Case Report Rapport de cas

The first case of porcine epidemic diarrhea in Canada

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Abstract — In January, 2014, increased mortality was reported in piglets with acute diarrhea on an Ontario farm. Villus atrophy in affected piglets was confined to the small intestine. Samples of colon content were PCR-positive for porcine epidemic diarrhea virus (PEDV). Other laboratory tests did not detect significant pathogens, confirming this was the first case of PED in Canada.

Résumé — Premier cas de diarrhée épidémique porcine au Canada. En janvier 2014, une mortalité accrue a été signalée chez des porcelets atteints de diarrhée aiguë dans une ferme de l'Ontario. L'atrophie des villosités chez les porcelets touchés a été confinée au petit intestin. Des échantillons du contenu du côlon étaient positifs par RCP pour le virus de la diarrhée épidémique porcine (VDEP). D'autres tests de laboratoire n'ont pas détecté d'agents pathogènes importants, ce qui confirme qu'il s'agit du premier cas de DEP au Canada.

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Porcine epidemic diarrhea virus (PEDV) is an *Alphacoronavirus* that was first associated with clinical disease of pigs in the United Kingdom in 1971 (1). The disease subsequently spread to many European countries, and also from Europe to Asia where it has become endemic in China, Japan, Korea, and Thailand (2). Since PED was first recognized in the United States in April 2013 (3), the disease has spread rapidly across the continental US and caused over 7111 confirmed cases in 30 states by May 31, 2014 (4). Early reports regarding genetic composition of the virus found that the US PEDV was very similar to a predominant PEDV strain/lineage circulating in China (5). However, a "variant" PEDV with < 90% nucleotide identity in the spike (S1) protein gene was also detected in Ohio (6). The Ohio "variant" PEDV was over 99% identical to a "variant" PEDV strain/lineage that emerged in China in 2010 (7).

Because of the explosive spread of PED in the US and the Canadian industry's connectedness to the US markets, in July 2013 the University of Guelph Animal Health Laboratory (AHL) developed a PCR test to rapidly detect PEDV in cases of acute enteritis in swine. The purpose of the work described herein is to document the first Canadian identification of PEDV as a cause of acute diarrhea affecting a swine herd in Ontario.

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Case description

History

In January of 2014, piglets from a 500 sow farrow-to-finish herd in southwestern Ontario experienced diarrhea, vomiting, and mortality over a 24-hour period. In the first 24 h, the clinical signs began in 1 farrowing room and spread to include almost all of the litters in this room. Affected piglets were < 1 wk of age. The other farrowing rooms were not yet affected. The herd had a past history of sporadic *Escherichia coli* diarrhea in piglets < 5 d of age. Injectable tylosin (200 mg/mL, Elanco, Greenfield, Indiana, USA) had been successfully used at 0.25 mL/piglet, once daily for 1 to 2 d. The diagnostic rule-outs by the referring veterinarian also included transmissible gastroenteritis (TGE) and PED. The producer was instructed to submit live, acutely affected piglets to the AHL.

The diarrhea did not respond to antibiotic treatment, and had escalated in 24 h to include most of the piglets in the affected farrowing room. The producer had noted vomiting in some litters. Piglet morbidity and mortality were 100%.

Autopsy, histopathology, and immunohistochemistry (IHC)

Four live piglets with acute diarrhea were submitted to the AHL and euthanized for autopsy. They ranged in age from 2 to 5 d, and in weight from 0.7 to 0.84 kg. Samples of stomach, small intestine, and colon were rapidly fixed in 10% buffered formalin for histologic examination. These were routinely processed,

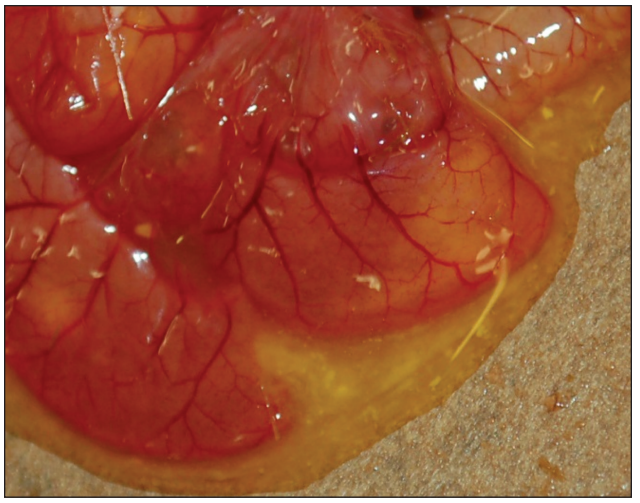

Figure 1. Small intestine of a PED infected pig, showing typical watery yellow intestinal content and congestion of serosal blood vessels.

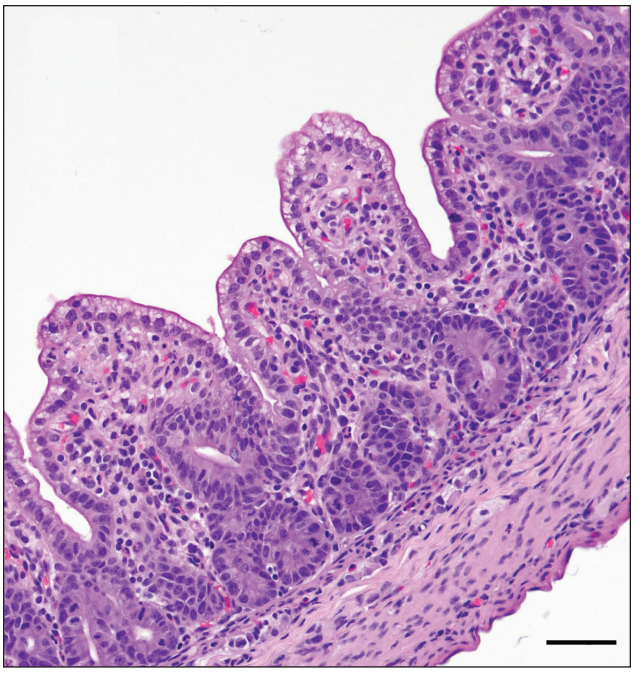

Figure 2. Histology of small intestine in a PED infected pig, showing marked villus atrophy and villus fusion. Bar = 50 μm

sectioned at 5 μm, stained with hematoxylin and eosin, and examined by light microscopy. For TGEV IHC, deparaffinized sections were stained using monoclonal antibody FIPV3-70 (Custom Monoclonals International, Sacramento, California, USA).

Gross pathological findings were similar in all 4 piglets. The piglets were transported together in a ventilated box and were mostly (80% of body area) covered in watery feces when they were received. Body condition appeared adequate for their age with signs of minimal dehydration. Stomachs contained milk, and the small and large intestine were distended with watery yellow translucent content (Figure 1). Intestinal serosae were congested. Significant lesions were confined to the small intestine, wherein all 4 pigs had moderate to marked atrophy and fusion of intestinal villi (Figure 2). Enterocytes on villi were sometimes attenuated and occasional neutrophils or pyknotic nuclear debris could be seen in the superficial lamina propria. In some piglets, villus tip epithelium was columnar but vacuolated. Occasionally at villus tips, clumps of 2 or 3 rounded epithelial cells could be seen exfoliating. No syncytial cells were seen. TGEV IHC was negative.

Bacterial culture and serotyping

Small intestinal samples from all piglets were submitted for *E. coli* F4, *Salmonella* spp., and *Clostridium perfringens* cultures. For *E. coli* and *Salmonella* spp. isolations, samples were plated onto MacConkey and Hektoen agars and incubated overnight at 35°C in atmospheric conditions. In addition, tetrathionate broth, incubated overnight at 42°C in atmospheric conditions, was used as a *Salmonella* enrichment medium followed by plating onto brilliant green sulfa agar plates. For isolation of *C. perfringens,* phenylethylalcohol plates were incubated at 37°C for 48 h in a Concept 400 anaerobic workstation (Ruskinn Technologies, Sanford, Maine, USA). Bacterial identifications were performed using matrix assisted laser desorption ionization time-of-flight mass spectroscopy. All morphologically different *E. coli* isolates were tested for the presence of F4 by slide agglutination using F4 specific antiserum purchased from the OIE reference laboratory for *E. coli* at the University of Montreal.

No *E. coli* F4 positive or *Salmonella* spp. were isolated from any of the samples tested. Moderate numbers of *C. perfringens* were isolated from 1 piglet and 1 *C. perfringens* colony was isolated from another piglet. *Clostridium perfringens* was not isolated from the 2 remaining piglets.

Nucleic acid extraction and polymerase chain reaction (PCR)

Colon samples were stab-swabbed and agitated in virus transport medium (VTM). Total nucleic acids were extracted from 50-μL aliquots of VTM using the MagMAX-96 Viral RNA Isolation Kit in a MagMAX Express-96 Magnetic Particle Processor (Applied Biosystems, Foster City, California, USA). Samples were tested by a triplex real-time PCR assay designed to detect PEDV, TGEV, and generic porcine coronavirus (PCoV). Primers and probes (Table 1) for PEDV nucleocapsid protein gene and conserved 3′ end of PCoV were designed for this study, while primers and the probe targeting the TGEV spike protein gene were previously described (8). A porcine deltacoronavirus (PDCoV) real-time PCR assay targeting the conserved 3′ end of the *Deltacoronavirus* genus was also developed for this study (Table 2), and samples were also tested for PDCoV at a later date. Real-time PCR assays were carried out in 25-μL reactions in a Light Cycler 480 (Roche, Laval, Quebec) using Ag Path-ID One-Step RT-PCR Kit (Applied Biosystems) under standard conditions (Table 3). Samples with crossing points lower than 37 were considered positive.

Four colon samples were positive for PEDV with crossing points of 20.47, 25.96, 22.92, and 21.85. Samples were negative for TGEV, PCoV, and porcine rotavirus (PoRV) A, B, and C. The tests for PDCoV were negative.

Table 1. Primer sequences for PEDV, TGEV, and PCoV

Target	Primer/probe name	Reporter	Primer/probe sequence
PEDV-NC	PEDV_1_NC_130524_Pr	FAM	AGCAATGCTGCAACATTTGGTGC
PEDV-NC	PEDV_1_NC_130524_F	n/a	TATGCTCAGATCGCCAGT
PEDV-NC	PEDV_1_NC_130524_R	n/a	AGCCACATTACCACCAAAG
TGEV-S	TGEV_Pr	HEX	YAAGGGCTCACCACCTACTAC
TGEV-S	TGEV_F	n/a	TCTGCTGAAGGTGCTATTATATGC
TGEV-S	TGEV_R	n/a	CCACAATTTGCCTCTGAATTAGAAG
PCoV 3′	PCoV_1_130524_Pr	CY5	AAAGATCCGCTACGACGAGCCAAC
PCoV 3′	PCoV_1_130524_F	n/a	GGAGGTACAAGCAACCCTATT
PCoV 3′	PCoV_1_130524_R	n/a	CCAGACGTTAGCTCTTCCATT

PEDV-NC — porcine epidemic virus nucleocapsid protein gene; TGEV-S — transmissible gastroenteritis spike protein gene; PCoV — porcine coronavirus; n/a — not available.

Table 2. Primer sequences for porcine deltacoronavirus (PDCoV)

Target	Primer/probe name	Reporter	Primer/probe sequence
PDCoV 3′	PDCoV _140214-3 _Pr	FAM	ATGCAAACTAGGGCTGGCTACTCT
PDCoV 3′	PDCoV _140214-3 _F	n/a	AGCCAGAGAGCCAGTCA
PDCoV 3′	PDCoV _140214-3 _R	n/a	TGTACCCTCGATCGTACTCC

n/a — not available.

Table 3. Cycling parameters for PCR assays

Step	Temperature	Hold (h:mm:ss)	Ramp	Number of cycles
RT	45°C	0:10:00	4.4°C/s	1
Activation	95°C	0:10:00	4.4°C/s	1
Denaturation	94°C	0:00:05	2.2°C/s	45
Annealing/Extension	60°C	0:01:00	2.2°C/s	
Cool	40°C	0:10:00	2.2°C/s	1

Sequencing

Conventional N and S gene PCR followed by sequencing was used to confirm the real-time PCR results. The primers used to amplify the partial N and S genes of PEDV were based on those obtained from National Veterinary Services Laboratory (NVSL), Ames, Iowa, with minor modifications. Forward primer PEDN253 — 5′-GGCATTTCTACTACCTCGGA-3′ and reverse primer PEDN992 — 5′-ATAGCCTGACGCAT CAACAC-3′ and forward primer PEDS218 — 5′-GCTAGTGG CGTTCATGGTAT-3′ and reverse primer PEDS442 — 5′-TAGGCAATTACGACCTGTTG were used to amplify 739 bp and 224 bp fragments of the N and S1 genes, respectively. These were then sequenced directly, and gel-purified or cloned prior to sequencing. Sanger sequencing reactions were performed using Big Dye Terminator chemistry version 3.1 and the products were resolved on an Applied Biosystems 3130xl genetic analyzer.

N and S gene conventional PCR assays produced amplicons of the predicted size for all 4 colon samples. Sequencing of these PCR products showed that this Ontario PEDV was 99% identical to recent PEDV isolates from the USA and China.

Discussion

The etiology of neonatal diarrhea in pigs involves various pathogens. An agent-specific diagnosis requires laboratory testing because clinical signs and autopsy are most often non-specific, and multiple pathogens may be involved (9). A sudden incursion of PEDV and extensive economic damage related to PED in the US has raised awareness and sensitized farmers, practitioners, and diagnostic laboratories in Canada.

There is extensive truck traffic that transports early-weaned piglets, market hogs, and cull sows from Ontario to the USA. Empty trucks then return to Ontario, with or without being washed, disinfected, and dried. The transport process has been shown to be a source of transmission of PEDV in the USA (10). Porcine epidemic diarrhea virus is transmitted by the fecal-oral route, and the original fear was that contaminated trucks would transfer the virus into Canada. However, the index case and the next 10 Ontario cases, as well as an isolated case in Prince Edward Island, were suspected to have a common link via contaminated feed, not transport. Bioassay testing of feed and spray-dried porcine plasma (SDPP) determined that piglets inoculated with feed did not shed PEDV, but piglets inoculated with SDPP excreted relatively high levels of PEDV. Therefore feed still could not be ruled out as a potential source of infection (11).

In the case presented in this report, clinical signs, autopsy, and histopathology were compatible with viral infection. The clinical signs, in conjunction with the lesions seen and supporting laboratory test results allowed us to conclude this was the first documented and laboratory-confirmed case of PED in Ontario and in Canada.

Acknowledgments

The authors acknowledge financial support from the Ontario Ministry of Agriculture, Food and Rural Affairs, and the many clerical and technical staff at the AHL on whom we relied during this busy period of increased surveillance and infection control.

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Book Review
Compte rendu de livre

The Soul of All Living Creatures: What Animals Can Teach Us about Being Human

Vint V. Broadway books (Crown Publishers), New York: 2013. 225 pp. ISBN 978-0-307-71887-7 (trade paperback) $17.99.

"The soul is the same in all living creatures, although the body of each is different."

With that Hippocratic epigraph Vint Virga introduces us to his personal odyssey illuminating the connections and reciprocal relationships of all living beings. Once in private small animal practice, he expanded his horizons into behavioral medicine, following an epiphany with a critically injured patient (Pongo) which will resonate with many whose life course has taken them down the veterinary path. Even before that, as those of us of a certain vintage will remember, like Virga, significant moments in our training, such as the lessons of our "surgery dogs," not just the honing of our surgical skills but also our compassion.

The book is a memoir of unforgettable patients in homes and zoos whose behavioral problems and their management illustrate the gamut of their emotional lives. Obsessive-compulsive disorder, feline hyperesthesia syndrome, and aggressive fits are but a few of the diagnostic rubrics treated. The book is meant for a general audience, so emphasis is on behavioral treatments without specifics of pharmaceutical interventions where indicated.

Via behavioral anecdotes, often accompanied by complementary parables, Virga demonstrates the communality of human and animal minds and emotions — from domestic settings with dogs and cats providing exemplars of sensitivity, mindfulness, adaptability, forgiveness and presence to zoos with ocelots exemplifying responsiveness to wolves and whales revealing their expressivity and clouded leopards manifesting integrity.

The author's language and turn of phrase are often quite lyrical which makes for a delightful read. Each chapter finishes with a philosophical flourish making the essential point.

A recent article by Alex Halberstadt (1) gives more insight into Virga's zoological ventures. To flesh out the characteristics of emotions and mental states, I would recommend Temple Grandin's *Animals Make Us Human* (2) as a definitive companion to Virga's treatise.

And to round out the discussion, especially to coax those who might still be hesitant to accept Virga's and Grandin's premises with respect to animal awareness and emotion despite decades of irrefutable evidence, I would like to proffer some readings by Donald R. Griffin, a pioneer in the field of cognitive ethology; his eminently readable and persuasive texts (3–5) would be excellent additions to anyone's reading list and intellectual armamentarium.

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