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# Early postnatal respiratory viral infection induces structural and neurochemical changes in the neonatal piglet brain

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ABSTRACT

Infections that cause inflammation during the postnatal period are common, yet little is known about their impact on brain development in gyrencephalic species. To address this issue, we investigated brain development in domestic piglets which have brain growth and morphology similar to human infants, after experimentally infecting them with porcine reproductive and respiratory syndrome virus (PRRSV) to induce an interstitial pneumonia Piglets were inoculated with PRRSV on postnatal day (PD) 7 and magnetic resonance imaging (MRI) was used to assess brain macrostructure (voxel-based morphometry), microstructure (diffusion tensor imaging) and neurochemistry (MR-spectroscopy) at PD 29 or 30. PRRSV piglets exhibited signs of infection throughout the post-inoculation period and had elevated plasma levels of TNFα at the end of the study. PRRSV infection increased the volume of several components of the ventricular system including the cerebral aqueduct, fourth ventricle, and the lateral ventricles. Group comparisons between control and PRRSV piglets defined 8 areas where PRRSV piglets had less gray matter volume; 5 areas where PRRSV piglets had less white matter volume; and 4 relatively small areas where PRRSV piglets had more white matter. Of particular interest was a bilateral reduction in gray and white matter in the primary visual cortex. PRRSV piglets tended to have reduced fractional anisotropy in the corpus callosum. Additionally, *N*-acetylaspartate, creatine, and myo-inositol were decreased in the hippocampus of PRRSV piglets suggesting disrupted neuronal and glial health and energy imbalances. These findings show in a gyrencephalic species that early-life infection can affect brain growth and development.



## 1. Introduction

Infectious disease is the most common cause of illness in children, with acute respiratory infection constituting the most prevalent reason for hospitalization in children under 1 year of age (Hall et al., 2009). This is a concern for neurodevelopment because the phase of rapid brain growth that begins in the late prenatal period continues into the early postnatal period due to dendritic growth, synaptogenesis, and intense glial cell proliferation (Dietrich et al., 1988; Huttenlocher, 1979; Rice and Barone, 2000). During infection, immune-to-brain signaling pathways activate microglia, causing neuroinflammation (Dantzer and Kelley, 2007). Developing and mature neurons, as well as glia, have numerous pro-inflammatory cytokine receptors (Sawada et al., 1993), and untimely inflammation in rodent models, especially in the prenatal period, is known to affect neural development and increase risk for behavioral disorders that manifest later (Meyer et al., 2011). Little is known, however, about peripheral infection in the neonatal period, especially in humans and other animals whose brain is gyrencephalic and experience major perinatal growth.

Quantitative magnetic resonance imaging (MRI) can provide important information on brain development in early childhood

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and adolescence but few studies have focused on the period from birth to 2 years of age when dramatic brain development occurs. Progress on understanding the influence of infection on brain development in human infants has been especially slow because separating the impact of infection from the many other factors impacting brain development is difficult. Furthermore, while studies in rodents have been critical in establishing the neuroimmune hypothesis of developmental disorders, translating the results to human infants can be challenging due to the differences in brain development and morphology. In this regard, the domestic piglet (*Sus scrofa domestica)* may be a useful translational model. Similar to humans, the major brain growth spurt in pigs extends from the late prenatal to the postnatal period (Dobbing and Sands, 1979). Gross anatomical features, including gyral pattern and distribution of gray and white matter of the neonatal piglet brain are similar to that of human infants (Dickerson and Dobbing, 1967; Thibault and Margulies, 1998). Moreover, their physical size allows neuroimaging instruments designed for humans to be used with piglets. Indeed, structural MRI, functional MRI, and positron emission tomography have all been conducted in pigs (Conrad et al., 2012b; Fang et al., 2005; Jakobsen et al., 2006). Additionally, piglets can undergo cognitive testing at a young age and show greater overlap with humans in genes involved in immunity compared to rodents (Dawson, 2011; Dilger and Johnson, 2010; Meurens et al., 2012). Thus, piglets represent a gyrencephalic species with brain growth similar to humans that can be used in highly controlled experiments to explore how infection and inflammation affects brain structure and function.

In the present study, we used MRI to assess macrostructure (voxel-based morphometry), white matter microstructure (diffusion-tensor imaging) and metabolites (spectroscopy) to test the hypothesis that neuroinflammation in the early postnatal period affects brain development in piglets. For this study piglets were given porcine reproductive and respiratory syndrome virus (PRRSV), which infects mononuclear myeloid cells in lungs inducing interstitial pneumonia (Done et al., 1996). PRRSV is a useful model for investigating neuroinflammation as it was recently reported to activate brain microglial cells (Elmore et al., 2014).

## 2. Materials and methods

### 2.1. Animals, housing, and feeding

Naturally farrowed crossbred piglets from three separate litters from the University of Illinois swine herd were used. Piglets remained with the sow for 24–48 h in order to receive colostrum which confers postnatal passive immunity. Importantly, sows were PRRSV-free and not vaccinated for PRRSV. Piglets were brought to the biomedical animal facility on PD 2, where upon arrival they were assigned to either the control group (3 males and 3 females) or the PRRSV infection group (4 males and 4 females) based on sex, litter of origin, and body weight. They were housed individually in cages (0.76 m L × 0.58 m W × 0.47 m H) designed for neonatal piglets (Elmore et al., 2014). Each cage was positioned in a rack, with stainless steel perforated side walls and clear acrylic front and rear doors within one of two separate but identical disease containment chambers that have been described (Elmore et al., 2014). Each cage was fitted with flooring designed for neonatal animals (Tenderfoot/NSR, Tandem Products, Inc., Minneapolis, MN, USA). A toy (plastic Jingle Ball™, Bio-Serv, Frenchtown, NJ, USA) was provided to each piglet. Room temperature was maintained at 27 °C and each cage was equipped with an electric heat pad (K&H Lectro-Kennel™ Heat Pad, K&H Manufacturing, LLC, Colorado Springs, CO, USA). Piglets were maintained on a 12-h light/dark cycle; however, during the dark cycle minimal lighting was provided.

Piglets were fed a commercial sow milk replacer (Advance Liqui-Wean, Milk Specialties Co., Dundee, IL, USA). Milk was reconstituted daily to a final concentration of 206 g/L using tap water and supplied at a rate of 285 mL/kg BW (based on daily recorded weights) to a stainless steel bowl via a peristaltic pump (Control Company, Friendswood, TX). Using this automated feeding system (similar to that described previously (Dilger and Johnson, 2010)), piglets received their daily allotted milk over 18 meals (once per hour from 0600 h to 2400 h).

Two of the PRRSV females developed severe diarrhea and were removed from the study. All animal experiments were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and approved by the University of Illinois at Urbana–Champaign Institutional Animal Care and Use Committee.

### 2.2. Experimental design and assessment of sickness

At PD 7, piglets were inoculated intranasally with either 1 mL of $1 \times 10^5$ 50% tissue culture infected dose (TCID 50) of live PRRSV (strain P129-BV, obtained from the School of Veterinary Medicine at Purdue University, West Lafayette, IN, USA) or sterile phosphate buffered saline (PBS). Daily body weights (kg) were obtained to monitor piglets' growth. In addition, daily rectal temperatures were obtained PD 7 through PD 28. The willingness of the piglets to consume their first daily meal was determined from PD 7 to PD 28 using a feeding score (1 = no attempt to consume the milk; 2 = attempted to consume the milk, but did not finish within 1 min; 3 = consumed all of the milk within 1 min). Body weight, rectal temperature, and feeding score data were analyzed as a two-way (treatment × day) repeated measures ANOVA using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC, USA). There were no significant differences due to sex, therefore it was not included in final analysis of body weight, rectal temperature, and feeding score data. Significance was accepted at $p < 0.05$.

The presence of PRRSV antibodies in the serum of all piglets at the end of the study was analyzed by the Veterinary Diagnostic Laboratory (University of Illinois, Urbana, Illinois) using a PRRSV-specific ELISA kit (IDEXX Laboratories, Westbrook, MD). Serum tumor necrosis factor alpha (TNF-α) levels at the end of the study were determined using porcine-specific sandwich enzyme immunoassays (R&D Systems, Minneapolis, MN). Data analysis was conducted using the MIXED procedure in SAS. Serum TNF-α concentrations were analyzed as a two-way (treatment × sex) ANOVA. Sex was not significant so it was removed from the model and data were reanalyzed as a one-way ANOVA to determine the effects of treatment. Significance was accepted at $p < 0.05$.

At PD 29 and PD30 control and PRRSV piglets were transported to the Biomedical Imaging Center at the Beckman Institute and MRI was conducted using a Siemens MAGNETOM Trio 3 T imager and a 32-channel head coil (Siemens, Erlangen, Germany). The MRI sequences have been previously described (Radlowski et al., 2014), and are briefly explained below. The total scan time per pig was roughly 50 min.

### 2.3. Structural MRI acquisition and analysis

For brain structure analysis, anatomic images were acquired using a 3D T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence and registered using Diffeomorphic Anatomical Registration using Exponentiated Lie Algebra (DARTEL). Before scanning, piglets were anesthetized using a telazol:ketamine:xylazine (100/50/50 mg/kg/BW; Fort Dodge Animal Health). Anesthesia was maintained by inhalation of isoflurane (98% oxygen/2% isoflurane). An MRI compatible pulse oximeter was used to monitor heart rate and oxygen saturation