Розділ 4. Інфекційні хвороби. Епізоотологія

TRANSMISSION OF BOVINE VIRAL DIARRHEA VIRUS TYPE 1A FROM EXPERIMENTALLY INFECTED WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) TO CALVES

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Despite the world-wide implementation of bovine viral diarrhea virus (BVDV) control programs; BVDV still constitutes a substantial financial burden to the cattle industry. Furthermore, the possibility of wildlife reservoirs presents a threat for already established and future BVDV control programs. The objective of this study was to assess the transmission of bovine viral diarrhea virus from experimentally infected white-tailed deer fawns to colostrum-deprived calves. Five white-tailed deer fawns and six Holstein colostrum-deprived bull calves were housed in an isolation facility. Fawns were inoculated intranasally with a non-cytopathic BVDV-1a isolate (2 ml containing $10^{6.7}TCID_{50}$/ml) and 2 days post-inoculation (DPI) animals were commingled until the end of the study. Whole blood and serum samples were collected on days -6, 0, 7, 14 and 21 DPI for buffy coat for RT-PCR and virus neutralization and BVDV specific antibodies ELISA. Nasal, oral and rectal swabs were collected on days 0, 3, 7, 14, 17 and 21 DPI for RT-PCR. By 21 DPIN, all animals were euthanized and necropsied and tissues were collected for histopathology, immunohistochemistry and virus isolation. All fawns became infected and shed the virus for up to 18 days. Evidence of BVDV infection as a result of cohabitation with acutely infected fawns was detected in four out of the six calves. To our knowledge, this is the first report assessing the transmission from acutely infected wildlife to cattle. Based on our study and previous findings, BVDV infected wildlife infected may constitute a risk for introduction of this disease into cattle farms.

Bovine viral diarrhea virus (BVDV) belongs to the genus *Pestivirus* in the family *Flaviviridae.* Despite world-wide implementation of BVDV control programs and the availability of multiple BVDV vaccines, this virus still constitutes a financial burden to the cattle industry. Besides infecting cattle, BVDV is also present in wild ruminants all around the world tessaro[11]. Even though, the possibility of wildlife reservoirs presents a threat to already established and future BVDV control programs, interspecies transmission was not studied until recently. Recent findings suggest that BVDV transmission from PI cattle to wildlife and vice-versa can occur [7], on the other hand, the potential transmission of BVDV by acutely infected animals is needs to be determine. The objectives of this study were to assess if bovine viral diarrhea virus type-1a, isolated from free ranging WTD, can be transmitted from experimentally infected WTD fawns to colostrum-deprived calves.

Materials and methods. This study was approved by the Purdue University Animal Care and Use Committee. Five 2 to 3 week old female WTD fawns and six colostrum-deprived male Holstein calves (free of BVD virus and antibodies) were used in this study. Animals were housed under biosecurity level 2 at the Purdue University Laboratory Animal Housing Facility. On day 0, all fawns were intranasally inoculated with BVDV isolated from hunter harvested WTD in Indiana [9]. On day 2 post inoculation (DPI), animals were allocated randomly to 5 groups: one group included one fawn and two calves and four groups included one fawn and one calf. Clinical evaluation was performed daily. On 21 DPI, all animals were humanely euthanized and necropsied and multiple tissue samples were collected for histopathology and virus isolation. For sample collection and diagnostics tests performed please refer to Figure 1.

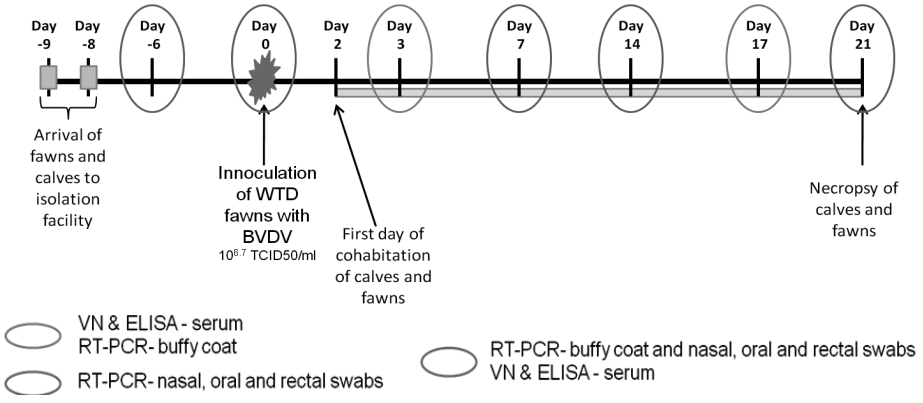

Figure 1

Results. Prior to inoculation, 4 fawns and one calf became sick due to causes other than BVDV infection. One fawn developed oral abscesses which resolved with antibiotic treatment (oxytetracycline, LA 200® at a dose of 20 mg/kg every other day; Pfizer Animal Health, PA, USA) and 3 fawns developed diarrhea which completely resolved in two of them. The one fawn with intermittent diarrhea throughout the study was euthanized and necropsied on 16 DPI due to the severity of clinical signs. No parasites were detected in fecal samples collected from the sick fawns. However, fecal swabs submitted for bacteriology were positive for *E. coli*. The sick calf developed septicemia based on hyperfibrinogenimia, neutropenia with left shift and hypopyon observed on left eye. Samples obtained from this calf were negative for the presence of bacteria or parasites. Fawns and calves tested negative for BVDV antibodies in VN and ELISA and buffy coat RT-PCR prior to the first day of the study. All fawns had evidence of BVDV as early as 3 DPI and shed the virus for up to 18 days based on RT-PCR of buffy coat and swabs (Table 1).

Table 1 – Results from RT-PCR and virus isolation (VI)

DPI[a]		0	3	7	14	17	21	
DPC[a]		-2	1	5	12	15	19	
Test		RT-PCR	RT-PCR[c]	RT-PCR	RT-PCR	RT-PCR	RT-PCR	VI[d]
Kennel #	Animal # [b]							
1	F63[e]	-	N, R	N, O, BC	N, O, BC	N, O, BC	ND	Int
	C53	-	-	-	-	-	BC	Ln, Int
	C91	-	-	-	-	-	-	-
2	F1504	-	N	N, R, BC	R, BC	-	-	-
	C51	-	-	-	BC	N	BC	Int
3	F56	-	N, O	N, R, BC	-	-	-	-
	C50	-	-	-	-	-	-	-
4	F1526	-	N, R	N, R, BC	N, R, BC	N, O, R	N, O, BC	Lg, Ln
	C92	-	-	-	-	-	O, BC	Lg, Ln, Int
5	F61	-	N, R	N, R, BC	O, BC	-	-	Ln
	C52	-	-	BC	BC	-	BC	Int, Lg

DPI - days post inoculation, DPC - days post cohabitation; [b] F - fawn, C – calf; [c] BC - buffy coat, NS - nasal swab, OS - oral swab, RS - rectal swab, (-) = negative results; [d] Int - intestine, Lg - lung, Ln - lymph node; [e] Fawn 63 was euthanized by day 16 DPI. Samples collected at necropsy included, tissues, buffy coat and nasal, oral and rectal swabs. ND- not done.

Following cohabitation, calves and fawns were commonly seen sharing the same pen area. Four out of six calves had positive buffy coat by RT-PCR for BVDV. Virus was detected in the buffy coat in one of the calves as early as 5 days post-cohabitation and the latest was by day 18 post-cohabitation (Table 1). Only one calf had evidence of the virus in a nasal swab sample and another calf had evidence of the virus in one oral swab sample.

Virus was isolated from intestines, lung and/or pooled lymph nodes in 3 out of the 5 fawns and in 4 calves by 21 DPI/19 days post cohabitation (Table 1).

VN titers and ELISA were positive in 4 out of the 5 fawns. One fawn was positive to ELISA by 14 DPI but VN was negative. Fawn # 63, euthanized on 16 DPI, was positive to ELISA but VN negative. By 21 DPI the remaining three fawns developed virus neutralization titers ranging from 1:4-1:8 and were positive on BVDV specific ELISA. Only the calf that was PCR positive for BVDV after 5 days of cohabitation developed antibodies against BVDV based on positive ELISA (Table 2).

On necropsy, no gross lesions were identified in any of the animals. All BVDV infected fawns and calves had marked lymphoid atrophy in the Peyer's patches. No other lesions characteristic of BVDV were observed. IHC was performed on an ileum sample from one of the BVDV infected calves. There was positive labeling of BVDV virus antigen as evidenced by low numbers of scattered positive cells in areas of lymphoid depletion/necrosis and in the lamina propia of villi.

Discussion. To the best of our knowledge, this is the first report assessing the transmission from acutely infected wildlife to cattle. Neither fawns nor calves developed any clinical signs related to the infection; this is consistent with results obtained previously in our laboratory using the same BVDV strain [10]. However, four fawns were sick within 24-48 hours after arrival: one fawn developed oral lesions, and three fawns developed diarrhea which resolved in two of them. The diarrhea present in the fawns could have been due to the stress of transportation.

All fawns were successfully infected with BVDV following inoculation and were actively shedding the virus as early as 3 DPI and as long as 18 DPI through feces, nasal and/or oral secretions. The two fawns sick throughout the study had higher levels of viremia (data not shown) and shed higher quantities of virus (data not shown) for a longer period of time.

Four out of the 6 calves were infected with BVDV as a result of direct contact with the fawns as evidenced by the presence of the virus in the buffy coat and in tissues collected at necropsy. Studies assessing BVDV transmission from acutely infected animals to in-contact animals are rare. One study demonstrated that elk in contact with acutely infected elk actively shedding BVDV resulted in infection of the in-contact animal [11]. Similar to the results of this study, the in-contact elk developed viremia, however, there was no evidence of shedding of the virus following infection. Two BVDV transmission studies between acutely infected calves and naïve calves failed to show successful transmission [4,5]. In both studies, evidence of infection was based on seroconversion and not virus detection.

The presence of antibodies in four fawns coincided with clearance of the virus and the inability to isolate the virus from tissues at necropsy. Only the calf with evidence of infection by 5 days post-cohabitating with the fawns developed antibody titers to BVDV by 21 DPI based on ELISA. There is a possibility that the infected calves did not have enough time to develop antibodies, although

seroconversion usually occurs within 14-30 days [1]. Microscopic findings agree with previous studies where the primary histopathologic lesions observed due to BVDV infection were lymphoid depletion of Peyer's patches and thymic atrophy [2,8,10,11]. When compared to previous experiments using virulent BVDV strains, in our study, the lack of clinical signs and the paucity of lesions may be due to the low virulence of the strain used [2,3,6].

Table 2 – Results from virus neutralization (VN) and ELISA

DPI[a]		0	7	14		21	
DPC[a]		-2	5	12		19	
Test		VN & ELISA[c]	VN &ELISA	VN	ELISA	VN	ELISA
Kennel #	Animal #[b]						
1	F63	-	-	-	-	- [d]	+ [d]
	C53	-	-	-	-	-	-
	C91	-	-	-	-	-	-
2	F1504	-	-	-	-	1:4	+
	C51	-	-	-	-	-	-
3	F56	-	-	-	-	1:8	+
	C50	-	-	-	-	-	-
4	F1526	-	-	-	-	-	-
	C92	-	-	-	-	-	-
5	F61	-	-	-	+	1:4	+
	C52	-	-	-	-	-	+

[a] DPI – days post inoculation, DPC - days post cohabitation; [b] F – fawn, C – calf; [c] (–) = negative results, (+) = positive ELISA; [d] Fawn 63 was euthanized by day 16 DPI.

Conclusion. To the best of our knowledge, this is the first report of transmission of BVDV from acutely infected deer to livestock. In this study, we demonstrate that BVDV-infected WTD can infect naïve calves with BVD 1a virus when commingled together for 21 days during infection. Based on our findings, wildlife acutely infected with BVDV may be a potential source of infection to susceptible cattle.

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ПЕРЕДАЧА ВІРУСНОЇ ДІАРЕЇ ТИПУ 1А РОГАТОЇ ХУДОБИ ВІД ЕКСПЕРИМЕНТАЛЬНО ІНФІКОВАНОГО БІЛОХВОСТОГО ОЛЕНЯ (*ODOCOILEUS VIRGINIANUS*) ДО ТЕЛЯТ

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Метою даного дослідження було виявити величину передачі вірусу вірусної діареї рогатої худоби від експериментально заражених молодих білохвостих оленів до телят, яких позбавили молозива. У дослідженні показано, що всі молоді олені, яких заражали, до 18 діб включно володіли вірусом. Експериментальними даними доведено, що вірус інфекції вірусної діареї рогатої худоби (BVDV) був виявлений у чотирьох із шести телят, які утримувались спільно з гостро інфікованими молодими оленями.