Evaluating Transmission of Bovine Viral Diarrhea Virus to Cattle by Exposure to Carcasses of Persistently Infected White-tailed deer (*Odocoileus virginianus*)

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KEY WORDS: Bovine viral diarrhea virus, carrion transmission, Odocoileus virginianus, persistent infection, white-tailed deer

ABSTRACT

Infections with bovine viral diarrhea virus (BVDV) clinically analogous to cattle are described in white-tailed deer (Odocoileus virginianus), but the epidemiologic role of persistently infected (PI) white-tailed deer is unknown. Persistently infected white-tailed deer shed BVDV, maintaining BVDV in groups of deer. Survival of PI white-tailed deer is reduced, and clinically ill or dead PI deer may be a source of BVDV. This study sought to determine if BVDV transmission could occur when cattle come in contact with carcasses of PI white-tailed deer. In two trials, performed in Auburn, Alabama, during November and December 2009, steers were exposed to the carcass of PI fawn A (BVDV 2) or PI fawn B (BVDV 1). Trials were designed with consideration of the influence of contact networks on disease epidemiology, and only one steer from each group was separated into a pen with the carcass. The number of contacts with the carcass was monitored. Following 8 hours, the single steer was commingled with four other steers for 28 days. Animals were tested for BVDV infection. Controls included one steer inoculated intranasally with spleen-homogenate from fawn A, and two steers inoculated intranasally or intravenously with spleen-homogenate from fawn B. Steers in both trials repeatedly contacted the carcasses, but BVDV transmission did not occur. The intranasally inoculated control for trial A and the intravenously inoculated control for trial B became viremic and seroconverted. Although both PI carcasses

Table 1.

Fawn ID	Time of sampling							
	Birth					Death[a]		
	Virus isolation		RT-PCR	ELISA	IHC	Virus isolation	RT-PCR	IHC
	WBC	Nasal swab	Serum	Ear notch[b]	Ear notch	Tissues	Tissues	Tissues
A	+	-	+	+	+	+	+	+
B	+	+	+	+	+	-	+	+

a Both fawns died at 10 days of age
b The sample-to-positive-ratios were: Fawn A: 1.518; Fawn B: 1.353, where the established cut-off for positive bovine samples is S/P >0.39 (WBC – white-blood cells; RT-PCR – reverse transcriptase polymerase chain reaction; IHC – immunohistochemistry)

were potentially infectious and steers made repeated contact, transmission of BVDV did not occur in this model.

INTRODUCTION

Bovine viral diarrhea virus (BVDV), genus Pestivirus, family Flaviviridae, is a bovine pathogen, but the virus lacks host specificity, and infections occur in many Artiodactyls.[1] Infection of free-ranging or captive wild ruminants with BVDV may threaten natural resources, including rare and endangered species. Contact of infected wildlife with susceptible cattle populations could present a risk to ongoing BVDV control and eradication programs.[2] The risk of BVDV transmission between wildlife and cattle is currently unknown, but because wildlife and cattle commonly share habitat, this could be a critical, yet poorly understood, aspect of BVDV control programs in the United States.[3]

BVDV infections clinically analogous to bovine infections are reported in white-tailed deer (Odocoileus virginianus), and include reproductive losses and birth of persistent infections.[4,5] Persistently infected (PI) white-tailed deer can shed BVDV at similar levels to PI cattle,6 and contact of pregnant does with PI fawns can result in BVDV transmission and birth of PI fawns.[5] In the United States, evidence of persistent infection in free-ranging white-tailed deer was demonstrated, and reported prevalence rates for PI white-tailed deer were 0.1 – 0.3%.[7-10]

Persistently infected white-tailed deer, born to experimentally infected does, had decreased survival and died before 10 months of age.[5] Early deaths of PI deer reduce potential for BVDV transmission to susceptible animals by nose-to-nose contact, but infection of cattle following contact with dying or dead deer may be possible, and werethe subject of this investigation.

MATERIALS AND METHODS

Experimental Design and Animals

This research was performed under the approval of the Institutional Animal Care and Use Committee of Auburn University (2009-1659). The study-design emulated natural exposure of a susceptible steer to BVDV by presence of a deceased PI deer in the grazing area with consideration of influences of social hierarchies and contact networks of cattle on disease transmission.[11] The study evaluated direct carcass-to-steer transmission with subsequent transmission to herd-mates. This study consisted of two separate trials, performed in November and December 2009, each using one of two carcasses of PI fawns (Table 1). Fawns were born to dams experimentally exposed to either BVDV 2 strain PA131 (Fawn A) or BVDV 1b strain AU526 (Fawn B) during gestation.12 A post-mortem examination was performed on the carcasses, which were subsequently frozen at -20 °C for approximately 2 years until inclusion in this study.

Two groups of five seronegative, BVDV-negative Holstein steers were established for carcass exposure experiments. Groups were

Table 2.

Fawn ID	BVDV		0 hr		2 hr	4 hr	6 hr	8 hr	
			Swab	Muscle	Swab	Swab	Swab	Swab	Muscle
A	Type 2 PA131	Virus isolation	+	+	+	+	+	+	+
		Titration[a]	$6.2x10^3$	$2x10^3$	BDT[b]	$2x10^2$	$3.5x10^3$	$2x10^2$	$3.5x10^4$
B	Type 1 AU526	Virus isolation	-	-	-	-	-	-	-
		Titration[a]	BDT	BDT	BDT	BDT	BDT	BDT	BDT

a Results given as 50% cell culture infectious doses/ml (CCID50/ml)
b BDT – Below detection threshold of 1x102 CCID50/ml

maintained in two-hectare pastures. Health was assessed once daily. For each trial, the carcass of a PI fawn was thawed at 5 °C for 12 hours. One steer was separated from its group into a pen of approximately 125 m2 with carcass exposure for 8 hours (Day 0). Every 2 hours, the pen size was reduced by approximately half. Six hours after first exposure, feed was placed in direct vicinity of the carcass. A contact with the carcass was defined as the muzzle of the steer approaching the carcass at a distance of less than 20 cm. A separate event of contact was defined as being at least 15 minutes subsequent to a prior event. The number of contacts of the steer with the carcass was monitored by time-lapse photography.

In order to limit behavioral alterations by presence of personnel, visual observations were not performed, except when samples were collected from the carcasses. To demonstrate infectious ability and evaluate survival of BVDV in tissues, two sites in thoracic and abdominal cavities of each carcass were swabbed with a Dacron polyester swab at 0, 2, 4, 6, and 8 hours. Additonally, muscle biopsy samples (1 cm3) were collected from carcasses at 0 and 8 hours. Following 8 hours of exposure, the separated steer was returned to its group. Blood samples for BVDV virology were collected from steers on days 0, 4, 6 - 11, 14 - 18, 21, 22, and 28 of each trial.

Following the exposure trials, steer that served as infection controls without contact with principal animals were inoculated with spleen homogenates from fawns. One steer was intranasally inoculated with 2 ml of spleen homogenate in minimal essential medium (MEM) containing 1x104 CCID50/ml of BVDV from fawn A. Two steers were inoculated with 2 ml of inoculum containing 0.25 ml of spleen homogenate from fawn B, one intranasally, and the other intravenously. While consistently positive by RT-PCR, virus isolation (VI) of tissues from carcass B were negative at death and subsequent retesting, making quantification by virus titration impossible.

Samples Analyses

Muscle samples were homogenized with a Tekmar Stomacher (Model 80, Tekmar Co, Cincinnati, OH, USA) and re-suspended with 3mL of MEM. White blood cells and sera were refrigerated for £ 72 hours before VI procedures were performed. Sera were removed and stored at -80º C for virus neutralization (VN) assays. To obtain the buffy coat, white blood cell samples were processed as described previously,13 except that samples were resuspended in 1 mL of MEM.

Serum, buffy coat, carcass swab media, and muscle samples were assayed for BVDV by passage though Madin Darby bovine kidney (MDBK) cells for 3 days.[13] The quantity of BVDV in carcass swab media and muscle samples was determined by virus titration performed in triplicate in 96-well plates containing MDBK cells. An immunoperoxidase

monolayer assay was performed to confirm the presence of non-cytopathic BVDV as previously described.[14]

A virus neutralization microtiter (VN) assay was used for quantification of antibodies in serum.[13] This assay utilized MDBK cells, and the BVDV strains used for VN were obtained from the carcass corresponding to each trial. Detection of BVDV in virologic assays was performed by an immunoperoxidase monolayer assay.[13]

A two-round rapid-cycle RT-PCR (RT-nPCR) assay was performed on aliquots of spleen homogenates of fawn carcasses and serum samples from infection control steers as previously described.15 Consensus sequences were determined using Align X® computer software (Vector NTI Suite 7.1, InforMax, Inc., Bethesda, MD). Results were used to compare the nucleotide sequences of BVDV strains in spleen homogenates of PI fawns and at the time of viremia in the infection control steers.

RESULTS

In both trials, the individually exposed cattle made contact with the PI carcass at regular intervals, often at distances less than 5 cm. In trial A, the steer investigated the carcass on seven separate events with a minimum of 15 minutes and a maximum of 158 minutes between two separate contacts. In the second trial, the steer investigated the carcass on 14 separate events, and the minimum and maximum time spans between events were 16 and 55 minutes, respectively.

The carcasses were assessed for BVDV by VI on muscle biopsy and surface swab samples (Table 2). Isolation of BVDV was successful only from the carcass in trial A, and, with the exception of one viral titration, both muscle biopsies and surface swab samples were positive. In contrast, neither sample type was positive for BVDV from carcass B.

Carcass exposure did not result in transmission of BVDV, and steers in both trials were consistently negative on VI and VN. Daily clinical examinations including rectal temperature and complete blood cell counts did not indicate BVDV infection, as clinical parameters remained within reference ranges (data not shown).

From the control steer inoculated intranasally with spleen homogenate from fawn A, BVDV was isolated from whole blood and serum on days 6 - 14 and 8 - 14, respectively. Seroconversion was demonstrated on post-inoculation day 28. Only the intravenously inoculated control steer for trial B became viremic. Virus was isolated from whole blood and serum samples from this steer on days 6 - 10 and 7 - 8, respectively. On post-inoculation day 28, this steer had an antibody titer of 1:512. In the steer that was intranasally inoculated with spleen homogenate from fawn B BVDV, infection could not be demonstrated. Sequence homology was demonstrated between the 5' UTR of BVDV PA131 in carcass A and control steer for trial A, and BVDV AU526 in carcass B and the intravenously inoculated steer of trial B.

DISCUSSION

While transmission of BVDV by exposure to carcasses was not observed in this study, the observed frequency and proximity of investigative contacts by steers with the carcasses emphasizes the potential for disease transmission to cattle by this route. Similar observations were made when alpacas, sheep, deer, and cattle were presented with possums or ferrets sedated to simulate behavior of terminal tuberculosis.16,17 In those studies, ruminants came into aerosol transmission distance of <1.5 m or had direct physical contact, and in at least one instance intensely licked the sedated marsupial.[17]

Within cattle herds, specific individuals have a greater likelihood of making contact with non-bovine species, and may have the potential to act as a hub in the transmission of diseases.[11] The present study sought to emulate these herd structures, and by limiting the exposure of a deer carcass to one steer, tried to explore if this animal could introduce BVDV into the remaining herd.

Observations of cattle suggest that animals ranked higher in social hierarchies and those with greater rates of intra-herd contacts are more likely to make interspecific contacts.[11,18] Cattle and deer in the highest ranks of the group hierarchy were most likely to investigate and make physical contacts with sedated badgers, and a significant positive correlation existed between dominance rank and a positive reaction on the tuberculin test.[18]

Chance for disease transmission from carrion is influenced by survival time of the pathogen, which is unknown for BVDV in tissues of deceased PI animals. In the present study, BVDV was successfully isolated from muscle biopsies and surface swabs collected from carcass A throughout the study period of 8 hours, after approximately 2 years in storage at -20˚ C. The survival of BVDV in tissues of dead PI animals has not been investigated, but in slaughtered PI cattle, BVDV remains infectious in muscle tissues for up to 60 days at refrigeration temperatures (Givens, M. D., personal communication). The related pestivirus, classical swine fever virus, remains infectious for up to 85 days in chilled pork and for 126 to 252 days in curing pork products.[19,20]

Failure to isolate BVDV from spleen of carcass B despite successful intravenous inoculation of a steer is noteworthy. Tissues collected from fawn B at death were positive by RT-PCR but negative by VI. At birth, fawn B was positive on multiple sample types, and skin biopsies resembled those of PI cattle and PI white-tailed deer on ELISA and immunohistochemistry. Possible explanations for the negative VI at death and from carcass tissues include the inability to successfully culture BVDV strains obtained from deer in cells of bovine origin, or the clearance of BVDV by fawn B during the time from birth to death. Our group and others have successfully cultured BVDV isolates from deer on bovine cells, including field strains isolated from sick deer.[7] The elimination of BVDV following prolonged postnatal viremia was reported in pigs[21,22], and clearance of the infection from blood was associated with seroconversion, 21 but this observation warrants further research.

The observed frequency and proximity of investigative contacts of steers with PI carcasses emphasizes the potential for disease transmission to cattle by this route. Although this study was limited by the low infectivity of carcass B, the frequent contacts made by the steer to carcass A without resulting BVDV transmission suggest that the introduction of BVDV into susceptible cattle herds from exposure to PI carcasses should be of limited concern. Complex factors determine the true risk for BVDV infection by contact with a carcass, including BVDV survival in tissues, likelihood of contact with the carcass, and contact network structures of cattle herds. The influence of wildlife on the control of BVDV is currently unknown, and further research is necessary to understand complex interactions at the wildlife-livestock interface.

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