunodeficient mice

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ABSTRACT

Molecular studies have provided convincing evidence that a unique deltaproteobacterium is the causative agent of epizootic bovine abortion (EBA). Bovine fetuses, infected following dam exposure, are the only identified susceptible mammalian host. The inability to cultivate the bacterial agent of EBA (*aoEBA*) *in vitro*, associated with the substantial cost of bovine experimentation, drove efforts to identify an alternative laboratory animal host. Mice with severe combined immunodeficiency (SCID) were chosen as a potential host after immunocompetent mice proved resistant to infection. SCID mice inoculated with *aoEBA*-infected bovine fetal thymus homogenates began to show clinical signs at 2 months and became increasingly cachectic over the next 1–2 months. Following a 2nd passage (P2) through SCID mice, three susceptible pregnant heifers were inoculated with P2 murine tissue homogenates. All three fetuses presented with lesions indistinguishable from naturally occurring EBA, confirming successful passage of the bacterial pathogen in SCID mice. All murine (P1 and P2) and bovine fetal tissues contained *aoEBA* as determined by PCR; 16S bacterial ribosomal nucleotide sequences were identical in all murine and fetal bovine tissues examined. Bacteria in fetal bovine tissues were determined to be heavily opsonized, based upon microscopic evaluation of tissues stained with either FITC-conjugated anti-bovine IgG or biotin-conjugated anti-bovine IgG in conjunction with avidin-FITC. Unlike the near-term bovine fetus, the absence of an antibody response in infected SCID mice permits harvest of unopsonized bacteria for development of serologic assays.

1. Introduction

Epizootic bovine abortion (EBA) is confined to the Western United States with incidences reported in the foothill ranges of California, Western and Northern Nevada and Southern Oregon; the range of the tick vector, *Ornithodoros coriaceus*, coincides with disease incidence (Hall et al., 2002; Howarth et al., 1956; Loomis et al., 1974; McKercher, 1969; Schmidtmann et al., 1976; Teglas et al., 2006). Economic impact of these late-term abortions continues to be significant (Howarth et al., 1956; McKercher, 1969, University of California Agriculture & Natural Resources Annual Report, 2003). Diagnosis of EBA is based upon gross and microscopic pathology, elevated fetal immunoglobulins, compatible history (susceptible dam in an EBA-endemic area during mid-gestation) and the exclusion of other abortifacients by routine microbiologic procedures.

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A reliable experimental challenge system, using select homogenized, cryopreserved EBA-diseased fetal thymic tissue homogenates as inoculum (Stott et al., 2002), facilitated antibiotic treatment studies that suggested a prokaryotic etiology (Stott et al., 2002). Subsequent molecular studies identified a previously undescribed bacterium (16S rRNA gene) within the delta group of proteobacteria (King et al., 2005). Successful application of an EBA-specific PCR to infected fetal bovine necropsy tissues and the tick vector provided convincing evidence that this unique organism was the etiologic agent of EBA (*aoEBA*) (King et al., 2005).

Mid-gestation fetuses in naïve heifers have been the only identified susceptible mammalian host for EBA; *in vitro* attempts at cultivation have been unsuccessful. Based upon assumption that the immunologic immaturity of the bovine fetus contributes to its susceptibility to infection with *aoEBA*, mice with severe combined immunodeficiency (SCID) were tested as potential hosts. The primary objectives of the present study were: (i) determine the susceptibility of immunocompetent and SCID mice to *aoEBA* infection and (ii) establish the SCID mouse as a laboratory animal system to facilitate EBA research.

2. Materials and methods

2.1. Cattle

Mixed-breed beef heifers were obtained from the Dakotas, an area in the United States in which EBA has never been reported and the tick vector (*O. coriaceus* Koch) has not been found. Cattle were maintained on irrigated pasture at the Main Station Field Laboratory, University of Nevada, Reno (UNR). All animal research was conducted in compliance with the United States Federal Animal Welfare Act and the Health Research Extension Act and under approval by the UNR Institutional Animal Care Use Committee.

Heifers were artificially inseminated (AI) or bull-bred and gestational age of fetuses estimated using a combination of observed breeding date, breeding period (1, 7 or 30 days) and rectal palpation at 60 days. Dams were inoculated at 85–125 days gestation. A diagrammatic representation of animals involved in tissue-passage experiments is provided in Fig. 1. Bovine fetuses F1 and F2 were derived from heifers inoculated with 0.5 g a*oEBA*-infectious bovine fetal thymus homogenates (Stott et al., 2002). Bovine fetus F3 was derived from a pregnant heifer inoculated with thymus homogenate derived from a healthy negative control fetus. Necropsy tissues from bovine fetuses F1–F3 were used for inoculation of appropriate groups of severe combined immunodeficient mice (C3H-*scid*) (1st-passage; P1). Following two serial passages of either negative control or *aoEBA*-infected tissues through C3H-*scid* mice, 2.75 g aliquots of pooled 2nd passage (P2) murine tissues were inoculated subcutaneously into each of 4 pregnant heifers. Three heifers were inoculated with pooled P2 murine *aoEBA*-positive tissue homogenates (H4–H6) and the fourth was inocu-

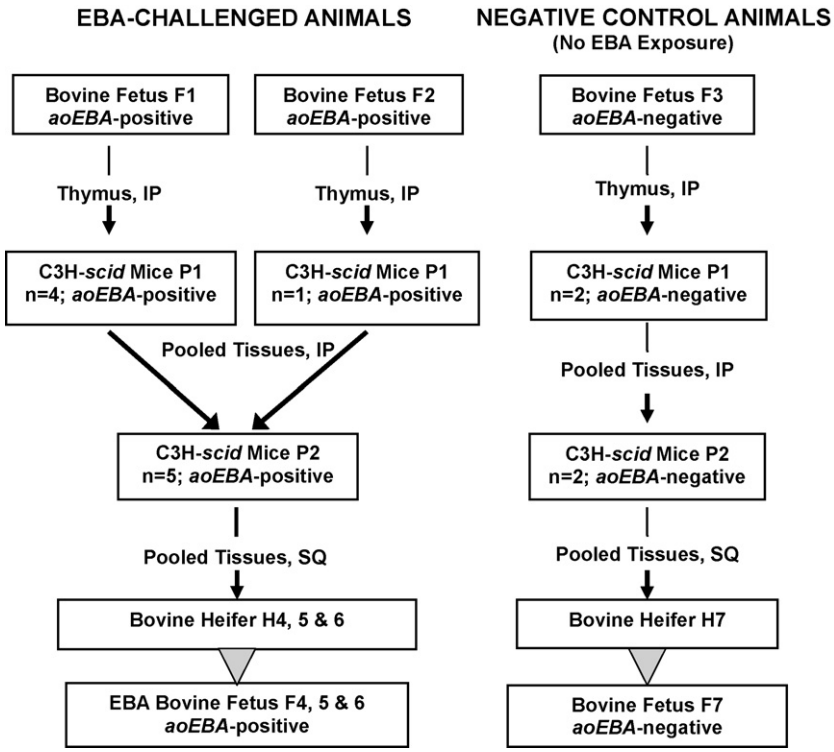

Fig. 1. Flow diagram depicting the animal inoculation protocol used to establish serial passage of *aoEBA* in C3H mice with severe combined immunodeficiency (C3H-*scid*). Inoculation of pregnant cattle with necropsy tissue homogenates was by the subcutaneous (SQ) route. Mice were similarly inoculated by the intraperitoneal (IP) route. P1 = Passage #1. P2 = Passage #2. *aoEBA*-positive refers to identification of bacteria in necropsy tissues using PCR. *aoEBA*-negative refers to an absence of bacteria in necropsy tissues as determined by PCR.