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EXPERIMENTALLY INDUCED DISEASE

Distribution of Bovine Viral Diarrhoea Virus Antigen in Persistently Infected White-Tailed Deer (*Odocoileus virginianus*)

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Summary

Infection with bovine viral diarrhoea virus (BVDV), analogous to that occurring in cattle, is reported rarely in white-tailed deer (*Odocoileus virginianus*). This study evaluated the distribution of BVDV antigen in persistently infected (PI) white-tailed deer and compared the findings with those from PI cattle. Six PI fawns (four live-born and two stillborn) from does exposed experimentally to either BVDV-1 or BVDV-2 were evaluated. Distribution and intensity of antigen expression in tissues was evaluated by immunohistochemistry. Data were analyzed in binary fashion with a proportional odds model. Viral antigen was distributed widely and was present in all 11 organ systems. Hepatobiliary, integumentary and reproductive systems were respectively 11.8, 15.4 and 21.6 times more likely to have higher antigen scores than the musculoskeletal system. Pronounced labelling occurred in epithelial tissues, which were 1.9–3.0 times likelier than other tissues to contain BVDV antigen. Antigen was present in >90% of samples of liver and skin, suggesting that skin biopsy samples are appropriate for BVDV diagnosis. Moderate to severe lymphoid depletion was detected and may hamper reliable detection of BVDV in lymphoid organs. Muscle tissue contained little antigen, except for in the cardiovascular system. Antigen was present infrequently in connective tissues. In nervous tissues, antigen expression frequency was 0.3–0.67. In the central nervous system (CNS), antigen was present in neurons and non-neuronal cells, including microglia, emphasizing that the CNS is a primary target for fetal BVDV infection. BVDV antigen distribution in PI white-tailed deer is similar to that in PI cattle.

Keywords: bovine viral diarrhoea virus; immunohistochemistry; *Odocoileus virginianus*; persistent infection

Introduction

Bovine viral diarrhoea virus (BVDV), a member of the genus *Pestivirus* within the family Flaviviridae, is a pathogen with substantial impact on the beef and dairy industries worldwide. Clinical manifestations associated with BVDV infection in cattle range from subclinical to severe disease (Baker, 1995). BVDV may cause clinical signs associated with reproductive, respiratory or enteric illness, and BVDV-induced immunosuppression contributes to polymicrobial disease, such as the bovine respiratory disease complex (Baker, 1995; Kapil *et al.*, 2005). Maintenance of BVDV within cattle populations and transmission to new susceptible hosts are mainly the result of exposure to persistently infected (PI) cattle that harbour and shed the virus throughout life (Brownlie *et al.*, 1987).

Persistent infection is the result of exposure of a developing fetus to a non-cytopathic biotype of BVDV before development of a functional immune system. The virus is recognized as self, resulting in widespread

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infection of tissues. In PI cattle, viral antigen is present throughout tissues of all three germinal layers (Cutlip *et al.*, 1980). The extent of antigen distribution varies among tissues and cells of the reticuloendothelial, lymphoid and gastrointestinal organs commonly contain the most BVDV antigen (Bielefeldt Ohmann, 1988; Shin and Acland, 2001). BVDV antigen may be present in cells without evidence of microscopical lesions or signs of inflammation; however, encephalitis and glomerulonephritis have been reported in PI cattle (Cutlip *et al.*, 1980; Bielefeldt Ohmann, 1988; Liebler-Tenorio *et al.*, 2002).

The pathophysiological mechanisms of morbidity and mortality in PI cattle are not completely understood. Viral infection of reproductive and endocrine organs and immune system cells are implied to play a role in the pathogenesis of BVDV-associated diseases (Bielefeldt Ohmann, 1988; Grooms *et al.*, 1996; Shin and Acland, 2001). The diversity of clinical signs observed in PI cattle has been implied to result from heterogeneous antigen distribution in the central nervous system (CNS) of affected animals (Montgomery, 2007).

In addition to cattle, BVDV is able to establish permissive infections in various domestic and free-ranging species in the mammalian order Artiodactyla (Passler and Walz, 2010). The implications of BVDV infections in species other than cattle are incompletely understood. Clinical disease is reported in domesticated small ruminants, swine and camelids, and the health effects of BVDV-associated disease are similar to those in cattle with the most severe effects on reproductive health (Taylor *et al.*, 1977; Stewart *et al.*, 1980; Loken and Bjerkas, 1991; Hegazy *et al.*, 1996; Goyal *et al.*, 2002; Carman *et al.*, 2005). Few reports of natural BVDV-associated disease in heterologous species exist, and the role of BVDV as the causative pathogen in some case reports is equivocal (Richards *et al.*, 1956; Romvary, 1965; Brass *et al.*, 1966; Nettleton *et al.*, 1980; Neumann *et al.*, 1980; Feinstein *et al.*, 1987; Diaz *et al.*, 1988). Among free-ranging species, BVDV infections of white-tailed deer have recently received much attention. The virus was identified in free-ranging white-tailed deer (Chase *et al.*, 2008; Duncan *et al.*, 2008a; Passler *et al.*, 2008; Pogranichniy *et al.*, 2008) and experimental infections resulted in clinical signs analogous to those in cattle (Ridpath *et al.*, 2007, 2008; Raizman *et al.*, 2009). White-tailed deer can become persistently infected with BVDV and shed the virus at similar levels to cattle (Passler *et al.*, 2007), which can result in efficient transmission to other deer (Passler *et al.*, 2009a). In one observational study involving two PI fawns, the BVDV antigen distribution was similar to that in cattle with broad distribution in different tissues, especially the epithelium and vascular endothelium (Duncan *et al.*, 2008a). The aim of the present study was to further characterize BVDV antigen distribution in PI white-tailed deer.

Materials and Methods

Animals

Six PI fawns are described in this study. All the fawns were born to white-tailed deer exposed experimentally to one of three strains of BVDV (Passler *et al.*, 2007, 2009b). These strains represented both species of BVDV (BVDV-1 strains AU526 and KY16; BVDV-2 strain PA131) and originated from PI cattle. Four of the fawns were born alive (numbers 1, 5, 6 and 7) and two were stillborn (SB1 and SB2). The median age at death for the fawns was 10 days (0—124 days, mean 28.17 days). The live-born fawns were hand-raised in an isolation facility. Fawn number 6 was weak and unable to rise after birth and colostrum intake was considered insufficient. This animal had a low birth weight and despite intravenous fluid therapy and antibiotic administration, it remained lethargic until it was humanely destroyed at 10 days of age. The other three live-born fawns appeared vigorous, nursed normally and did not show signs of disease until they died peracutely. Virological testing for BVDV infection was performed at birth and at the time of necropsy examination (Table 1).

Necropsy Examination

A complete necropsy examination was performed on each animal within 24 h of death and samples of spleen, thymus, mesenteric lymph node, lung, liver and ileum were collected for virus isolation. Representative samples of tongue muscle, trachea, oesophagus, thyroid gland, thymus, lung parenchyma, bronchial lymph node, mediastinal lymph node, diaphragm, spleen, kidney, adrenal glands, liver, bladder wall, testes or ovaries, mesenteric lymph node, rumen, reticulum, omasum, abomasum, duodenum, pancreas, jejunum, ileum, caecum, colon, skin, brain, eye and bone marrow were collected into 10% neutral buffered formalin and fixed for 5—7 days. Following fixation, tissues were processed routinely and embedded in paraffin wax for routine histopathology and immunohistochemistry (IHC). Sections (4 μm) were cut on silane-coated slides and dried. Sections were then dewaxed and rehydrated by sequential immersion in xylene followed by graded concentrations of ethanol, then tap water. IHC was performed with a commercial autostainer (Dako North America Inc., Carpinteria, California, USA). Blocking of endogenous peroxidase activity was performed with

Table 1
Virological assessment of PI fawns

Fawn ID	*Sampling at birth*					*Sampling at time of death*		
	Virus isolation		*RT-PCR*	*ELISA*	*IHC*	*Virus isolation*	*RT-PCR*	*IHC*
	WBC	*Nasal swab*	*Serum*	*Ear notch*[*]	*Ear notch*	*Tissues*	*Tissues*	*Tissues*
1	+	+	+	ND	+	+	+	+
5	–	–	–	+	+	–	+	+
6	+	–	+	+	+	+	+	+
7	+	+	+	+	+	–	+	+
SB1	ND	ND	ND	ND	+	+	+	+
SB2	ND	ND	ND	ND	+	+	+	+

ND, not determined, WBC, white blood cells.
Infecting BVDV strains were BVDV-1 AU526 for fawns 5 and 7, BVDV-1 KY16 for SB1 and SB2 and BVDV-2 PA131 for fawns 1 and 6.
[*]Determination of positive result was based on cut-off for bovine samples of sample to positive ratio >0.39.

3% H_20_2 and sections were pretreated with proteinase K prior to application of primary antibody. With the exception of the IHC on fawn 1, for which the monoclonal antibody (MAb) 15C5 (Syracuse Bioanalytical, East Syracuse, New York, USA) was utilized, MAb 3.12F1 (Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, Oklahoma, USA) was used for detection of BVDV antigen. This MAb shows high agreement with MAb 15C5 that is used widely for IHC on ear-notch samples and has been utilized with samples from white-tailed deer (Montgomery, 2007; Passler *et al*., 2008). Following incubation with the primary antibody, BVDV antigen was detected using a biotinylated link antibody followed by peroxidase-labelled streptavidin (Dako). The substrate was NovaRED (Vector Laboratories, Burlingame, California, USA). The sections were counterstained with haematoxylin and coverslipped under non-aqueous mounting medium. Each BVDV-labelled tissue section was accompanied by a negative control slide in which BVDV antibody was replaced with primary antibody diluent. The location of antigen distribution was evaluated and the intensity of antigen labelling within tissues was graded (0–5) as shown in Table 2 (Montgomery, 2007).

Statistical Analysis

For statistical analysis, organs were assigned to one of the 11 organ systems (alimentary, cardiovascular, endocrine, haemolymphatic, hepatobiliary, integumentary, musculoskeletal, nervous, renal, reproductive or respiratory). Additionally, tissues were assigned to the traditional classification of tissue type (i.e. connective tissue, epithelium, muscle or nervous tissues) or classified as comprising immune cells. Odds ratios were calculated for positive antigen labelling for each organ system and tissue combination. Data were also analyzed using a cumulative multinomial model as implemented in PROC LOGISTIC of SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA). Because observed antigen labelling scores >2 were uncommon, often resulting in cell counts ≤5, we combined antigen staining classes ≥2. The score test in the above mentioned PROC resulted in $P = 0.095$ of observing a larger χ^2 for tissue analysis and $P = 0.372$ for organ system analysis, thereby confirming that the proportional odds model was adequate. We used the descending option and calculated odds ratios between tissues or organ systems and their associated 95% Wald's confidence intervals. These ratios thus indicate the odds of obtaining a higher antigen labelling score.

Results

BVDV antigen was distributed widely throughout multiple tissues and cell types of all live-born fawns and stillborn fetuses. Although individually variable

Table 2
Scoring criteria for BVDV antigen expression

Grade	*Character of BVDV antigen expression*
0	No antigen expression
1	Weak labelling of a small percentage of cells, difficult to appreciate at low to moderate magnification
2	Generally weak labelling as indicated for grade 1, but with patchy areas of more intense labelling analogous to grade 3
3	Moderate labelling in up to 50% of cells, easily detected at low magnification
4	Generally moderate labelling in up to 50% of cells as indicated for grade 3, but with patchy areas of intense labelling analogous to grade 5
5	Moderate to intense labelling in >50% of cells, easily detected at low magnification

Scoring of antigen expression as adapted from Montgomery (2007).

in extent and distribution (Fig. 1), antigen expression was present in all 11 organ systems investigated (Table 3). In six of the 11 organ systems examined (alimentary, endocrine, integumentary, musculoskeletal, nervous and urinary), the most pronounced antigen labelling was of epithelial tissues. Comparison of tissue types across all organ systems by the proportional odds model demonstrated that epithelial tissues were 1.9–3.0 times more likely to contain BVDV antigen than other tissue types (Fig. 2). Greatest individual scores within the alimentary tract were assigned to epithelial samples from the tongue, oesophagus, abomasum and small intestine in all fawns with the exception of fawn 1 in which only small intestinal epithelium was scored 1–2. Antigen was present in >90% of epithelial samples of liver and skin, and the lowest frequency of positive epithelial antigen labelling was in the musculoskeletal system. Within the skin, individual antigen labelling scores ranged from 2 to 5 for hair follicles and epidermis of all fawns and 0 to 2 for glandular epithelia. Individual antigen labelling scores in the hepatobiliary system were greatest for biliary epithelium, ranging from 1 to 2.

Within the haemolymphatic, hepatobiliary and respiratory systems, the frequency of antigen labelling was greatest for immune cells. While immune cells were not detected in all organ systems, the frequency of labelled cells ranged from 0.25 in the cardiovascular system to 1.0 in the hepatobiliary system. Within immune cells, the greatest individual scores (ranging from 0 to 5) were detected in macrophages and neutrophils within the thymus and lymph nodes.

For those organ systems in which muscle tissues were present, the frequency of positive antigen labelling of muscle tissues ranged from 0.1 to 0.83. Within the cardiovascular system, muscle tissues had the greatest frequency of positive labelling, in contrast to other organ systems where muscle tissues contained comparatively little antigen.

The frequency of positive labelling was generally less for connective and nervous tissues, with the exception of reproductive organs, where connective tissues were the tissue type most often positive for BVDV antigen. For the remainder of the organ systems in which connective tissues were present, the frequency of antigen expression ranged from 0.38 in the musculoskeletal system to 0.78 in neurological organs. The frequency of antigen labelling within nervous tissues ranged from 0.3 in endocrine organs to 0.67 in cardiovascular organs. Antigen expression was detected in neurons and non-neuronal cells (Fig. 3), including microglia within the CNS, and individual antigen labelling scores ranged from 0 to 2. Cerebrum and cerebellum of all fawns contained antigen-positive neurons and endothelial cells.

Results of the comparison of BVDV antigen distribution within organ systems using the proportional

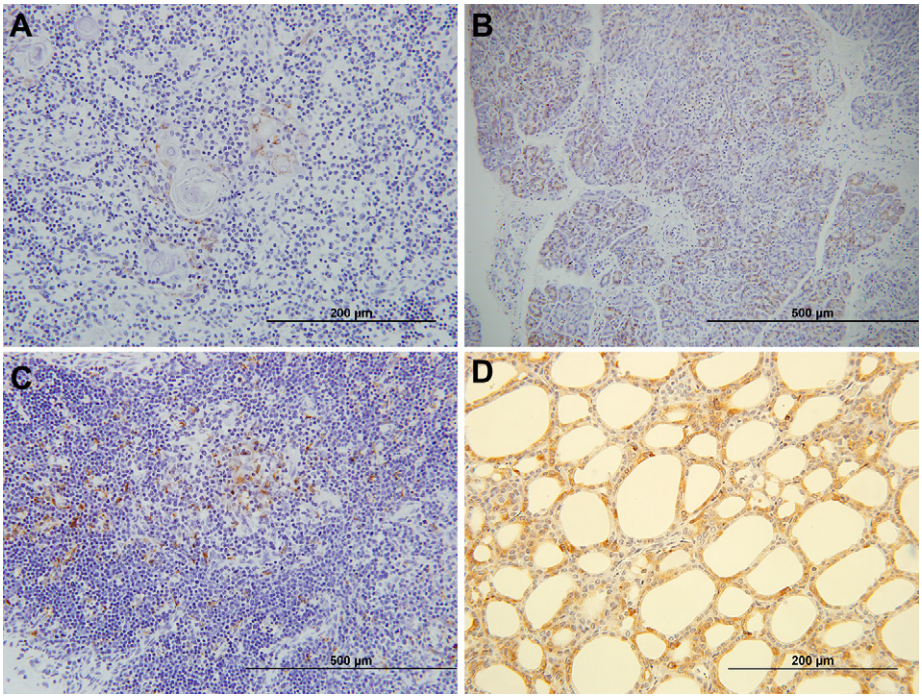

Fig. 1. Immunohistochemical labelling of BVDV antigen in tissues from white-tailed deer. (A) Thymus, class 2 labelling showing sparse antigen in the epithelial reticulum. Note lymphoid depletion. (B) Pancreas, class 3 labelling showing moderate immunoreactivity in exocrine pancreas. (C) Thymus, class 4 labelling showing multifocal, moderate to intense immunoreactivity in the cortex with no evidence of lymphoid depletion. (D) Thyroid, class 5 labelling showing moderate to intense expression in follicular epithelial cells.

Table 3
Antigen distribution in tissues, frequency of positive samples and odds of obtaining positive labelling for a particular tissue within an organ system

Organ system/tissue	*Antigen labelling score*						*Antigen positive*	*Odds positive*	*Total sample* n=
	0	*1*	*2*	*3*	*4*	*5*			
Alimentary									
Connective	0.55	0.34	0.10	—	—	—	0.45	0.81	29
Epithelium	0.32	0.28	0.23	0.08	0.08	0.02	0.68	2.12	53
Immune cell	0.73	0.27	—	—	—	—	0.27	0.38	11
Muscle	0.50	0.41	0.09	—	—	—	0.50	1.00	22
Cardiovascular									
Connective	0.50	0.20	0.30	—	—	—	0.50	1.00	10
Epithelium	0.46	0.18	0.32	0.04	—	—	0.54	1.15	28
Immune cell	0.75	0.25	—	—	—	—	0.25	0.33	4
Muscle	0.17	0.67	0.17	—	—	—	0.83	5.00	6
Nervous	0.33	0.50	0.17	—	—	—	0.67	2.00	6
Endocrine									
Connective	0.50	0.40	0.10	—	—	—	0.50	1.00	10
Epithelium	0.36	0.36	0.21	—	0.07	—	0.64	1.80	14
Nervous	0.70	0.20	0.10	—	—	—	0.30	0.43	10
Haemolymphatic									
Connective	0.44	0.34	0.16	0.06	—	—	0.56	1.29	32
Epithelium	0.39	0.36	0.21	—	0.04	—	0.61	1.55	28
Immune cell	0.30	0.18	0.33	0.12	0.03	0.03	0.70	2.30	33
Hepatobiliary									
Epithelium	0.07	0.60	0.33	—	—	—	0.93	14.00	15
Immune cell	—	—	0.80	0.20	—	—	1.00	Infinity	5
Integumentary									
Connective	0.50	—	0.50	—	—	—	0.50	1.00	6
Epithelium	0.04	0.25	0.42	0.17	0.04	0.08	0.96	23.00	24
Immune cell	0.33	0.17	0.50	—	—	—	0.67	2.00	6
Musculoskeletal									
Connective	0.63	0.25	0.13	—	—	—	0.38	0.60	16
Epithelium	0.50	0.50	—	—	—	—	0.50	1.00	4
Immune cell	0.67	—	0.33	—	—	—	0.33	0.50	3
Muscle	0.90	0.10	—	—	—	—	0.10	0.11	10
Nervous									
Connective	0.22	0.56	0.22	—	—	—	0.78	3.50	18
Epithelium	0.20	0.41	0.39	—	—	—	0.80	4.11	46
Immune cell	0.70	0.27	0.03	—	—	—	0.30	0.43	30
Nervous	0.49	0.27	0.24	—	—	—	0.51	1.02	89
Renal									
Connective	0.48	0.43	0.10	—	—	—	0.52	1.10	21
Epithelium	0.46	0.12	0.24	0.17	—	—	0.54	1.16	41
Reproductive									
Connective	—	0.50	0.50	—	—	—	1.00	Infinity	2
Epithelium	0.13	0.13	0.63	—	—	0.13	0.88	7.00	8
Respiratory									
Connective	0.59	0.24	0.18	—	—	—	0.41	0.70	17
Epithelium	0.23	0.45	0.29	0.03	—	—	0.77	3.43	31
Immune cell	0.10	0.60	0.30	—	—	—	0.90	9.00	10
Muscle	0.36	0.45	0.18	—	—	—	0.64	1.75	11
Total sample number									709

—, score not assigned.

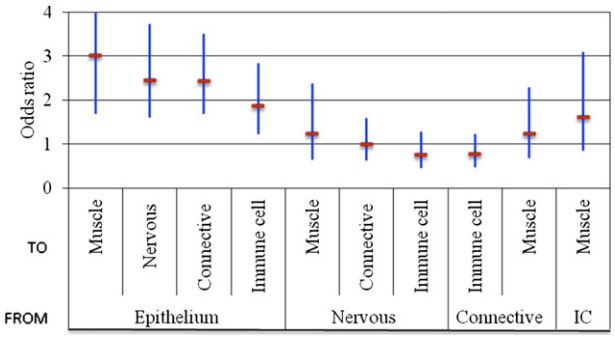

Fig. 2. Odds ratios between 'from' and 'to' tissues resulting from fitting a proportional odds model to antigen labelling classes of tissue types. The horizontal red line indicates the point estimate for the odds ratio and the vertical blue line indicates the 95% confidence interval for a given estimate. IC, immune cell.

odds model are displayed in Table 4. Antigen was most likely to be detected in the hepatobiliary, integumentary and reproductive organs of the PI fawns. All other organ systems were more likely to contain antigen than the musculoskeletal system.

Evidence of moderate to severe lymphoid depletion was present in all fawns. Depletion of lymphocytes was moderate to severe in thoracic and intestinal lymph nodes as well as in Peyer's patches. Thymic lymphoid depletion was also present and ranged from mild to severe. Histopathologically, evidence of enteritis (fawn 5), pneumonia (fawns 6 and 7) and pyelonephritis (fawn 1) was detected.

Discussion

The present study employed descriptive and analytical methods to characterize the distribution of BVDV antigen in PI white-tailed deer. The fawns studied were persistently infected with either BVDV-1 or BVDV-2 strains of cattle origin, complementing the findings of a previous study that described immunohistochemical findings in two twin PI fawns infected with BVDV-2 R03-20663 strain of cervine origin (Duncan *et al.*, 2008a). To our knowledge, comparison by use of a proportional odds model of the BVDV antigen distribution between organ systems and tissue types has not been used previously. This method may provide greater conceptual understanding of the data and allow more valid conclusions compared with simple recording of observational data as performed in previous similar studies in cattle (Fredriksen *et al.*, 1999; Shin and Acland, 2001; Liebler-Tenorio *et al.*, 2004; Confer *et al.*, 2005; Montgomery, 2007) and deer (Duncan *et al.*, 2008a).

The results of the present study demonstrate widespread distribution of BVDV antigen in PI fawns. Although variable in intensity, antigen was detected in all organ systems corroborating previous descriptions of the distribution of BVDV antigen in PI cattle (Bielefeldt Ohmann, 1988; Shin and Acland, 2001; Liebler-Tenorio *et al.*, 2004) and PI white-tailed deer (Duncan *et al.*, 2008a). The most pronounced antigen expression was in the epithelial tissues of different organ systems, which is also in agreement with previous descriptions of PI cattle and white-tailed deer and further substantiates that BVDV infections in white-tailed deer are largely analogous to those of cattle.

The results of this study suggest that the greatest analytical sensitivity in detecting PI white-tailed deer could be achieved by collecting samples from the hepatobiliary, integumentary and reproductive organs. Skin samples are commonly collected for the diagnosis of PI cattle by IHC or antigen capture enzyme-linked immunosorbent assay (ACELISA) and both assays possess high rates of analytical sensitivity and specificity (Brodersen, 2004; Edmondson *et al.*, 2007). Recent surveillance studies in free-ranging deer also used these antigen detection assays

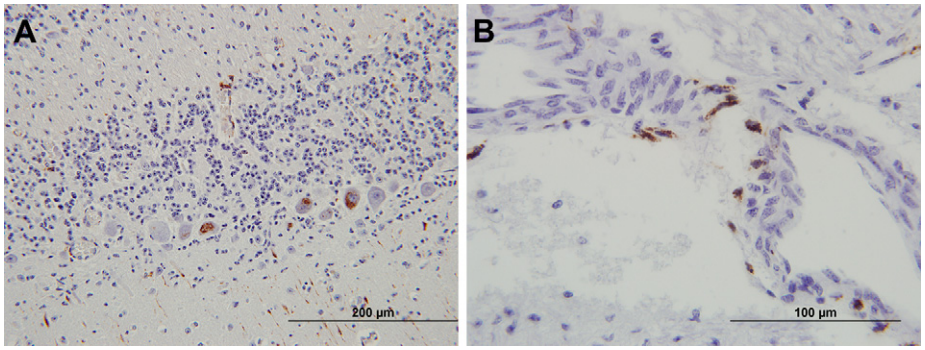

Fig. 3. Expression of BVDV antigen in the cerebellum of white-tailed deer. (A) Antigen in Purkinje cells, endothelial cells, the molecular layer and sparsely in the granular layer. (B) Endothelial antigen was common in all fawns, but in this cerebellar region there is no endothelial antigen expression, but prominent perivascular microglial labelling.

Table 4
Odds ratios between organ types resulting from fitting a proportional odds model to antigen labelling scores

From/to	*Alimentary*	*Cardiovascular*	*Endocrine*	*Haemolymphatic*	*Hepatobiliary*	*Integument*	*Musculoskeletal*	*Nervous*	*Renal*	*Reproductive*	*Respiratory*
Alimentary		0.9	1.2	0.7	0.2	0.2	2.8	0.9	0.9	0.1	0.7
Cardiovascular	1.1		1.4	0.7	0.3	0.2	3.1	1.0	1.0	0.1	0.8
Endocrine	0.8	0.7		0.5	0.2	0.1	2.2	0.7	0.7	0.1	0.5
Haemolymphatic	1.5	1.3	1.9		0.4	0.3	4.2	1.4	1.3	0.2	1.0
Hepatobiliary	4.3	3.8	5.3	2.8		0.8	11.8	3.9	3.8	0.5	2.9
Integumentary	5.5	4.9	6.9	3.7	1.3		15.4	5.0	4.9	0.7	3.7
Musculoskeletal	0.4	0.3	0.4	0.2	0.1	0.1		0.3	0.3	0.0	0.2
Nervous	1.1	1.0	1.4	0.7	0.3	0.2	3.1		1.0	0.1	0.7
Renal	1.1	1.0	1.4	0.7	0.3	0.2	3.1	1.0		0.1	0.8
Reproductive	7.8	6.9	9.7	5.1	1.8	1.4	21.6	7.1	6.9		5.2
Respiratory	1.5	1.3	1.8	1.0	0.3	0.3	4.1	1.3	1.3	0.2	

The ratio above the diagonal is the inverse of the corresponding ratio below the diagonal, (e.g. the odds ratio of integumentary versus alimentary = 5.5 below the diagonal and correspondingly the odds ratio of alimentary versus integumentary = 1/5.5 = 0.2 above the diagonal).

on cervine skin samples to detect PI animals (Duncan *et al*., 2008b; Passler *et al*., 2008; Pogranichniy *et al*., 2008). Based on these studies, the prevalence of persistent infection in the deer populations examined was 0.2—0.3%. Although confirmation of infection was performed in all three studies, bovine IHC and ACELISA assays have not been formally evaluated for use in heterologous hosts. While the present study confirms that skin is a suitable diagnostic sample for detection of BVDV antigen in white-tailed deer, much disagreement between results of IHC, ACELISA and reverse transcriptase polymerase chain reaction (RT-PCR) assays was detected when ear-notch biopsies from approximately 1,500 hunter-harvested white-tailed deer were examined for evidence of BVDV infection (Passler and Walz, unpublished observations).

For diagnosis of BVDV infection in cattle, samples in addition to skin are commonly collected, including samples from the spleen, lymph nodes, Peyer's patches and thymus, the lungs, thyroid and the gastrointestinal tract (Radostits *et al*., 2007). Although overall, antigen expression was also detected in these tissues in the present study, alimentary and haemolymphatic tissues of PI white-tailed deer were less likely to contain BVDV antigen, suggesting that BVDV diagnostics in white-tailed deer should not rely solely on samples from these organ systems. Specifically, samples from the upper gastrointestinal tract, including forestomach and small intestine, contained more antigen than in the lower gastrointestinal tract. Lower than expected antigen expression in lymphoid tissues likely resulted from the often severe lymphoid depletion observed in Peyer's patches, thymus and lymph nodes. Prominent lymphoid depletion was also detected in a previous description of PI white-tailed deer (Duncan *et al*., 2008a) and white-tailed deer fawns following acute experimental infection (Raizman *et al*., 2011). While decreases in circulating lymphocytes and lymphoid depletion are common in acutely infected cattle, lymphoid depletion is not a common feature in PI cattle, contrasting with the findings in deer (Walz *et al*., 2001; Liebler-Tenorio *et al*., 2002, 2004).

Previous studies have shown that the CNS is a primary target for BVDV infection of fetal cattle and widespread antigen expression was reported in nervous tissues of PI cattle (Fernandez *et al*., 1989; Hewicker *et al*., 1990; Montgomery, 2007). Although BVDV antigen was detected exclusively in neurons in one study (Fernandez *et al*., 1989), another study demonstrated viral antigen in neurons and non-neuronal cells (Montgomery, 2007). In the current study, viral antigen was detected in neurons and non-neuronal cells, including blood vessels and meninges, corroborating findings of a previous report (Duncan *et al*., 2008a). Antigen expression was detected in microglia of three of the fawns. This is in contrast to PI cattle in which antigen detection in microglia has not been reported, suggesting differences of viral strains or pathophysiology of BVDV infection in deer. In the present study, the most pronounced antigen labelling was detected in the cerebrum and cerebellum, which is in agreement with a previous report (Duncan *et al*., 2008a). While the cerebral cortex and hippocampus were considered predilection sites of BVDV infection in PI cattle in one study (Fernandez *et al*., 1989), antigen was detected in the hippocampus of only two of the fawns evaluated here.

The present study has demonstrated widespread antigen distribution in the tissues of PI white-tailed deer. Variations in location of antigen labelling compared with that in cattle may be the result of virus strain differences and variations of cell tropism, or differing pathophysiological mechanisms of BVDV

infection in cattle and deer. However, findings of this study largely correspond to those in PI cattle and emphasize the promiscuous nature of BVDV. The results of this study suggest that species that are related phylogenetically to cattle should be considered a component of the ecology of BVDV.

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The first two authors contributed equally to this work.

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