1 ABSTRACT (SYNOPSIS)

Title	A Point Prevalence Study to Evaluate the Prevalence of Antibodies to Selected Porcine Viruses in Patients with Cystic Fibrosis Who Are Receiving Porcine-Derived Pancreatic Enzyme Replacement Therapy: A Harmonized Protocol Across Sponsors
Keywords	Point prevalence, HEV, PPV and PCV2
Rationale and Background	Pancreatic enzyme replacement therapy (PERT) with porcine-derived pancreatic enzyme products is the cornerstone of nutritional management of pancreatic exocrine insufficiency due to cystic fibrosis (CF) or other conditions. The approval of currently marketed PERTs in the United States (US) requires sponsors to evaluate the risk of transmission of selected porcine viruses in patients taking PERT. Therefore, this study was conducted in the US to establish the point prevalence for HEV, PPV, and PCV2 antibodies in CF patients taking PERT compared to age- and geography-matched controls.
Research Question and Objectives	The primary objective of this study was to evaluate the seroprevalence of antibodies to selected porcine viruses in patients exposed to porcine-derived PERT compared with a matched control group of patients who have not been exposed to PERT. The target viruses were HEV, PPV and PCV2.
Study Design	This is a multicenter, non-interventional point prevalence study conducted in the US to determine the seroprevalence of antibodies to selected porcine viruses in PERT-exposed patients and in an unexposed control group matched for age and geographic region of residence. **Study Procedures** Patients who met all the inclusion criteria and none of the exclusion criteria for either the PERT-exposed or control patients were included in the study. After the patient or legal representative has signed the Informed Consent/Assent form, the study-related information was collected: demographic and medical history, PERT therapy, transfusion history, and history of potential exposure to pig viruses. In addition, a single 4.0-mL blood sample was obtained as part of a planned blood collection as standard of care and processed for serum. Serum (1.5 mL) was divided into 3 aliquots of equal volume, frozen within 2 hours of blood collection, and subsequently stored frozen at -70°C or below until assay for antibodies to the selected porcine viruses. The aliquots were analyzed at a central laboratory with validated assays to determine the presence of antibodies to the HEV, PPV, and PCV2. Patients and / or parents of the patients were requested to fill out the 'Porcine Virus Risk Questionnaire' on paper and give it back to the physician or site personnel. The study period was defined as the period from the signed, written informed consent until 24 hours after the blood sample collection. There was no follow-up contact with participating patients as part of this study. Study participation did not in any way influence the patient's course of treatment as determined by their physician.
Setting	All efforts were made to enroll predominantly CF sites that could recruit an adequate number of PERT-exposed patients and matched unexposed control

	patients. Non-CF sites within the proximity of the CF recruiting sites were added to recruit control patients. A total of 50 sites participated in the study throughout the US.
Patient selection	**Inclusion criteria:** Each potential study patient had to satisfy all of the following criteria to be enrolled in the study: 1. Be male or female aged 2 years or older; 2. Had a blood draw planned as part of their standard of care following enrollment into the study, documented with the signing of the informed consent document; and 3. Signed (or their legal representative(s) had signed) an informed consent form indicating that they understood the purpose of the study and were willing to participate in the study. Assent was also required of children capable of understanding the nature of the study (typically 7 years of age and older). *Patients in the PERT-Exposed Group had to meet the following criteria:* 1. Had been diagnosed with CF; and 2. Had received PERT for a minimum total duration of 6 months (at any point in the patient's medical history). *Patients in the PERT-Unexposed Group had to meet the following criteria:* 1. Be under medical management for chronic disease; 2. Never received any PERT product; 3. Matched an enrolled PERT-exposed patient based on age and region-of-residence; and 4. Had a blood draw planned to be performed within 180 days of the matched PERT-exposed patient blood draw. **Exclusion criteria:** Any potential patient (PERT-exposed and unexposed group) who met the following criteria was excluded from participating in the study: 1. Has a porcine heart valve, a porcine-derived graft, or had been exposed to porcine-derived insulin. This exclusion did not apply to previous porcine-derived heparin exposure. 2. Refused blood collection; or 3. Had any condition that, in the opinion of the investigator, would make participation not be in the best interest (e.g., compromise the well-being) of the patient or that could prevent, limit, or confound the protocol-specified assessments.

Study size (including dropouts)	Overall, 1,266 patients were enrolled in the study; 753 PERT-exposed and 513 PERT-unexposed patients. All PERT-unexposed patients completed the study, while 10 PERT-exposed patients (1.3%) did not: investigator was unable to obtain a blood sample from 4 patients, 1 patient did not return for blood sample and 5 patients had reasons other than those pre-listed. Overall, 1,020/1,266 patients (80.6%) were included in the Full Analysis Set (FAS); 510/753 PERT-exposed patients (67.7%) and 510/513 PERT-unexposed patients (99.4%).
Variables and Data Sources	**Clinical Data** The following information was collected: informed consent/assent prior to the initiation of any study-related procedures, inclusion and exclusion criteria validation, matching of PERT-exposed patients with PERT-unexposed patients, demographic data, medical history, PERT history (for PERT-exposed patients), CF history, current and prior other porcine-derived enzyme supplements, and specific data on blood sample collection and storage No study-related, product-related or procedure-related adverse events were to be collected. **Patient Questionnaire** The Porcine Virus Risk Questionnaire is a 31-item questionnaire including questions on the patients' diet and exposure to pigs and other animals to be completed on paper. For patients 18 years and older, the questionnaire was completed by the patients; for patients under 15 years of age, the questionnaire was completed by the parents on behalf of the patient; and for patients 15-17 years of age, parents completed questions 1-4 of the questionnaire on behalf of the patient and patients completed the remaining questions. The questionnaire collected information on occupational and recreational exposure to animals, travel to Africa, Latin America, or East Asia for greater than one week, diet, frequency of eating poultry, pork, eggs, beef, wild boar, other game, fish/shellfish, and organ meat, drinking water from a spring river or private well, vegetarian or vegan diet and duration of diet, use of injectable drugs, frequency of dietary supplements containing organ extracts such as pancreas, liver, and brain. **Serology of target viruses** A single blood sample was obtained and separated into 3 equal aliquots of serum to be frozen and stored to test the presence of anti-HEV, anti-PPV and anti-PCV2 antibodies. Screening assays were performed with results reported as either 'positive' or 'negative' for each antibody type (anti-HEV, anti-PPV and anti-PCV2).
Results	**Patient Characteristics** The mean (SD) age of patients included in the FAS population was 17.9 years (12.79) and there were 49.6% females. Most of patients were white (83.6%) and 7.8% were black or African American. They were equally distributed across the U.S. territory with patients enrolled in most of the U.S. states;

	patients were most frequently included (>5%) in the following states: Illinois (10.6%), New York (9.8%), Pennsylvania (5.9%), Texas (5.9%), Massachusetts (5.4%), Michigan (5.4%), Florida (5.3%), and California (5.1%). Approximately three quarters of patients were living in urban neighborhoods (77.8%). The median (Q1-Q3) weight of PERT-exposed patients was 46.2 kg (27.2-58.3). As expected because of matching, PERT-exposed and PERT-unexposed groups were similar in terms of age and geographic region of residence. Both groups were also similar in gender distribution. There were fewer white patients in the PERT-unexposed group compared with the PERT-exposed group (73.3% vs. 93.9%) and more black or African American patients (13.7% vs. 2.0%). Overall, 2.9% (95% CI: 1.9% - 4.0%) of patients were HEV-positive with 4.3% (95% CI: 2.6% - 6.1%) in the PERT-exposed group and 1.6% (95% CI: 0.5% - 2.6%) in the PERT-unexposed group. The percentage of HEV-positive patients increased across age categories. None of the patients aged ≥2 to <5 years were HEV-positive. Among those aged ≥5 to <12 years, 2.5% in the PERT-exposed group were HEV-positive compared with 0.8% in the PERT-unexposed group. In patients aged ≥12 to <18 years 3.8% in the PERT-exposed group were HEV-positive compared with 1.3% in the PERT-unexposed group. Among the patients aged ≥18 years, 6.9% in PERT-exposed group were HEV-positive compared with 2.8% in the PERT-unexposed. None of the 508 matched pairs, i.e. 1016 patients, with valid PPV test results were found to be PPV positive. Valid results for PCV2 antibody status were available for 509 matched pairs, i.e. 1018 patients. Overall, the percentage of patients who were seropositive for PCV2 was 1.4% (95% CI: 0.7% - 2.1%), with 1.6% (95% CI: 0.5% - 2.7%) and 1.2% (95% CI: 0.2% - 2.1%) in the PERT-exposed and PERT-unexposed groups, respectively. None of the patients aged ≥2 to <5 years were PCV2-positive. In those aged ≥5 to <12 years 1.7% and 3.2% patients in the PERT-exposed and PERT-unexposed groups, respectively, were PCV2-positive. In those aged ≥12 to <18 years, 2.6% and 1.3%, respectively, were PCV2-positive. In the patients aged ≥18 years, 1.1% of patients in the PERT-exposed group were PCV2-positive compared with none in the PERT-unexposed group. **Conditional Logistic Regression** The seroprevalence of HEV, PPV and PCV2 was much lower than anticipated when study was designed, i.e. HEV seroprevalence was 2.9% (n=30) in the study compared with the anticipated prevalence of 10%. As such, no confounders, except age, were identified by the unblinded team, who examined the distribution of the potential confounders and HEV seroprevalence in the PERT-exposed group before unblinding. After unblinding, Directed Acyclic Graph (DAG) analysis was performed based on published epidemiological and clinical evidence to identify potential confounders for HEV status and PERT exposure, and tables for the propensity score analyses were generated. The potential confounders identified in the DAG analysis were age (years), regions by continental US, urban vs. rural, sex, race, ethnicity, and use of injectable drug (yes, no). No DAG analyses were performed for PCV2 or PPV since only 14 and 0 seropositive subjects were identified, respectively.

	The planned propensity score analysis and conditional logistic regression were performed to assess the effect of PERT exposure on HEV serostatus. HEV status was regressed on PERT exposure and a categorical representation of propensity scores, based on the quintiles of the distribution. The propensity scores were calculated using logistic regression, for the confounders identified in the DAG analysis. Based on the graphical examination of propensity scores in the PERT-exposed and PERT-unexposed groups it was considered that good common support had been achieved and trimming was not done for the propensity score analyses, taking into consideration the low HEV seroprevalence. Final conditional regression model controlling for matched pairs, fitted with quintiles of the propensity score (5 level) and the treatment term without additional adjustment for individual confounders, were done, to avoid over-parameterization, even if there was a confounding variable for which the balance between the treatment groups, adjusted by propensity score analysis, was not reached at the pre-specified statistical level of 5%. Results from the final conditional logistic regression model showed the odds ratio for HEV positive serostatus for the PERT-exposed vs. PERT-unexposed patients was 3.22 (95% CI: 1.19 – 8.70), p=0.0215. In the sensitivity analysis, without adjustment for any confounders or propensity score in the conditional logistic regression model, the odds ratio for HEV positive serostatus for the PERT-exposed vs. PERT-unexposed patients was 3.33 (95% CI: 1.34 – 8.30), p=0.0097, controlling for matched pairs only. Due to low prevalence of PCV2 positive serostatus, no confounders were identified, and no propensity score analyses were done. Without adjustment for any confounders or propensity score, preplanned conditional logistic regression controlling for matched pairs only was performed to assess the effect of PERT exposure on PCV2 serostatus. Results showed that the odds ratio for PCV2 positive serostatus for the PERT-exposed vs. PERT-unexposed patients was 1.33 (95% CI: 0.46 – 3.84), p=0.5943. No analyses for PPV serostatus were performed because since there were no positive anti-PPV antibody assay results. **Logistic Regression – PERT-Exposed Group Only** In the logistic regression analyses for the PERT-exposed patients, only age was statistically significantly associated with HEV positive serostatus: odds ratio (95% CI), 1.06 (1.03 – 1.09), p <0.0001 for each 1-year increase in age. In the logistic regression analyses for the PERT-exposed patients, none of the variables were significantly associated with PCV2 positive serostatus.
Discussion	In this cross-sectional study of matched PERT-exposed (N=510) and PERT-unexposed (N=510) patients, 2.9% (95% CI: 1.9% - 4.0%) of patients were HEV-positive; [4.3% (95% CI: 2.6% - 6.1%) in the PERT-exposed group and 1.6% (95% CI: 0.5% - 2.6%) in the PERT-unexposed group. Age was the only variable associated with HEV positive serostatus [(odds ratio (95% CI), 1.06 (1.03 – 1.09), p <0.0001]. Without adjustment for the propensity score, the odds ratio (95% CI) for HEV positive serostatus for the PERT-exposed vs. PERT-unexposed patients was 3.33 (1.34 – 8.30), p=0.0097, controlling for matched pairs only. After adjustment for confounders identified through DAG analysis (as a propensity score) the odds ratio (95% CI) for HEV seropositivity

	in the PERT-exposed vs. PERT-unexposed patients was 3.22 (1.19 – 8.70), p=0.0215. The overall seroprevalence of PCV2 was 1.4% (95% CI: 0.7% - 2.1%); [1.6% (95% CI: 0.5% - 2.7%) in the PERT-exposed and 1.2% (95% CI: 0.2% - 2.1%) in the PERT-unexposed group]. The odds ratio (95% CI) for PCV2 positive serostatus for the PERT-exposed vs. PERT-unexposed patients was 1.33 (0.46 – 3.84), p=0.5943, controlling for matched pairs only. No confounders were identified and no propensity score analyses were done. Finally, none of the patients were seropositive for PPV. The HEV IgG seroprevalence of 1.6% among individuals in the unexposed population in the VTPP study is, therefore, consistent with the seroprevalence estimated among those 6-29 years old in the 2009-2010 National Health and Nutrition Evaluation Survey population. The prevalence of HEV seropositivity in the PERT exposed group is far lower than the overall prevalence of HEV seropositivity in the US (6%-16%) and in Europe, where anti-HEV IgG seroprevalence is even higher. A recent meta-analysis of 26 studies including 63,828 individuals reported an average seroprevalence of 19% (95%CI: 14%–25%), which may be as high as 52% in some regions. The clinical risks associated with isolated seroprevalence of anti-HEV IgG, a marker of past exposure, are not well known, particularly in places where the incidence of acute hepatitis E infection is rare, as few cases of locally-acquired acute HEV infection have been documented in the US. Notably, no case reports suggesting viral transmission (HEV, PPV and PCV2) coincident with use of PERT products were retrieved from the Global Safety Database search within each sponsor. Therefore, the overall risk of HEV seropositivity in patients exposed to PERT remains low. The seroprevalence of PCV2 was low and not different between PERT exposed and unexposed patients. This was consistent with results from several serological studies that failed to detect serological evidence of PCV2 infection in humans. In a recent study among 40 patients with CF, all of whom used PERTs, negative PCV2 serological results further supports a low risk of PCV2 infection in relation to the long-term ingestion of potentially contaminated PERTs and other porcine supplements. Using previously published data, it had been assumed that the overall HEV seroprevalence rate would be about 10% and was age-dependent. However, in the study population, the overall observed seroprevalence of 2.9% was much lower, with 4.3% in the PERT-exposed group and 1.6% in the PERT-unexposed group. This lower prevalence, with only 30 HEV seropositive individuals, limited our ability to perform logistic regression analyses to identify potential risk factors as this analysis is sensitive to low prevalence rates. Other limitations may include the potential misspecification of the model forms for the propensity score analysis and primary analysis with no adjustment for the imbalance of one covariate (race) between PERT-exposed vs PERT-unexposed patients when using a logistic regression due to low prevalence of HEV seropositivity. The variables recorded as part of the study may not have been comprehensive (for example, consuming specific items or global travel), and, thus the estimates/outcomes may be subject to unmeasured confounding. Potential significant recall bias (or more specifically

	failure to recall) for dietary information, such as frequency of eating undercooked meat (or perhaps not even realizing the meat was undercooked), or shellfish. It is also possible that participants did not know if the heparin or nutritional supplements they were taking were porcine-derived, or taken from organ extracts (pancreas, liver and brain). Another limitation of this study is that the study design did not allow for comparison of HEV, PPV, or PCV2 seroprevalence in patients using different PERT products or different dosages of any given PERT.
Conclusion	This study was conducted to evaluate the seroprevalence of antibodies to selected porcine viruses in patients exposed to porcine-derived PERT compared with a matched control group of patients who had not been exposed to PERT. The target viruses included were HEV, PPV and PCV2. The study found a very low overall seroprevalence of HEV compared with that reported for the general population. The seroprevalence of PCV2 was extremely low, and no patients were seropositive for PPV. Although a small imbalance in HEV seroprevalence was observed between PERT exposed and PERT-unexposed patients, the long-term clinical implications of these findings are expected to be minimal. In addition, a vast, long-standing collective safety database across multiple PERT products supports the fact that no risk to patient safety associated with seroconversion has been reported over decades of use. Based on the important clinical benefit that PERT products provide to patients, the low seroprevalence of selected porcine viruses identified in this study, and the lack of a clinically identifiable safety concerns, the Joint Sponsors support the continued favorable benefit-risk profile of PERT products.
Marketing Authorization Holder(s)	AbbVie Inc. Allergan Sales LLC Vivus, Inc (since 8 June 2018)