Porcine Group C Rotavirus as a Cause of Neonatal Diarrhea in a Quebec Swine Herd

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ABSTRACT

A porcine group C rotavirus was found to be the unique cause of a problem of enzootic neonatal diarrhea in a minimal disease herd composed of 190 sows on a continuous farrowing program. During the outbreaks of diarrhea, 10 to 80% of the litters were affected with a morbidity rate of 100% and case fatality rates of 5 to 10%. Clinical signs began 24 to 48 h after birth and were characterized by a profuse yellow diarrhea lasting a few days. Piglets from different outbreaks of diarrhea were necropsied. They had multifocal villous atrophy in the small intestine, especially in the ileum. Group C rotavirus was demonstrated by direct immunofluorescent staining of frozen intestinal sections and by polyacrylamide gel electrophoresis of viral RNA extracted from the intestinal contents of diarrheic piglets. The infection with clinical illness and lesions was reproduced experimentally in newborn piglets by oral inoculation of a suspension prepared from a pool of intestinal contents from diarrheic piglets.

RÉSUMÉ

Un rotavirus atypique du groupe C a été incriminé comme cause unique d'un problème de diarrhée néonatale enzootique dans une maternité de 190 truies assainies sur un programme de mise bas continuelles. Durant les épisodes de diarrhée, 10 à 80% des portées étaient affectées avec un taux de morbidité de 100% et un taux de mortalité variant de 5 à 10%. Les signes cliniques débutaient 24 à 48 h après la naissance et se caractérisaient par une diarrhée jaunâtre profuse durant quelques jours. Des nécropsies ont été pratiquées sur plusieurs porcelets lors de différents épisodes de diarrhée. Des lésions d'atrophie villositaire multifocale ont été observées dans l'intestin grêle et tout particulièrement dans l'iléon. Un rotavirus du groupe C a été démontré à l'aide de la technique d'immunofluorescence directe appliquée sur des sections congélées d'intestins grêles et par électrophorèse en gel de polyacrylamide de l'ARN viral extrait du contenu intestinal de porcelets diarrhéiques. La maladie a été reproduite expérimentalement par l'inoculation orale de porcelets nouveau-nés avec une suspension de contenu intestinal provenant de porcelets diarrhéiques.

INTRODUCTION

Up to 1980, strains of rotavirus known to cause diarrhea in pigs and many other species all had a common group antigen detectable by serological methods such as the immunofluorescence and the enzyme-linked immunosorbent assay (ELISA) techniques (1,2). Since that time, however, viral agents resembling rotaviruses morphologically, but without the common group antigen have been described in many animal species including humans (3-10). These viruses have been called rotavirus-like, pararotaviruses, nongroup A viruses or atypical rotaviruses. Based on comparative antigenic and nucleic acid analyses, five distinct groups of rotaviruses have been proposed (11,12). Typical rotaviruses belong to group A and the so-called atypical rotaviruses to groups B, C, D and E. In pigs, groups A, B, C and E have been reported (1,2,5,9,10,12), and group A seems to be the most common (13-16). Outbreaks of neonatal diarrhea in pigs caused by group A rotaviruses are well described (17), but this is not the case for those caused by atypical rotaviruses. The main purpose of this paper is to describe clinical, pathological and microbiological observations made on an outbreak of enzootic neonatal diarrhea in pigs caused by a group C rotavirus. Experimental reproduction of the disease is also reported.

MATERIALS AND METHODS

SWINE HERD

This was a minimal disease herd on total confinement composed of 190 sows on a continuous farrowing program.

DIAGNOSTIC PROCEDURES

Necropsies were performed on ten diarrheic piglets from different episodes of diarrhea. They were submitted alive at the beginning of diarrhea. Segments from the small intestine and colon were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin, phloxin and safran (HPS). Routine bacteriology was performed on the ileums and the methods used for detecting enterotoxigenic *Escherichia coli* have been reported (18). The direct immunofluorescent staining of frozen sections of the duodenum, jejunum and ileum was used for the demonstration of transmissible gastroenteritis (TGE) virus,

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rotavirus group A (conjugates provided by Mr. Robert Assaf, Institut Armand-Frappier, Laval Québec), rotavirus group C (conjugate provided by Dr. K.W. Theil, Ohio Agricultural Research and Development Center, Wooster, Ohio), and the coronavirus CV777 (conjugate provided by Dr. P. Debouck, Faculty of Veterinary Medicine, Gent, Belgique) using methods already described (19).

Direct electron microscopic examination was performed on the intestinal contents (small and large intestines) of five pigs after negative staining with 3% phosphotungstic acid (PTA) (20).

Rotaviral RNA obtained from intestinal contents was analyzed in comparison with that of porcine rotavirus strains A/OSU and C/Cowden (obtained from the American Type Culture Collection, Rockville, Maryland). The A/OSU isolate was propagated in MAl04 cells in the presence of 1 μg/mL trypsin (Worthington Biomedical Corp., Freehold, New Jersey). Purified virus (cesium chloride fractions with densities ranging from 1.36 to 1.38 g/mL) resuspended in TEN buffer (50 mM Tris pH 7.4, 1 mM EDTA and 150 mM NaCl), was used for further analysis. The porcine C/Cowden isolate was propagated by inoculating orally one-day old specific pathogen-free piglets with 2 mL of a 10% suspension of intestinal content in phosphate buffered saline (PBS) containing 100 U of penicillin and 100 μg of streptomycin per mL. Piglets were euthanized when showing diarrhea or shortly before and intestinal contents were collected and diluted fivefold in TEN buffer prior to partial purification (ultracentrifugation through 40% sucrose). Virus in the intestinal contents of piglets with the natural infection was partially purified for genome profile analysis in the same manner. Rotaviral double-stranded (ds) RNA was extracted as follows. Specimens containing 0.5 M NaCl and 1% (w/v) sodium dodecyl sulfate were extracted twice using phenol-chloroform-isoamyl alcohol and once with chloroform only. Double-stranded RNA was precipated from supernatants overnight at -70° C using 2.0 volumes of ethanol. Extracts were run on 8% polyacrylamide 0.75 mm thick gels for 2 h at 150 volts in a mini Protean II apparatus (Bio-Rad Laboratories, Mississauga, Ontario). Gels were silver stained (Bio-Rad silver stain kit) according to manufacturer's instructions.

EXPERIMENTAL INOCULATION OF PIGLETS

The inoculum was a bacteria-free filtrate of a 10% dilution prepared in PBS of a pool of intestinal contents from four of the diarrheic piglets diagnosed as having group C rotavirus infection. Seven colostrum-deprived piglets were each orally inoculated at one day of age with 2 mL of inoculum. They were observed for clinical signs and euthanized between 15 and 24 h postinoculation (PI); diagnostic procedures used on these pigs were similar to those described for the field cases.

RESULTS

CASE HISTORY AND CLINICAL FINDINGS

The herd experienced several cyclic episodes of neonatal diarrhea over a period of six months. During these outbreaks, 10 to 80% of the litters were affected with a morbidity rate of 100% and case fatality rates of 5 to 10%. Clinical signs were characterized by a profuse yellow diarrhea beginning 24 to 48 h after birth and lasting a few days. Vomiting was observed occasionally and the sows were not affected.

PATHOLOGICAL FINDINGS

Colonic contents of the ten piglets necropsied were abundant, liquid and yellow colored. Gross changes in the intestinal mucosa were unremarkable. All piglets had a multifocal villous atrophy in the small intestine, especially in the ileum (Figs. 1 and 2). Atrophic villi were covered by cuboidal or low columnar vacuolated enterocytes and the crypts were elongated. Focal desquamation of enterocytes at the tip of some villi was also observed. Coccidial organisms were not observed.

MICROBIOLOGICAL FINDINGS

Enterotoxigenic *E. coli* were not demonstrated; the piglets were also negative for TGE virus, group A rotavirus and the coronavirus CV777. They were however positive for group C rotavirus and villous enterocytes throughout the small intestine were infected (Figs. 3 and 4). Immunofluorescence was observed only in the cytoplasm of the infected cells and was not detected in the enterocytes lining the crypts. Direct electron micro-

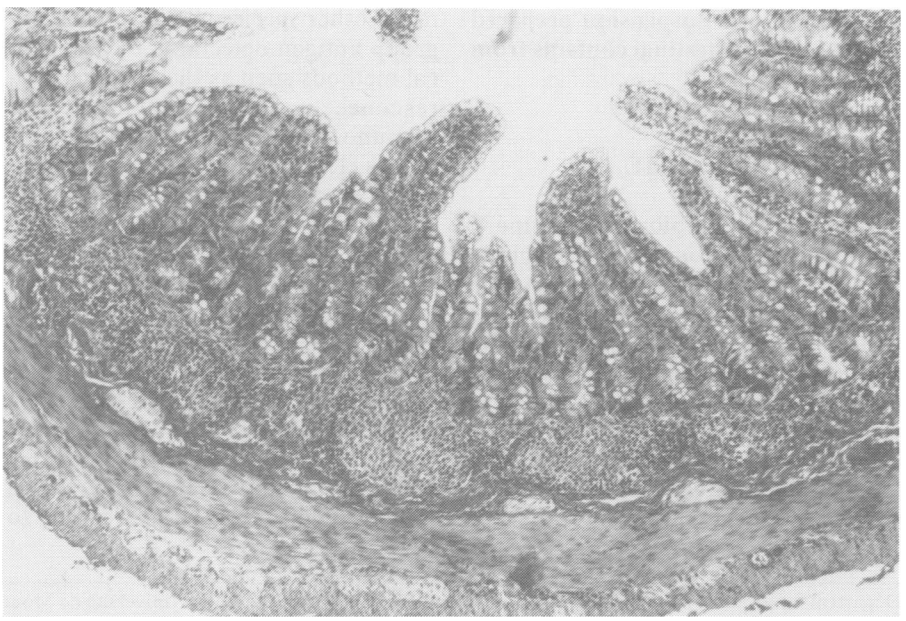

Fig. 1. Ileum of a two day old piglet with group C rotavirus infection. Villous atrophy and elongation of the crypts. HPS X40.

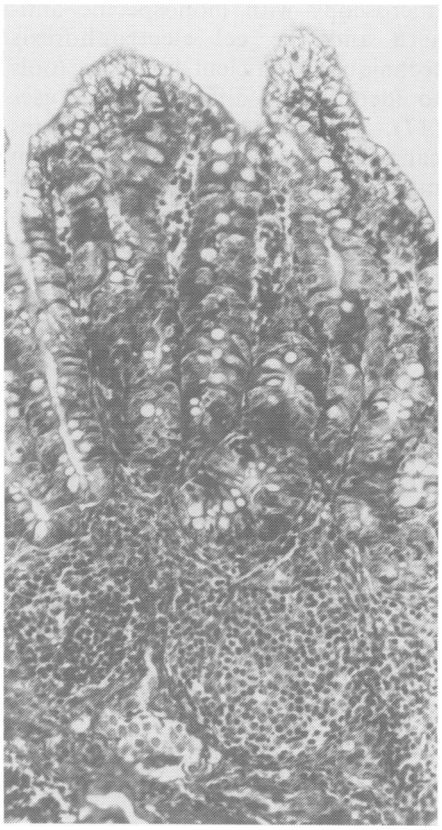

Fig. 2. Ileum of a one day old piglet with group C rotavirus infection. Atrophic villl are covered by low columnar vacuolated enterocytes. HPS X125.

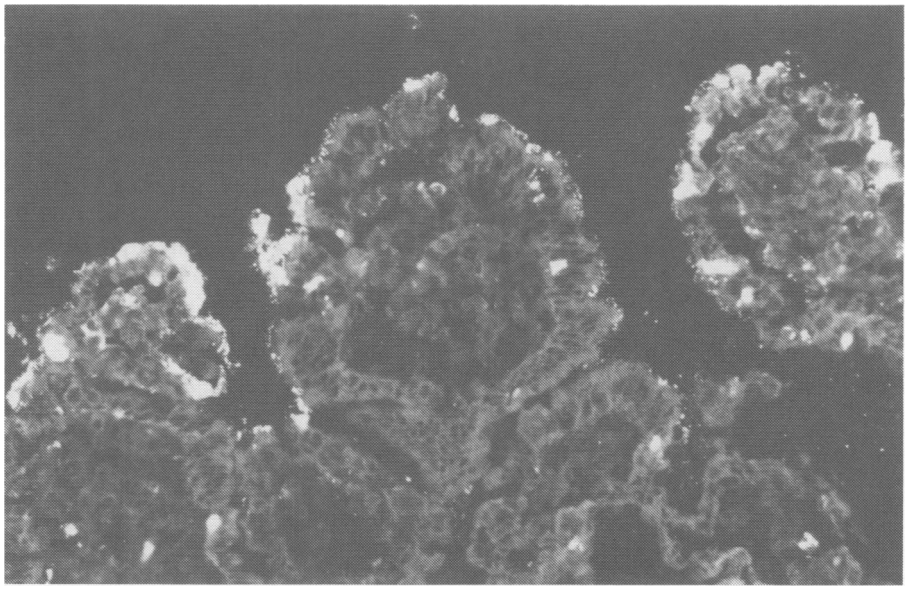

Fig. 3. Fluorescent antibody stained frozen section of the jejunum of a two day old piglet with group C rotavirus infection. Several enterocytes lining the villi are infected. X50.

scopic examination of intestinal contents of diarrheic piglets revealed viral particles indistinguishable in size and morphology from rotaviruses (Fig. 5). The diameter of double-shelled particles was calculated to be approximately 71 nm. Single-shelled, damaged and penetrated particles were also observed.

GENOME PROFILE ANALYSIS

The electrophoretic profile of viral ds RNA extracted from the intestinal contents of diarrheic piglets had a pattern similar to the 4-3-2-2 class size electropherotype of group C rotaviruses and clearly different from the 4-2-3-2 migration pattern of group A rotaviruses (Fig. 6). When compared to the C/Cowden electropherotype, the electrophoretic mobility of RNA segment 5 appeared slower with our clinical isolate (C/St-H).

EXPERIMENTAL INOCULATION OF PIGLETS

Inoculation of piglets resulted in the appearance of yellowish liquid feces as early as 15 h PI in certain piglets. Piglets were infected by group C rotavirus as indicated by the demonstration of infected villous enterocytes by the immunofluorescence technique. They were negative for rotavirus group A and TGE virus. Control PBS inoculated piglets were negative for all these agents. Fluorescence was most prominent in the lower small intestine, but could sometimes be seen throughout the small intestine. Five of the seven infected piglets had a mild to moderate villous atrophy localized mainly in their lower small intestine. Atrophic villi had a hypercellular lamina propria and were covered by cuboidal enterocytes. The crypts were hyperplastic and focal desquamation of enterocytes was noted at the tip of some villi These lesions were not observed in control piglets. Viral particles observed in the feces of the infected piglets displayed typical rotavirus morphology, and the electrophoretic profile of the viral RNA extracted from intestinal contents was identical to that obtained from the original field cases.

DISCUSSION

This outbreak of enzootic neonatal diarrhea shows clearly that porcine group C rotavirus can cause significant diarrheic problems in neonatal piglets. In a recent study, rotaviruses were demonstrated in about 46% of

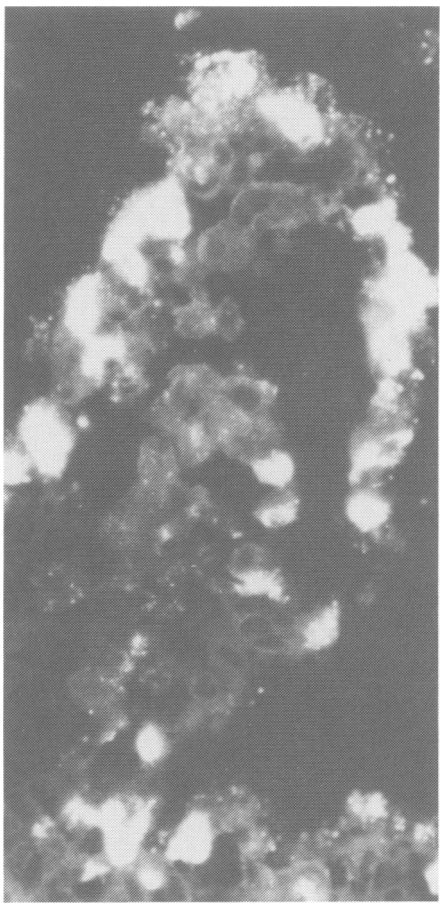

Fig. 4. Same piglet shown in Fig. 3. Villous enterocytes have large amounts of viral antigen in their cytoplasm. X150.

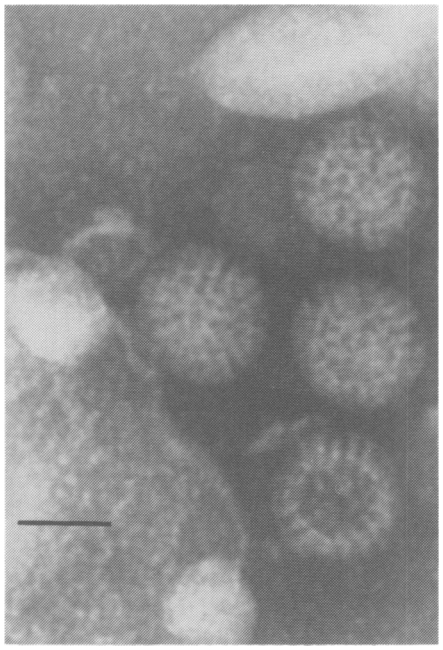

Fig. 5. Electron micrograph of rotavirus particles present in the intestinal content of diarrheic piglets. Three double-shelled particles and one partially damaged particle can be seen. Bar represents 50 nm. PTA stain.

the preweaning diarrhea cases studied and nearly 50% of these rotaviruses were type B or type C (15). In this study however, detailed histopathological examinations of the intestinal tracts were not performed or reported, and the absence of correlation between compatible intestinal lesions and the viruses demonstrated makes it difficult to determine their real implication in the diarrheic cases. Asymptomatic carriers of these viruses surely exist and a clear distinction should be made between infection and clinical disease. Results of other studies using demonstration of rotaviruses in feces (14,16) or serological surveys (21) also suggest that nontype A rotaviruses could be involved in some outbreaks of preweaning diarrhea of pigs. At present, immunological methods readily available to demonstrate rotaviruses detect group A rotaviruses only and for this reason, several of the nontype A diarrheic cases are probably not diagnosed (17). Immune electron microscopy with monospecific antisera and the gel electrophoresis technique are efficient diagnostic tools to identify the different rotaviruses (17). However, these techniques cannot be used on a routine basis in many diagnostic laboratories, and for this reason, immunological reagents for the ELISA, the immunofluorescence technique and others that can detect all types of rotaviruses are urgently needed.

Gross and microscopic lesions which were observed in the naturally infected piglets were similar to those reported in gnotobiotic piglets infected with the Cowden strain of group C rotavirus (5). Lesions of villous atrophy were also similar to those caused by porcine group A rotavirus (22), TGE virus (23), and the coronavirus CV777 (24). Reproduction of the infection with clinical illness and lesions in newborn piglets confirms the enteropathogenic role of this virus.

The enzootic nature of the diarrheic problem in this herd was related to breaks in cleaning and disinfection of farrowing pens. The continuous farrowing program was probably another predisposing factor. Enzootic diarrheic problems are common in farrowing operations (13) and group C rotavirus should be added to the other possible causes of the syndrome.

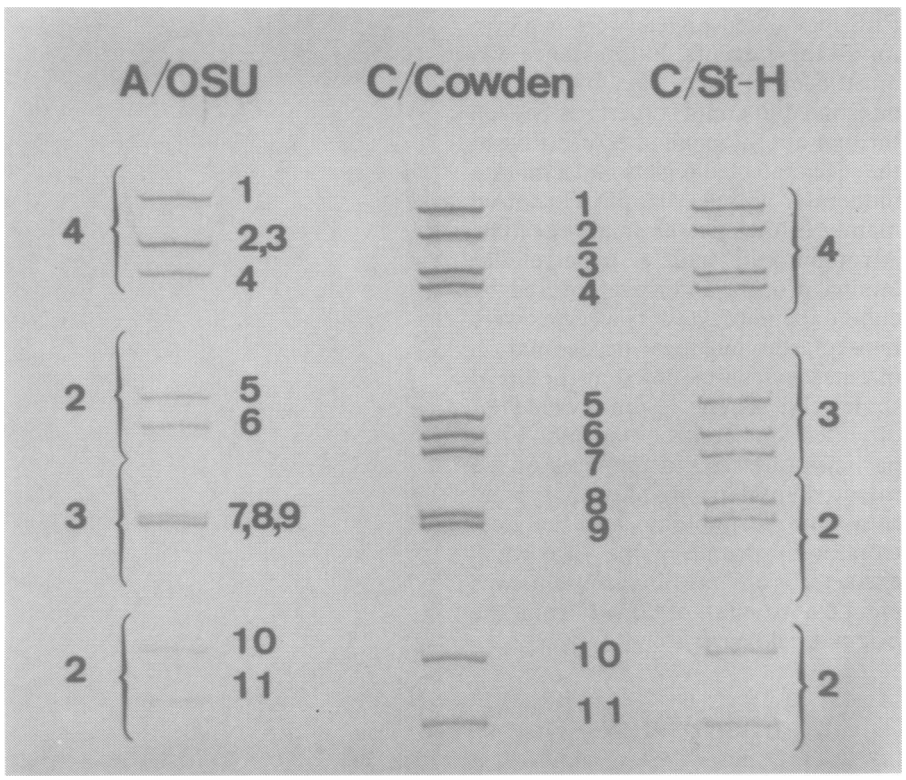

Fig. 6. Comparative electrophoretic profiles of ds RNA extracted from clinical field isolate (C/St-H) and reference A/OSU and C/Cowden strains of rotavirus. Individual ds RNA segments are numbered from one to eleven. Migration patterns are represented in brackets. Silver stained 8% acrylamide gel.

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