Infectious dose of PEDV

Courtesy of Harsha Verma & Sagar Goyal, University of Minnesota

This week we would like to highlight some important work being done with cell-adapted PEDV which may prove helpful to researchers planning challenge or bioassay studies.

The infectious dose of field prototype PEDV has already been determined via bioassay of serial 10-fold dilutions of intestinal homogenate of an infected pig. These results were reported in SHMPs from 10/18/13 & 11/01/13 and are reproduced below in Table 1. For comparison, the infectious dose of cell culture adapted prototype PEDV was recently determined using the same bioassay method. In brief, serial 10-fold dilutions of cell culture adapted PEDV were prepared in PBS and were tested by rRT-PCR. These dilutions were then inoculated in 11-day-old piglets via gavage with a feeding tube. The piglets were monitored for 48 hours post-inoculation for the appearance of clinical signs of diarrhea, dehydration, body condition, and lethargy. At 48 hours post-inoculation, piglets were euthanized and sections of their jejunum were tested by PEDV rRT-PCR at the University of Minnesota. These results are shown in Table 2.

Table 1: Minimum infectious dose (MID50) of PEDV using mucosal scrapings from PEDV-infected pig as the inoculum.

Virus dilution	Initial Ct value of virus dilutions	Diarrhea score[a]	Ct value of mucosal samples of inoculated pigs
Undiluted	16.39	ND	ND
10^{-1}	17.73	ND	ND
10^{-2}	20.53	3	17.24
10^{-3}	23.55	3	16.92
10^{-4}	27.04	3	15.32
10^{-5}	29.94	2	17.10
10^{-6}	32.06 / 33.65	1	16.02 / 15.52
10^{-7}	35.60 / 37.83	2	15.70 / 15.52
10^{-8}	-	1	16.03
10^{-9}	-	0	30.29
10^{-10}	-	0	-
10^{-11}	-	0	-
10^{-12}	-	0	-

Gray shading = initial study after which further serial dilutions were performed to identify MID50
ND = not done.
[a] Diarrhea score 0 = pigs showing no clinical signs and presenting with normal body condition, demeanor, and well-formed solid feces; Score 1= soft poorly formed feces; score 2= presence of watery liquid feces; score 3= watery diarrhea and dehydration.

As we remember from the infectious dose study with field prototype PEDV, it is a highly infectious virus with a very low MID50 since clinical signs of PED were seen at 10-8 dilution (inoculum negative by RT-PCR) and significant replication was still seen without clinical signs at 10-9 dilution (inoculum negative by RT-PCR). This information was originally useful for producers and researchers alike as it highlights the importance of using multiple disinfection and biosecurity processes in order to be confident in the inactivation of all viral particles.

Table 2: Minimum infectious dose (MID50) of PEDV using cell culture adapted PEDV as inoculum.

Virus dilution	$TCID_{50}$/mL	Initial Ct value of virus dilutions	Diarrhea score[a]	Ct value in mucosal samples from inoculated piglets
Undiluted	6.8×10^4	17.69	ND	ND
10^{-1}	6.8×10^3	20.96	2	17.49
10^{-2}	6.8×10^2	24.23	2	17.63
10^{-3}	6.8×10^1	28.37	2	22.40
10^{-4}	6.8×10^0	30.89	1	27.09
10^{-5}	<6.8	-	0	34.92
10^{-6}	<0.68	-	0	-
10^{-7}	<0.068	-	0	-
10^{-8}	<0.0068	-	0	-
10^{-9}	<0.00068	-	0	-
10^{-10}	<0.000068	-	0	-

ND= not done.

These more recent results using cell culture adapted PEDV are informative as a comparison of infectivity to field strains as well as a resource for researchers performing challenge experiments and bioassays. Unlike the field strain, dilutions past approximately 30 Ct did not produce clinical signs in inoculated piglets. One ten-fold dilution from the last PEDV PCR positive sample (30.89 Ct) did still cause significant replication in the inoculated piglet without appearance of clinical signs. Also, it is interesting that in this pilot data the cell culture adapted virus seems to not replicate as quickly over 48 hours as we see a steady increase in Ct value of mucosal samples from piglets with increasingly weaker inoculations (10-3/28 Ct and on). This is compared to the field strain which caused consistent and extensive replication to between 15 and 17 Ct after inoculation with each dilution until the weakest inoculum from which replication was detected (10-9/negative). One reason could be that the virus is somehow protected by the organic material present in intestinal homogenate. Also, the strains of the virus used in these studies are different, and the cell culture adapted virus may have been innately of low virulence.