CONTROL ID: 2022102

TITLE: Rapid Isothermal Detection of *Porcine Epidemic Diarrhea Virus* (PEDV) RNA

ABSTRACT BODY:

Narrative: Recent emergence of *porcine epidemic diarrhea virus* (PEDV) in the United States has caused significant economic concern to the swine industry. Since its first detection in April 2013, this virus has rapidly spread across the United States. While many diagnostic tests are available for PEDV detection, they cannot be used at penside, highlighting the need of such a rapid diagnostic test. This project is intended to address the need for a sensitive, specific, affordable, and fast solution for penside detection of PEDV. As a first step in providing a penside test for PEDV, we have developed an isothermal molecular detection assay. This test is based on reverse transcription loop-mediated isothermal amplification (RT-LAMP) using OmniAmp DNA polymerase. Detection is based on signal generation by a fluorescent intercalating dye that binds to the double stranded RT-LAMP DNA product. This chemistry is intended to be used eventually with a low-cost, portable, and easy to operate instrument, which is being developed concurrently to facilitate penside detection of PEDV and other agricultural pathogens. LAMP primers were designed to target nucleocapsid (N) gene of PEDV and reaction conditions were optimized for specific detection of PEDV. The time to result was under 30 minutes with no false positives in the negative control. Analytical sensitivity (50 copies of RNA/µl) of the assay was found to be comparable to real time RT-PCR. Analytical specificity of LAMP assay was evaluated using nucleic acid extracts from other swine pathogens such as TGEV, PCV-2, PRRSV, and SIV that are commonly found in swine production systems. We also developed a simple heat lysis protocol without the use of any expensive equipment as a method of sample preparation for low resource settings. This sample preparation method and RT-LAMP assay were used to test a cohort of residual clinical samples previously tested positive by RT-PCR, resulting in clinical sensitivity of 80% and 100% specificity. The combination of performance, time to result, ease of operation and interpretation, low cost and compatibility with less complex instrumentation suggest that this test platform can be used in testing laboratories or penside along with other diagnostic assays to provide timely results to swine producers.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021264

TITLE: Development and Evaluation of a Colloidal Gold Particles-Based Immunochromatographic Strip for Specific Detection of *Porcine Epidemic Diarrhea Virus*

ABSTRACT BODY:

Narrative: *Porcine epidemic diarrhea virus* (PEDV), a member of the *Coronaviridae* family, causes acute diarrhea and dehydration with significant morbidity and mortality in pigs. The economic impact of the PEDV has substantially increased, particularly in Korea, China, Japan, and the United States. It is difficult to diagnose PEDV simply by clinical signs and histopathological lesions. Although many techniques can be used for the detection of PEDV including enzyme-linked immunosorbent assays (ELISA), immunofluorescence (IF) tests and RT-PCR, they are time consuming and must be tested in laboratory.
In the present study, a rapid immunochromatographic (ICG) strip based on a conjugate of colloidal gold and monoclonal antibody (mAb) was developed for the rapid, sensitive

detection of PEDV in samples from the field. The two specific monoclonal antibodies (mAbs) against PEDV were produced and used as the capture and the detection mAbs, respectively. The detection limit of the ICG strip for PEDV is 105 TCID50/ml, and the assay can be completed in 10 minutes.
A cross-reactivity test indicated that the ICG strip was highly specific to PEDV showing no cross-reactivity with *porcine transmissible gastroenteritis coronavirus* (TGEV), *rotavirus* (RV), *porcine reproductive and respiratory syndrome virus* (PRRSV), *classic swine fever virus* (CSFV), and *porcine pseudorabies virus* (PRV) The results of the recovery test from the fecal samples in the field were in good agreement with those obtained by ELISA. The correlation between the two methods was K=0.93, 95% and CI: 0.90-1.00 when testing the fecal samples. Accordingly, the use of ICG technology provided an efficient, effective, and rapid means of detecting the presence of coronavirus PED antigen in field samples and indicates that it is a very useful tool for diagnosis in the field.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS: Trainee Travel Award

Trainee Letter: Stating role in work_REVISED.pdf

CONTROL ID: 2016455

TITLE: Development and Evaluation of a Duplex Real-Time RT-PCR for Detection and Differentiation of Classical and Variant Strains of *Porcine Epidemic Diarrhea Viruses* from the United States

ABSTRACT BODY:

Narrative: *Porcine epidemic diarrhea virus* (PEDV) was identified in May of 2013 in the United States (US). Since then, PEDV has been detected in 29 US states, causing significant economic losses to US swine industry. A new variant strain of PEDV was identified in late January 2014. Compared with classical PEDV, the variant PEDV strain contains three deletions, one insertion, and several nucleotide variations in first 1170 nt of the S1 domain in the spike gene. Variant PEDV has been detected in samples from multiple states by our laboratory as well as other laboratories in the US. It is critical to detect and differentiate variant PEDV from the classical PEDV during outbreaks to enhance control and prevent PED associated disease. Here, we report the development and validation of a duplex real-time RT-PCR assay for detection and differentiation of the variant and the classical strains of PEDV. The primers and probes were designed by targeting the conserved and deletion regions of the first 1170 nt of S1 region, respectively. The developed duplex real-time RT PCR has a high sensitivity (1 genome copy detection limit for both variant and classical PEDV) and specificity (no cross reaction with other porcine viruses). In addition, the positive field samples detected by this new assay were further confirmed by subsequent DNA sequencing. The duplex real-time RT-PCR offers a rapid and sensitive method to detect both classical and variant PEDV from clinical samples, and will allow differentiation of variant strains from classical PEDV strains.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS: AAVLD Laboratory Staff Travel Award

Trainee Letter: Letter-Role and Significance.pdf

CONTROL ID: 2022087

TITLE: Real-Time RT-PCR comparison to Ensure Accurate Detection of PEDV and TGEV

ABSTRACT BODY:

Narrative: *Porcine epidemic diarrhea virus* (PEDV) is major cause of severe diarrhea and dehydration in pigs. Belonging to the *Coronaviridae* family, PEDV is an enveloped, positive-sense, single-stranded RNA virus with a genome size of approximately 28kb. The first detection of PEDV was reported in 1971 from England while Japan, China, South Korea, and Thailand also have reported PEDV infections. The United States first detected PEDV in May 2013. The veterinary diagnostic laboratories quickly developed sensitive and specific real time RT-PCR (RRT-PCR) assays to detect PEDV in a variety of porcine and environmental samples. In this study, we compared the PEDV-TGEV multiplex RRT-PCR assay developed at the University of Minnesota (UMN) to a commercial TGEV-PEDV multiplex RRT-PCR assay.

Porcine intestinal samples, fecal samples, fecal swabs, oral fluid samples, and environmental samples are routinely submitted to UMN Veterinary Diagnostic Laboratory for enteric pathogen testing. Sample homogenates were extracted with the MagMax 96 Viral RNA Isolation Kit (Thermo Scientific), according to manufacturer's instructions. The commercial TGEV-PEDV multiplex RRT-PCR assay was preformed, according to manufacturer's instructions, while the UM RRT-PCR assay utilized the Path-ID Multiplex One-Step RT-PCR kit (Thermo Scientific, according to manufacture's instructions.

A total of 396 samples, consisting of porcine oral fluids (n=39), intestinal homogenates (n=107), fecal (n=136), fecal swabs (n=47), feedback (n=12) and environmental samples (n=55), were compared with the UMN TGEV-PEDV multiplex RRT-PCR and the commercial TGEV-PEDV multiplex RT-PCR assays. The UMN TGEV-PEDV multiplex RRT-PCR assay had lower Ct values compared to the commercial TGEV-PEDV multiplex RRT-PCR assay. The UMN TGEV-PEDV multiplex RRT-PCR assay detected 53 more positive PEDV samples (oral fluids (n=6), intestinal homogenates (n=9), fecal (n=6), fecal swabs (n=13), and environmental samples (n=19)) compared to the commercial TGEV-PEDV multiplex assay. The additional positive PEDV samples as indicated by the UMN TGEV-PEDV multiplex assay, but negative by the commercial TGEV-PEDV multiplex assay, were confirmed positive by a secondary UMN PEDV RRT-PCR assay, which targeted the N gene. The UMN TGEV-PEDV multiplex RRT-PCR assay detected 11 more positive TGEV samples (intestinal (n=4) and fecal (n=7)) compared to the commercial TGEV-PEDV multiplex RRT-PCR.

The UMN TGEV-PEDV RRT-PCR assay had superior performance over the commercial TGEV-PEDV multiplex RRT-PCR assay. Accurate detection of PEDV and TGEV in clinical samples is important to minimize the spread of these two viruses. The role of the clinical diagnostic laboratories is to provide high sensitivity and specificity assay to help prevent and control pathogens and many assays must be evaluated before choosing the best assay to support the swine industry.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2022640

TITLE: Kinetics of the *Porcine Epidemic Diarrhea Virus* (PEDV) Humoral Immune Response

(IgM, IgA, and IgG) in Serum and Oral Fluid Specimens from Pigs Infected Under Experimental Conditions

ABSTRACT BODY:
Narrative: Since first isolation in April 2013, *porcine epidemic diarrhea virus* (PEDV) has becomes more endemic in U.S. herds causing tremendous economic losses to the swine industry. The key to preventing the spread of PEDV is to control the movement of animals and fomites contaminated with the virus combined with surveillance. Therefore, serology is a vital tool used to control and to further understand this disease. Anti-PEDV antibodies have been detected in sera from swine with naturally occurring or experimentally induced PED by indirect ELISA2, IFA test3, and serum-virus neutralization test. However, the kinetics of the antibody response against PEDV has not been described to date.
We described the ontogeny of the whole virus-specific IgM, IgA, and IgG responses in serum and oral fluid from pigs experimentally inoculated with a 2013 U.S. PEDV isolate.
Fifty-six 3-week old pigs were inoculated with a PEDV isolate (USA/Iowa/18984/2013) via gastric gavage. Serum samples were collected on day 0 post inoculation (DPI) and every 7 days thereafter for 56 DPI, while pen-based oral fluid were collected daily for the first week, and twice a week thereafter for 56 DPI using methods previously described4.
The same PEDV isolate was propagated in Vero cells, pelleted, processed and used as whole virus antigen for ELISA plate preparation. ELISA conditions such as coating conditions, reagent concentrations, incubation time and buffer compositions were optimized for simultaneous detection of anti-PEDV IgM, IgA, and IgM antibodies in serum and oral fluid specimens.
Anti-PEDV IgM, IgA, and IgG responses over time in serum and oral fluid after experimental inoculation showed that, in a short-term, low-level IgM response was detected first (between 7 to 10 DPI) which was followed by a strong IgA and a moderate IgG response. Both IgA and IgG antibodies started to gradually decline after 4 weeks post inoculation.
In this study, we addressed the significance of whole virus-based IgM, IgA, and IgG responses at different stages of post-infection. In conclusion, whole virus-based IgM, IgA, and IgG responses were detectable in both serum and oral fluid over time post inoculation with a strong IgA response and a moderate IgG response that could correlate with immune protection against PEDV infection.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021181

TITLE: Disease Investigation and Virus Isolation of *Porcine Deltacoronavirus*

ABSTRACT BODY:
Narrative: Recently viruses closely related to deltacoronaviruses in China have been identified in multiple locations in the United States by PCR and sequence analysis. In early March, a breed to wean multiplier facility located in Illinois with approximately 2500 sows and 4000 suckling pigs started to experience inappetence and diarrhea in the gestating sows. Diarrhea and high mortality was subsequently observed in nursing piglets. An investigation was initiated to identify the source of the deltacoronavirus introduction. Samples were collected and submitted to the National Veterinary Services Laboratories and the University of Minnesota for diagnostic testing. Intestinal samples were found to be positive by PCR for *porcine deltacoronavirus* (PDCoV) and *rotavirus C*. PDCoV virus was isolated from one of

the intestinal samples. Whole genome sequencing was performed on the isolate and was 99% identical to PDCoV HKU15 strain IN2847 from Ohio and PDCoV 8734/USA-IA/2014.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2018969

TITLE: Detection and Characterization of a *Porcine Deltacoronavirus* from Pigs with Diarrhea

ABSTRACT BODY:

Narrative: During the late January and early February in 2014, a diarrheal disease occurred in several pig farms in Ohio. The clinical signs were similar to those caused by *porcine epidemic diarrhea virus* (PEDV), including watery diarrhea in sows and death in piglets. However, the mortality in piglets was lower (30%–50%) than that typically observed with PEDV infection. Fecal and intestinal samples from one farm were negative for PEDV, *transmissible gastroenteritis virus*, rotaviruses, and *Salmonella*. Examination of the samples by electron microscopy showed *coronavirus*-like virus particles. However, all samples were negative by a pan-*coronavirus* PCR. A *deltacoronavirus* was detected in all samples. Histological lesions were moderate comparing with that caused by PEDV currently circulating in the US. The virus is closely related (99% identity) to the *porcine coronavirus* HKU15 reported in Hong Kong in 2012. This is the first time that the virus has been detected in diarrheal disease. To date, this virus has been detected in at least 10 states in US.

CURRENT CATEGORY/DISCIPLINE: Virology

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AWARDS:

Trainee Letter:

CONTROL ID: 2021051

TITLE: Characterization of Genotypically Distinct Enteric and Respiratory *Bovine Coronaviruses*

ABSTRACT BODY:

Narrative: *Bovine Coronavirus* (BCoV) causes enteric and respiratory diseases in cattle and is a component of the bovine respiratory disease complex (BRD). The Kansas State Veterinary Diagnostic Laboratory (KSVDL) routinely performs quantitative PCR surveillance on the respiratory viruses associated with BRD. Surveillance over a 3 year period demonstrated that 13% of 1,135 tissue samples and 29% of the 776 swab samples submitted were positive for BCoV. BCoV was the sole pathogen detected in 34% of tissue samples and 56% of swab samples. The spike gene on a subset of positive samples was sequenced.

The Spike glycoprotein is believed to be responsible for host range and is the leading mediator of viral entry. Along with the data provided by KSVDL, genomic information of the spike gene was compared with data publicly available in Genbank. Looking at the phylogenic tree, the spike genomes fell into two distinct clades; Clad one being the vaccine strains and Clade two being the current respiratory disease. The virus isolates from KSVDL were primarily respiratory samples but there was one enteric sample and all samples align in Clade 2 in sub Clade B. The enteric also fell in this range along with the respiratory samples. Future work will investigate the antigenic relationship between enteric and respiratory viruses however our results suggest that BCoV is one of the major contributors to BRD. Further studies of complete BCoV genomes are needed to elucidate the genetic basis for host tropism and the underlying mechanisms of pathogenesis as well as provide information to assess the serological differences between the respiratory and enteric viruses.

CURRENT CATEGORY/DISCIPLINE: Virology

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