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# Identification of Pulmonary Infections with Porcine Rotavirus A in Conventional Pigs with Respiratory Diseases

April Nelsen, Ben Hause, and Chun-Ming Lin

Animal Disease Research and Diagnostic Laboratory, South Dakota State University, Brookings, SD 57007, USA

## INTRODUCTION

- Rotavirus (RV) is a significant global cause of acute enteritis in young children and animals, including neonatal and nursing piglets
- RVs infect mature enterocytes in the small intestine leading to disruption of the intestinal mucosal barrier and development of diarrhea
- While RV principally replicates in intestinal epithelial cells, several studies have detected RV in serum and multiple visceral organs, suggesting viremia
- Rotaviruses have been detected in nasopharyngeal secretions in infants with respiratory illness, as well as neonatal piglets in experimental settings
- In addition to enteritis, piglets inoculated with porcine RVA subtypes G9P[23] and G9P[7] also displayed interstitial pneumonia
- Knowledge regarding respiratory infection of RVs is still limited

## OBJECTIVES

- Determine the prevalence of RV in lungs obtained from pigs with respiratory disease
- Characterize RV lung tissue distribution and its possible role in porcine respiratory disease complex (PRDC) pathogenesis.

## EXPERIMENTAL DESIGN

Approach 1:

1. qRT-PCR on frozen lung tissue homogenates
2. Histopathology on FFPE of RVA qRT-PCR positive lungs
3. TEM on respiratory epithelial cells positive for RVA by histopathology

Approach 2:

1. Histopathology on TMA of piglets with rotaviral enteritis
2. Histopathology on TMA of randomly selected PRDC-affected cases

## RESULTS

### RVA PREVALENCE IN PIG LUNG

- 28 out of 91 (30.8%) frozen lung tissue homogenates qRT-PCR positive for RVA
- High RVA prevalence in suckling pigs (7/15, 46.7%) and nursery pigs (8/18, 44.4%)
- 11 paired intestine diagnostic results. 8 out of 11 had corresponding positive lung results

### RVA DISTRIBUTION

- Bronchial Epithelial Cells (n = 2)
- Macrophages/ Other Cells (n = 12)

### TRANSMISSION ELECTRON MICROSCOPY

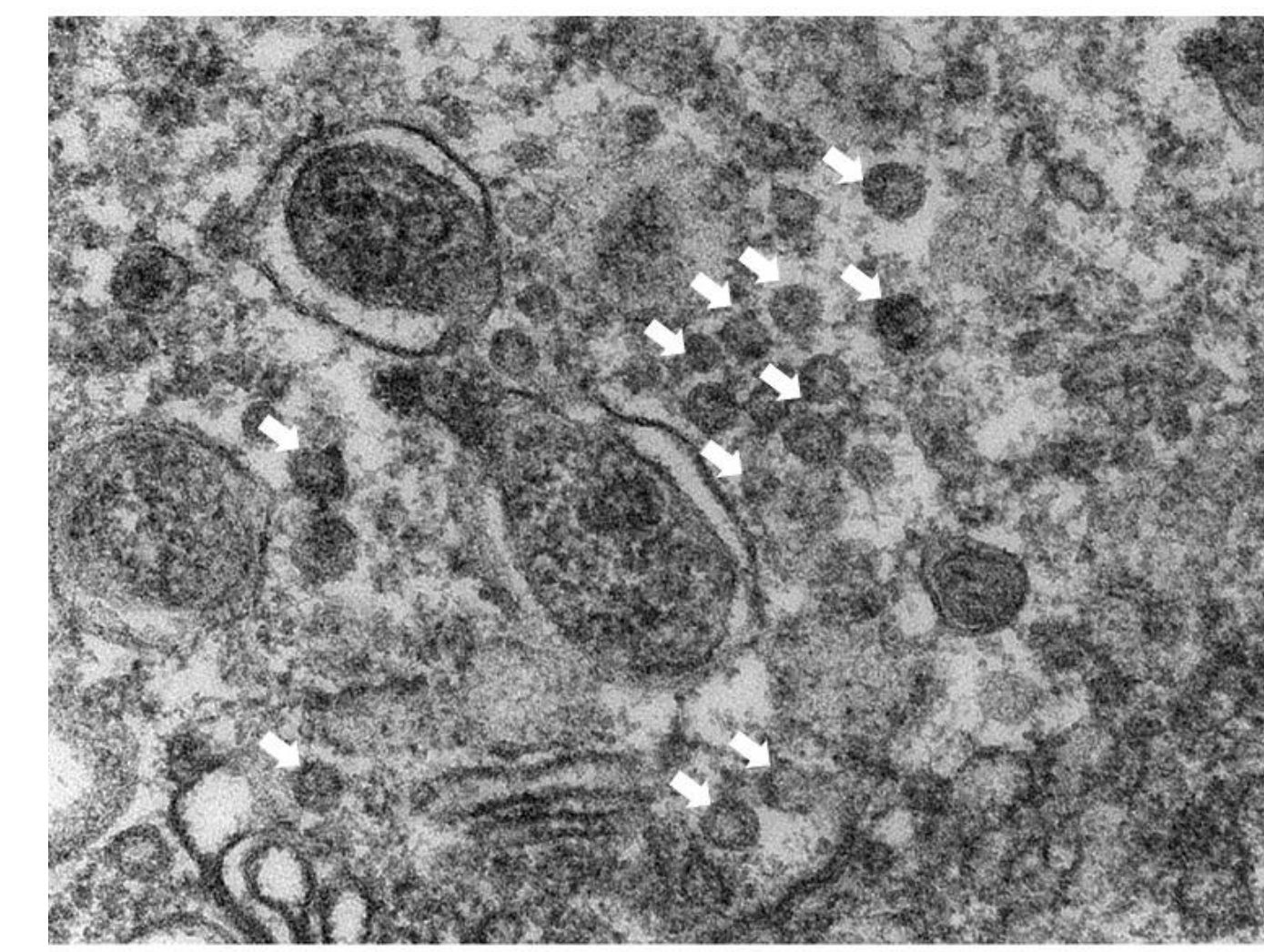


Figure 1. Visualization of rotavirus-like particles (VLP) in bronchial epithelial cells using transmission electron microscopy. VLP (arrows) ranging from 60 nm to 72 nm were observed near the apical border of the bronchiolar epithelial cells. Bar = 200 nm.

### HISTOPATHOLOGY

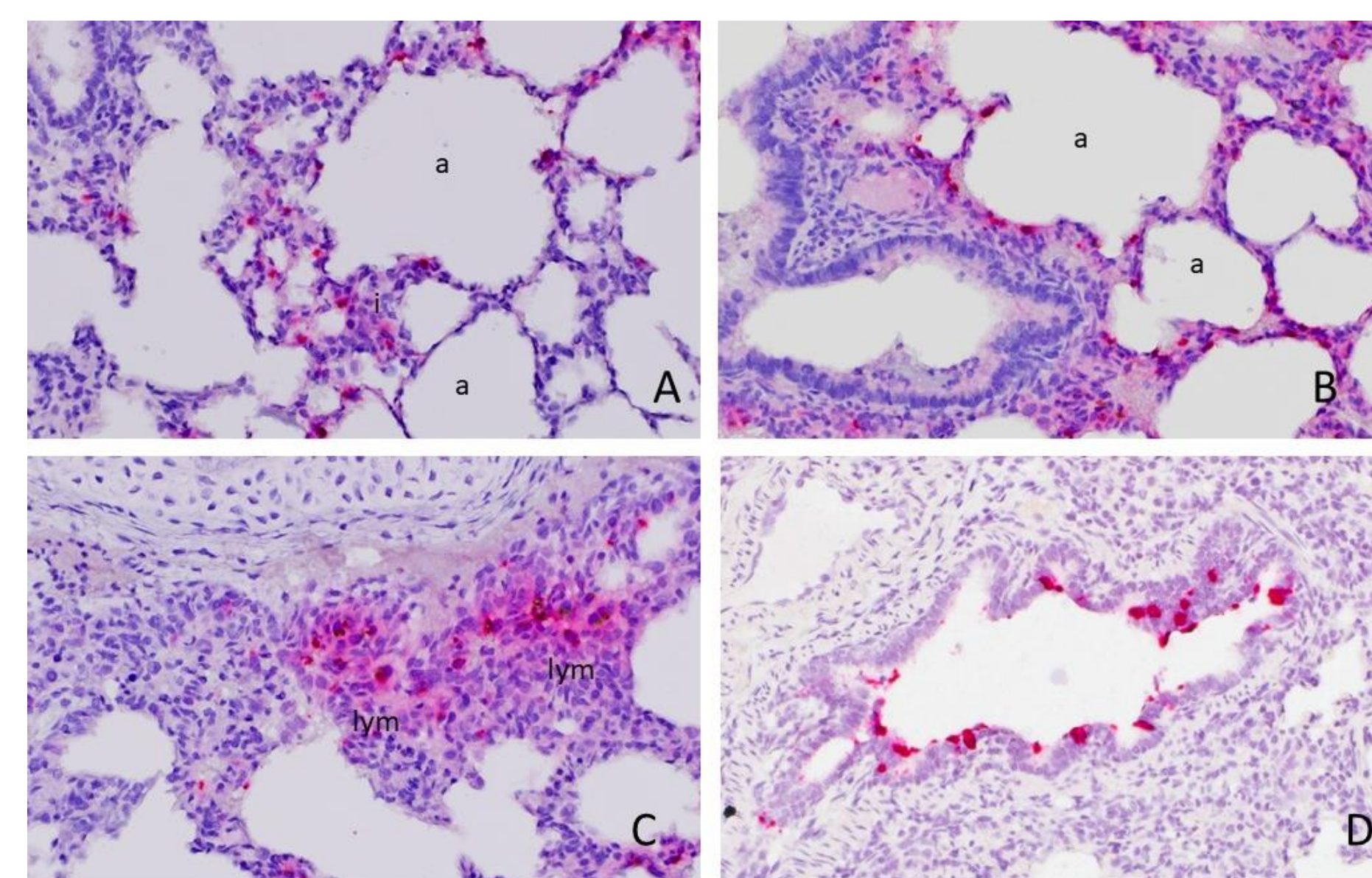


Figure 2. Detection of rotavirus A (RVA) nucleic acid in the lung by in situ hybridization (ISH). Positive red signals were detected in round cells resembling macrophages in the interstitium (i) or alveolar macrophages and pneumonocytes lining the alveoli (a) (A and B). Focally, they were also detected in monocyte-macrophage lineage cells in peripheral lymphoid aggregates (lym) (C) and bronchiolar epithelial cells (D). Images were taken under 400x magnification.

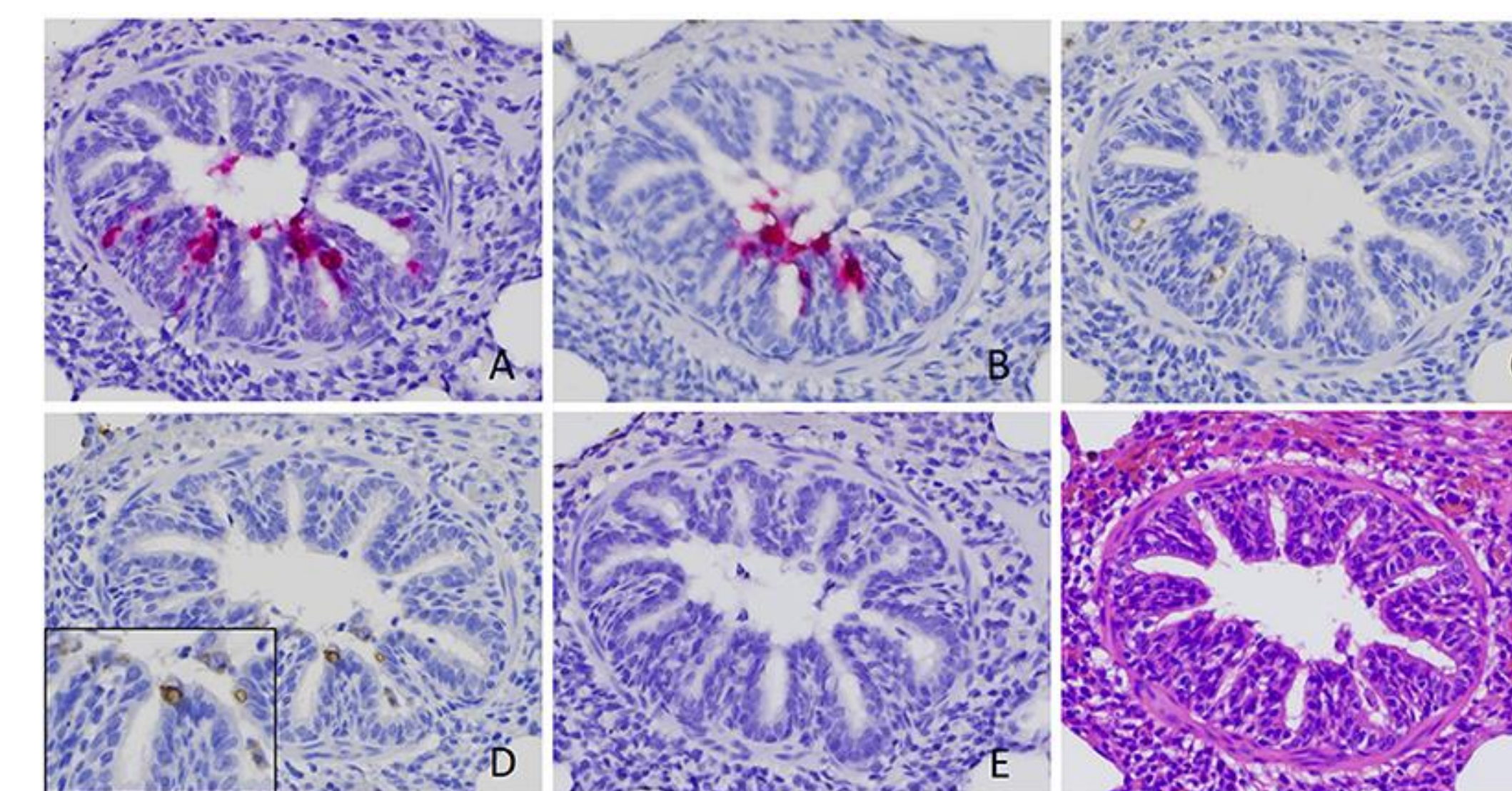


Figure 3. Verification of rotavirus A (RVA) infection in bronchiolar epithelial cells. Positive signal of RVA nucleic acid was detected by in situ hybridization (ISH) using probe targeting VP6 (A) and NSP3 (B). Positive signals of immunohistochemistry staining using antibody against RVA VP6 (C) and dsRNA (D), although weaker, were also detected in the serial sections obtained from the same case. No signal was detected by ISH using a probe targeting rotavirus B (E) (18). Serial section with routine hematoxylin and eosin stain (F) was performed. Images were taken under 400x magnification.

## CONCLUSIONS

- High portion of lung submissions tested RVA positive by RT-qPCR
- The positive cases are mainly, but not limited to, neonatal and nursing piglets
- In cases where both lung and intestine diagnostic results were available, RVA was detected in 8 out of 11 intestine samples from cases with RVA-positive lungs suggesting respiratory and enteric RV infections can be independent events, although they frequently occurred in the same cases.
- Signals of the RVA genome and antigen were detected in macrophages and bronchiolar epithelial cells, as well as leukocytes and endothelial cells in vascular capillaries, and cells resembling the pneumonocytes in the lungs
- Intensive RVA ISH and IHC signals in the macrophages could result from phagocytosis and accumulation of rotaviral materials filtered through the bloodstream, whereas detection of RV in other cell types suggests infection
- An intensive ISH signal of RVA in bronchial epithelium was detected in two cases and are evidence of infection

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Contact Information: [april.nelsen@sdstate.edu](mailto:april.nelsen@sdstate.edu)

