Rotavirus C: prevalence in suckling piglets and development of virus-like particles to assess the influence of maternal immunity on the disease development

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ABSTRACT

Rotavirus C (RVC) has been detected increasingly in humans and swine in different countries, including the US. It is associated with significant economic losses due to diarrheal disease in nursing piglets. In this study we aimed: (1) to determine the prevalence of RVC in healthy and diarrheic suckling piglets on US farms; and (2) to evaluate if maternal antibody (Ab) levels were associated with protection of newborn suckling piglets against RVC. There was a significantly higher prevalence ($p = 0.0002$) of litters with diarrhea born to gilts compared with those born to multiparous sows. Of 113 nursing piglet fecal samples tested, 76.1% were RVC RNA positive. Fecal RVC RNA was detected in significantly ($p = 0.0419$) higher quantities and more frequently in piglets with diarrhea compared with healthy ones (82.5 vs. 69.9%). With the exception of the historic strain Cowden (G1 genotype), field RVC strains do not replicate in cell culture, which is a major impediment for studying RVC pathogenesis and immunity. To circumvent this, we generated RVC virus-like particles (VLPs) for Cowden (G1), RV0104 (G3) and RV0143 (G6) and used them as antigens in ELISA to detect swine RVC Abs in serum and milk from the sows. Using RVC-VLP Ab ELISA we demonstrated that sows with diarrheic litters had significantly lower RVC IgA and IgG Ab titers in milk compared to those with healthy litters. Thus, our data suggest that insufficient lactogenic protection provided by gilts plays a key role in the development of and the increased prevalence of clinical RVC disease.

INTRODUCTION

- Rotavirus C (RVC) is an important cause of gastroenteritis in neonatal piglets. Increased porcine RVC prevalence has been reported recently in the US and globally
- Recent RVC outbreaks in swine farms across the US have caused significant economic losses due to weigh loss, sluggish growth and even death of neonatal piglets
- Currently there is no RVC vaccine or diagnostic tools for serosurveillance

OBJECTIVES

- To screen healthy and diarrheic suckling piglets for RVC using RT-qPCR
- To develop genotype specific RVC VLP-based ELISA that can detect swine RVC antibodies to study RVC lactogenic immunity

MATERIAL AND METHODS

RVC Screening

Fig. 1 Flowchart of sample collection and RT-qPCR to determine RVC RNA prevalence in piglets

Development of RVC VLP-based ELISA

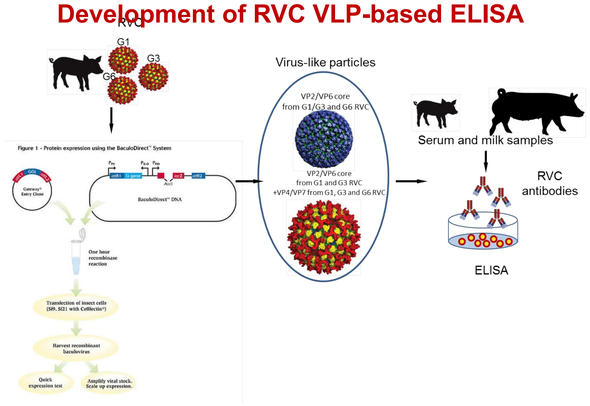

Fig. 2 Flowchart of RVC VLP Ab ELISA development to be used in screening Abs in swine field samples

RESULTS

RVC prevalence and RNA shedding titers

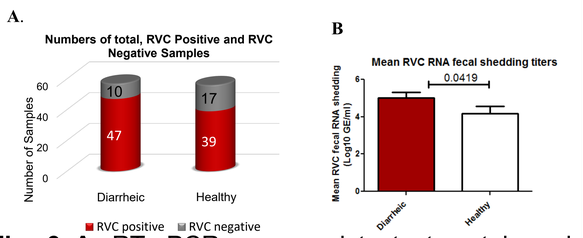

Fig. 3 A. RT-qPCR was used to test rectal swab samples obtained from diarrheic and healthy piglets for RVC RNA with overall RVC prevalence of **76.1%** (scours = 82.5%, healthy = 69.9%).**B.** CT values from RT-PCR were converted to log10 GE/ml using a standard curve. Diarrheic piglets had significantly higher ($p=0.0419$) RVC RNA fecal shedding titers when compared with healthy piglets

Scour litters vs. parity correlation

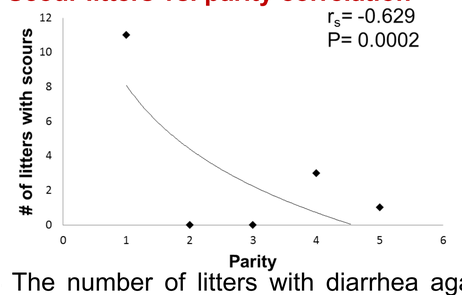

Fig. 4 The number of litters with diarrhea against maternal parity and correlation determined using the spearman rank test (r_s = -0.629). Gilts (1st parity) were significantly (P=0.0002) associated with having diarrheic piglets

Amplification of RVC structural genes

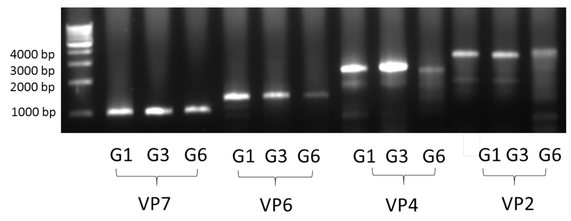

Fig. 5 Amplification of porcine RVC G1, G3 and G6 structural genes; VP7, VP6, VP4 and VP2 using specific primers. Amplicons were analyzed using 1% agarose gel. Expected sizes were 1054 bp for VP7, 1293 bp for VP6, 2432 bp for VP4 and 2724 bp for VP2 structural genes

VLP expression and antigenicity of RVC VLP-based ELISA

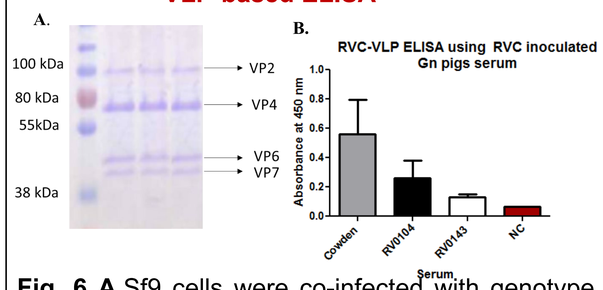

Fig. 6 A. Sf9 cells were co-infected with genotype specific (G1, G3 and G6) combinations recombinant baculovirus expressing RVC core and outer capsid protein genes. Analysis of semi-purified VLPs was done using a 12% precast protein gel. **B.** Antigenicity of VLPs using serum from Gn piglets inoculated with G1, G3 or G6 porcine RVC strains in a VLP-based indirect Ab ELISA

RVC Ab titers in field samples

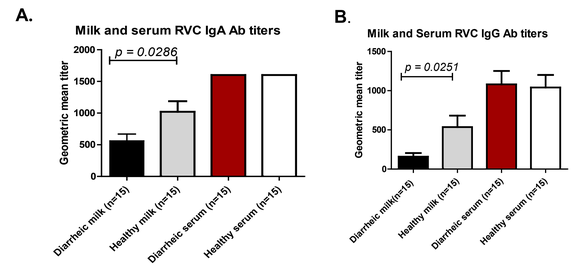

Fig.7 A. Milk and serum from sows/gilts with diarrheic or healthy piglets were tested for RVC IgA and IgG Ab titers **B.** Comparative analysis of milk RVC IgA and IgG Ab titers in sows vs. gilts.

CONCLUSIONS

1. Higher prevalence of diarrhea significantly negatively correlated with parity number

2. Insufficient lactogenic protection provided by gilts may play the key role in the development of and increased prevalence of clinical RVC disease

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