

Third Strain of Porcine Epidemic Diarrhea Virus, United States

To the Editor: In April 2013, porcine epidemic diarrhea virus (PEDV) was first reported (1). Since PEDV was detected, the virus has continued to spread throughout the United States and has now been reported in Mexico and Canada (2). PEDV was first reported in Europe in the 1970s and was later reported in Asia during the 1980s (3,4). PEDV outbreaks in Asia were more acute and severe than the PEDV outbreaks in Europe (4). In 2010, an increase, up to 100%, in illness and deaths in piglets was reported in China associated with PEDV infection (4–6). The original North American PEDV strains identified in 2013 caused severe illness and deaths in piglets and had a 99.5% nucleotide identity with Chinese strain AH2012 (1,7,8).

Recently, a North American PEDV variant-INDEL strain was identified, which has been previously described (9). This spike gene deletion has been described in global PEDV strains, which may correlate with a less severe clinical presentation of PEDV. Although the PEDV variant-INDEL strain (OH851) was first reported in February 2014 by the Ohio Department of Agriculture, the PEDV variant-INDEL strains were first detected in June 2013, which suggests that the original PEDV strain mutated or that 2 different PEDV strains were introduced concurrently into the United States (2).

The University of Minnesota Veterinary Diagnostic Laboratory has tested clinical samples from thousands of case-patients to determine the presence of PEDV by real-time reverse transcription PCR. Some of the PEDV-positive samples from case-patients were selected for PEDV spike gene sequencing per veterinarian's request, whereas other samples

were selected for complete genome sequencing to fulfill a grant objective. When PEDV was first detected in the United States, the University of Minnesota Veterinary Diagnostic Laboratory was only sequencing the PEDV spike gene segment to clarify the phylogenetic relationship between PEDV strains.

In February 2014, after the identification and analysis of PEDV variant-INDEL strains, we decided to sequence the complete PEDV genome by using next generation sequencing to clarify the phylogenetic relationship of the US PEDV strains (7). In January 2014, intestines from a deceased neonatal piglet, which had severe diarrhea, were positive for PEDV by real-time reverse transcription PCR, and PEDV was sequenced per request of the veterinarian. The sample was processed for next generation sequencing and analyzed by mapping to reference strain USA/Colorado/2013 (7). During the assembly of PEDV strain USA/Minnesota188/2014 (GenBank accession no. KM077139), a region of the spike only had 5× coverage and ambiguity bases in the consensus contig whereas the coverage across the remaining PEDV genome was 50×. The consensus contig was separated at this region, and the 2 separate contigs were created. After remapping, the 2 contigs were aligned back into a new consensus contig, and the new consensus contig was remapped to verify the accuracy of the contig, which had 50× coverage.

The PEDV strain Minnesota188 was aligned with the other complete genome of PEDV available in GenBank ($n = 113$); it had a 99.9% nucleotide percentage identity to Colorado/2013 and clustered in the North American clade II (Figure, panel A, <http://wwwnc.cdc.gov/EID/article/20/12/14-0908-F1.htm>). The spike gene segment had a 99.7% nucleotide percentage identity (99.4% amino acid identity) with Colorado/2013 and clustered with the

non-North American INDEL strains (Figure, panel B). The alignment indicated a spike gene nucleotide deletion at positions 164–169 (TTG-GTG), which corresponded to amino acid deletion at positions 55 and 56. The spike gene amino acid alignment identified substitution at positions 23 (I), 31 (H), 57 (K), and 59 (E), which have not been identified in the complete PEDV genomes available from North America.

Thus far, 3 naturally occurring US PEDV strains have been identified: the original PEDV, the PEDV with changes in the spike gene (INDEL), and the PEDV strain (S2aa-del) reported in this article. The role of genetic changes in the US PEDV strains to clinical disease has yet to be reported. The clinical presentation of diarrhea in this case was reported as equally or more severe than such presentation in cases caused by the prototype PEDV Colorado/2013. Other factors such as concurrent infections and the rate of group exposure, which is rapid in most PEDV cases affecting neonatal piglets, may influence the clinical presentation.

Documenting PEDV variation is vital to understanding the natural evolution of the virus and possibly identifying portions of the genome associated with different clinical disease features. Animal studies are required to define the effects of these mutations on clinical disease, pathogenesis, immunity; these studies will be conducted in the future with the S2aa-del strain. A consistent model to properly evaluate these differences is required to control PEDV infection. The most compelling need is to understand how exposure by sows to different PEDV strains correlates with protection of piglets from clinical disease. Whether the PEDV S2aa-del strain will circulate in the North American swine population is not known.

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Schistosomiasis in Cattle in Corsica, France

To the Editor: The origin of the human cases of urinary schistosomiasis observed in France was recently identified (1,2). None of these patients had traveled to a disease-endemic area, but all had vacationed in Corsica and had swum in the Cavu River, near Porto-Vecchio in southern Corsica. The letter by Berry et al. to *Emerging Infectious Diseases* (2) reminded us that bovine schistosomiasis had been reported in Corsica in the past, up through the 1960s, in the same area.

In cattle, *Schistosoma bovis* has been found in Africa and the Middle East (Iraq, Israel), as well as in the Mediterranean Basin, especially in Sicily and Sardinia in Italy, and Corsica, France, where cases were reported as early as 1929 by Emile Brumpt (3,4). In addition, certain *Schistosoma* blood fluke species, especially *S. haematobium* and *S. bovis*, can share the same definitive hosts (humans or animals) and the same intermediate hosts, i.e., *Bulinus contortus* snails. Cattle, sheep and goats, horses, wild ruminants and rodents can all be definitive hosts of *S. bovis*.

In cattle, the clinical manifestations of infestation are poorly documented. In experimental animals, intermittent diarrhea has been observed, sometimes containing blood or mucus,

in addition to a loss of appetite, progressive anemia, and, especially, blood eosinophilia, a sign which, as in humans, indicates that the infestation is recent. Under natural conditions, the disease is believed to be mainly sub-clinical and chronic. It should be noted that the acute form of the disease is more common in sheep (5). With regard to lesions, the disease is closer to intestinal schistosomiasis (caused by *S. mansoni*) than to urinary schistosomiasis. The lesions are characterized by the formation of gray-white granulomas ≈ 5 mm in diameter, or by polyps, and intestinal hemorrhaging due to bleeding of the granulomas formed during migration of the parasite's eggs to the intestinal lumen. In the liver, granulomas may also be observed, as well as fibrosis of the portal vein. Hepatomegaly and cirrhosis may also be present. These lesions are caused by adult parasites in the mesenteric vessels and the portal vein.

The presence of *Bulinus truncatus contortus* (Michaud) (*Mollusca, Gastropoda, Hygrophila*) snails was mentioned as early as 1832, and the species was formally identified in 1922 in Corsica. Since that time it has been assumed that this mollusc could be a potential intermediate host for human (6) or bovine (3,7) schistosomiasis.

In 1963, Gretillat studied bovine schistosomiasis in Corsica (8). Investigations of *Bulinus* snails were conducted solely in the southern part of the island, in the area where Brumpt had described their presence 30 years earlier. *Bulinus* snails were identified in 4 rivers, the Rizzanese, Baraci, Ortole and Spartano, especially in residual ponds of waterways sometimes quite close to the sea. At 2 sites, unidentifiable cercaria larvae were revealed through dissection (5 of 70 *Bulinus* snails in the Rizzanese, 26 of 50 in the Baraci). As part of the same study, slaughterhouse examination of 15 cattle from regions where *Bulinus* snails had been discovered revealed adult *Schistosoma* in the mesenteric