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Tissue distribution of bovine viral diarrhea virus antigens in persistently infected cattle

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The tissue distribution and cellular localization of viral antigens in three cattle with persistent bovine viral diarrhea virus (BVDV) infection was studied. In three cases, necropsy findings of oral ulcers, abmasal ulcers and necrosis of Peyer's patches were suspected have been caused by BVDV infection. Non-cytopathic BVDV was isolated from a tissue pool of liver, kidneys and spleen. Immunohistochemical detection of BVDV showed that BVDV antigens were detected in both epithelial and non-epithelial cells in all examined organs, including the gastrointestinal tract, liver, pancreas, lung, lymphatic organs (spleen, lymph nodes), adrenal gland, ovary, uterus, and the mammary gland. These findings support the hypothesis that animals with persistent BVDV infection spread BVDV through all routes, and that infertility in BVDV infection is associated with the infection of BVDV in the ovaries and uteri.

Key words: Bovine diarrhea virus, cattle, diagnosis

Introduction

Bovine viral diarrhea virus (BVDV) is a positive-sense single-strand RNA virus. BVDV is one of the most important viral pathogens of cattle and its control and prevention are of worldwide concern. Moreover, BVDV infection has been associated with enteric disease, mucosal disease [2], diabetes [12] and reproductive failure [1,8,9].

The reproductive effects of BVDV infection include early embryo loss, abortion, and congenital defects [9]. Several studies have shown that a variety of organs including the lymph nodes, spleen and liver are preferred sites of viral replication in fetuses and adult cattle [3,4,7,10]. Recently, ovaries have been shown to be one of the possible sites of BVDV replication and this could lead to abnormal ovum development [1,4-6,11].

Little is known about the distribution of BVD viral antigens in the ovary, uterus and mammary glands, of persistently BVDV-infected infertile heifers. The mammary gland is an potent important route of BVDV transmission in cattle, because somatic cells are continually excreted in milk.

Although previous studies have shown the distribution of viral antigens in experimental BVDV infections, little is known of the tissue distribution of viral antigens in naturally occurring BVDV infections. The aim of the present study was to investigate the distribution pattern of viral antigens in three fulminating natural cases of BVDV infection.

Materials and Methods

Case history

Two Holstein cattle (21 months old and eight years old) were submitted to the Pennsylvania Veterinary Laboratory, Harrisburg, PA. The heifer (case 1, 21 months old) was produced by embryo transfer, and born 11 days prematurely. She was small, and had always been smaller than her herd mates. Between 5 and 15 months of ages, she was given 4 injections of multivalent vaccine that included killed BVD virus. The heifer was artificially inseminated on 4 occasions (3 natural estrus cycles and 1 induced), but returned to estrus each time. Approximately, 2 weeks before the animal was presented for necropsy, loose feces were noted, and this progressed to severe diarrhea with blood and mucus in the feces. The animal was euthanized due to a poor prognosis. The second animal (case 2, 8 years -old) was submitted for necropsy and showed severe hemorrhages in the intestines without particular gross findings in other organs. Selected tissues including, intestines, liver, kidney, adrenal gland, pancreas, mammary gland, uterus, ovary, lung, heart, and skeletal muscle were fixed in 10% buffered formalin and processed for paraffin embedding. Five micron sections were stained with

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hematoxylin and eosin. Selected sections were immunohistochemically stained for BVDV antigen.

Virus isolation

Virus isolation was carried out on the tissue samples using established methods [1]. Tissue homogenates of pooled liver, kidneys, and spleen were inoculated onto 90% monolayers of cultured MDBK cells. Cells were grown for 72 hours at 32°C in 5% CO_2, and cultures were immunostained with monoclonal anti-BVDVandevaluated for the presence of BVDV.

Immunohistochemistry

The monoclonal antisera to BVDV (15.c.5, ascites) used in this study was donated by Dr. Edward Dubovi, New York State College of Veterinary Medicine, Veterinary Diagnostic Laboratory, Cornell University, Ithaca, N.Y. Immunohistochemical staining was done using a semiautomated capillary system (Microprobe® staining system, Fisher Biotech, Fisher Scientific, St. Louis, MO.) using a Mouse Histostain Plus Kit (Zymed Laboratory Inc., San Francisco, CA.). In brief, deparaffinized sections were blocked with 3% hydrogen peroxide in distilled water for 15 min., and then treated with 0.05% protease (Sigma, St. Louis, MO) in phosphate buffered saline (PBS). PBS was added with 30% BRIJ 35 (Sigma) (2.5 ml/liter). After washing with PBS containing BRIJ 35, sections were reacted sequentially with normal blocking sera and primary antisera (diluted in 1 : 1000) for 60 min. Biotinylated secondary antisera and streptavidin-peroxidase were then applied according to the manufacturer's recommendations. All of the reactions were performed in a humid chamber at 36°C. Normal mouse serum was substituted for primary antiserum as a negative control. After color development was completed, sections were counterstained with hematoxylin and mounted using Aquamount (Zymed).

Results

Gross and histological findings

The heifer (case 1) was thin but had some body fat reserves, and had about 20 irregular dorsal lingual ulcers and erosions ranging in size from 2 mm to 1 cm. There were no other abnormalities in the mouth, pharynx, esophagus or forestomachs, but 100 or more abomasal ulcerswere observed, which were 2-10 mm in diameter with a fibrous base and irregular fibrous rim. Peyer's patches were well defined, sunken, hemorrhagic with adhering surface flecks of mucus and debris. In the large intestine, mild edematous thickening of the wall was evident over its entire length, caused by edema. The large intestinal mucosa contained moderate numbers of ecchymotic hemorrhage. All other body systems with the exception of the central nervous system were examined and were unremarkable. Case 2 showed severe hemorrhages in the intestines without other significant gross lesions.

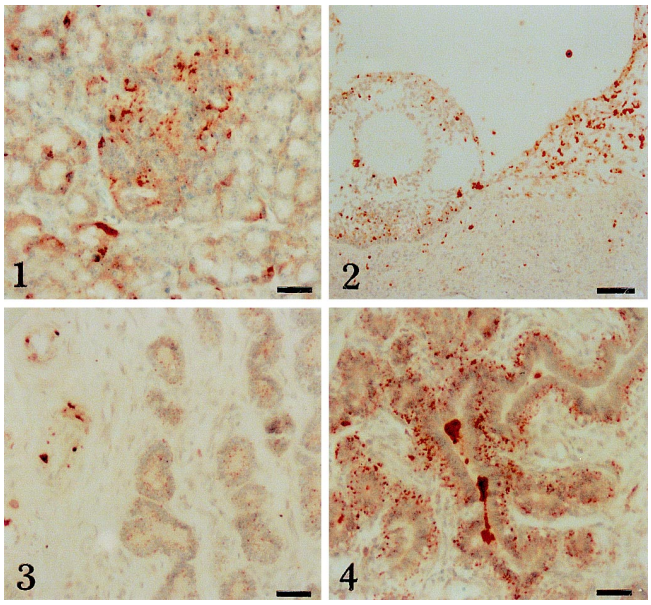

Fig. 1. Immunostaining of BVDV in the pancreas. BVDV positive cells were found in the islets and acini. Counterstained with hematoxylin. Scale = 50 μm.
Fig. 2. Ovary of cow, showing degenerating Graafian follicle. Cumulus oophorus, follicular cells and theca internal cells show staining for the BVD virus. Counterstained with hematoxylin. Scale = 100 μm.
Fig. 3. Uterus of cow, BVDV-immunoreactivity was recognized in the endometrial glandular and luminal epithelia, and occasionally within arterial walls and uterine smooth muscle Counterstained with hematoxylin. Scale = 50 μm.
Fig. 4. Immature mammary gland of cow. Staining for BVD virus is present in epithelial cells lining ducts. Counterstained with hematoxylin. Scale = 50 μm.

Immunohistochemical localization of BVDV antigen

Non-cytopathic BVDV was isolated from the examined tissue pools. We further examined the tissue distribution of BVDV in various tissues including the ovaries, intestines, liver, pancreas, adrenal gland, mammary glands, etc, as described as below.

Oval BVDV-positive cells were found in all connective tissues in the body including the lamina propria of the intestine . These cells are probably macrophages, and may contribute to viral spread in the body. Glomeruli in the kidneys were also positive for BVDV. Immunoreaction for BVDV was found consistently in the smooth muscles and some polyhedral cells, presumably macrophages of necrotic vessel walls. Small number of vascular endothelial cells were positive for BVDV antigen. Immunostaining for BVDV was localized in the cells of pancreatic islets and in the exocrine glandular acini (Fig.

Table 1. Summary of anatomic sites and immunohistochemical intensity of BVDV antigen-containing cells in a persistently infected heifer with infertility (21 month old)

Tissue	Mucosa/ parenchyma	Connective tissue	Macrophages/ histiocytes	Blood vessels	Others
Reproductive organ					
Mammary gland	+++	-	+	++	cells in lumen ++
Uterus	+	-	+	++	smooth muscle +
Ovary	+	+	+	++	cumulus cells ++
Lymphatic system					
Spleen	-	-	red pulp +++	+	
Lymph node	-	-	medullary ray ++	+	
Digestive organ					
Stomach	++	+	++	+	
Intestine	+++	+	+++	+++	
Pancreas	acini +++	-	+	-	Islets +++
Liver	+	-	Kupffer +++	+	
Other organs					
Lungs	bronchial +	-	alveolus +	+	
Kidney	+	-	+	+	
Adrenal gl	cortex ++	-	-	+	Medulla +

*The intensity of immunostaining and number of BVDV antigen-containing cells: -= no staining: += faint minimal staining: ++= moderate staining: +++= intense staining.

1). Kupffer's cells also contained BVDV antigen and moderate number of hepatocytes were BVDV-positive. Widespread infection by BVDV was recognized in parenchymal cells in all layers of the adrenal cortex and medulla, which suggests that adrenal hormone production might be affected in BVDV infection (Table 1).

In the ovaries, BVDV-positive oval cells, probably hematogenous macrophages, were scattered within the ovarian stroma. BVDV-immunoreactivity was also localized on the follicular epithelia in the tertiary, but not in primary, ovarian follicles with varying intensity (Fig. 2). Antigens was not detected in ova in primordial and secondary follicles. In addition, the walls of small arteries in the ovaries were positive for BVDV antigen. In the uterus, BVDV-immunoreactivity was recognized in the endometrial glandular and luminal epithelia, and occasionally within arterial walls and uterine smooth muscle (Fig. 3). Immunoreactivity for BVDV was observed along the bases of the mammary gland epithelial cells (notably ductules), with some clumps of BVDV antigens in the lumen of alveoli (Fig. 4) (Table 1). No immunoreaction was identified in the control slides of serial sections treated with normal mouse sera.

Discussion

In these cattle with fulminate BVD, many tissues containing the virus could have been a source of viral excretion. The BVD virus has a tropism for epithelial cells, including those of the intestine and its accessory glands. Virus in glandular secretions could be a source of viral dissemination in a herd.

We found consistent damage to arterial walls, which may explain the hemorrhage in this cow. BVDV is reported to cause infertility in cattle, and has been isolated from ovarian follicles [4,11], oviducts [1] and uterus [4]. We confirmed by immunohistochemistry that uterine tissue harbors viral antigens in the epithelium, arteries, and smooth muscle. The presence of BVDV in the ovary and uterus in this study is entirely consistent with results of previous studies [4,5,11]. In the present study, we also confirmed that viral antigens are present in the lumen and in the glandular epithelium of the mammary gland. This implies that milk could be a source of BVDV infection for calves, if persistently infected heifers survived long enough to calve and lactate.

Bovine pestivirus has been known to infect the endocrine cells of pituitary glands and pancreatic islets [10]. The involvement of the adrenal gland in BVDV infection has not been previously. Our study shows that the adrenal gland is in fact one of targets of BVDV, which implies that the production of adrenal hormones may be impeded by BVDV infection.

Our findings support the conclusion that all reproductive organs are vulnerable to BVDV infection and that infection of the reproductive organs may be one of the causative factors of repeated infertility. We also found that the mammary gland may be a source of virus excretion from persistently infected cows.

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