RESEARCH REPORT
SWINE HEALTH

Title: Investigation Into the Pathogenesis of Post-weaning Multisystemic Wasting Syndrome **- NPB #98-225**

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Abstract:

Post-weaning multisystemic wasting syndrome (PMWS) is a recently recognized entity in swine. Its presence has been reported on several continents. A consistent finding with these reports is identification of a porcine circovirus in affected pigs. The objective of this study was to attempt to develop an experimental PMWS model involving inoculation of conventionally raised pigs. Inoculua included a field isolate of porcine circovirus-type 2 (PCV2) in the form of a tissue homogenate from a naturally occurring case of PMWS and from cell-culture-propagated PCV2 isolated from a clinical case of PMWS. Pigs were inoculated intranasally. Fifty-six conventionally raised pigs were divided into non-inoculated control, virus-inoculated and contact control groups. Non-inoculated pigs were kept isolated from other treatment groups. Contact control pigs were non-inoculated, but housed in rooms with inoculated pigs. Pigs were serially sacrificed weekly. Sera were serially collected for determination of humoral response to infection. Tissues were collected for histopathologic examination, *in situ* hybridization, and virus isolation.

Clinical signs suggestive of PMWS were not observed. Histologic lesions consistent with PMWS were seen in pigs from all treatment groups, excluding pre-inoculation control pigs. A distinct serologic response was detected in pigs approximately two weeks after inoculation. Contact control pigs as well as strict control pigs became infected based on the results. Results indicate inoculation of pigs with either cell culture-propagated virus or tissue homogenates from natural cases of PMWS can produce a neutralizing antibody response, which is comparable to natural horizontal infection observed in the contact control pigs. Histologic lesions of PMWS can be reproduced by PCV infection.

Introduction:

A newly described syndrome, post-weaning multisystemic wasting syndrome (PMWS), has been recognized in North America and Europe.[3,6,8] This syndrome usually affects pigs five to nine weeks of age. Lesions seen in cases of PMWS include enlarged lymph nodes and interstitial pneumonia.[2] Further reports have shown porcine circovirus (PCV) to be

associated with this syndrome.[4] Recently, the PCV associated with PMWS has been shown to be a new type of pathogenic circovirus and has been referred to as type 2 porcine circovirus (PCV2).[7] Experimental reproduction of PMWS has not yet been achieved in conventional pigs.[1,5] Understanding the pathogenesis of PMWS is essential to developing management strategies for controlling this syndrome. Developing a disease model for PMWS would aid in study of the pathogenesis of PMWS and in vaccine development. Since PMWS is purported to be caused by PCV2, vaccination against PCV2 may be of benefit in control of PMWS.

Objectives

The objective of this work was to develop an experimental model in conventional swine for PMWS using PCV2 etiologic agent. This work also describes the kinetics of humoral immunity against PCV, and examines the role of humoral immunity in protection against PCV infection (specifically the role of neutralizing antibodies).

Procedures:

Fifty-six pigs approximately 5 weeks of age and serologically negative to PCV, porcine reproductive and respiratory syndrome virus, and pseudorabies virus were divided into six treatment groups**.** Treatment groups consisted of: 1 - pretrial controls (n=4); 2 - strict controls (n=6); 3 - tissue-homogenate-circovirus-inoculated (THV, n=20); 4 - THV-contact control (THV-CC, n=2); 5 - cell-culture-propagated-circovirus-inoculated (CCV, n=20); 6 - CCV-contact control (CCV-CC, n=4). Strict control pigs were non-inoculated pigs, which were kept in a room separate from all other treatment groups. Contact control pigs were non-inoculated, but kept in rooms in direct contact with inoculated pigs.

Tissue homogenate inoculum consisted of lymphoid tissue homogenate that had been heat-treated at 56C for 60 minutes and then chloroform extracted. Final concentration of PCV2 in the tissue homogenate inoculum was 3.0 $\log_{10}$ $TCID_{50}$/ml of inoculum. Three milliliters of tissue-homogenate inoculum was aerosolized into each nostril using a three-ml. syringe equipped with a tip nebulizer. Cell culture propagated PCV2 inoculum contained 4.4 $\log_{10}$ $TCID_{50}$/ml of inoculum. Two milliliters were administered per nostril as noted above. Inocula were tested for the presence of extraneous viruses.

Tissues and sera were collected from pretrial control pigs three days prior to initiation of the study. Pigs were inoculated with tissue homogenates from pigs with PMWS or with laboratory cultured PCV2. Rectal temperatures were measured daily in all pigs. All pigs were monitored daily for clinical signs of lethargy, inappetance, respiratory distress, and fecal consistency for up to 42 days. Serum was collected serially and pigs were sacrificed at regular intervals and examined for evidence of PCV2 infection, lesions of PMWS, and kinetics of PCV-specific humoral immune response. Whole blood, serum, and nasal swabs were collected serially on days –3, 0, 3, 6, 9, 12, 15, 21, 28, 35, and 42. Packed cell volume was measured on whole blood. Indirect fluorescent antibody (IFA) tests and serum virus neutralization (SVN) tests were performed on serum samples to measure humoral response to PCV2 infection.

Tissues were collected from one pig from the strict control group and three pigs each from the THV and CCV groups weekly up to day 42-post inoculation. On day 42-post inoculation, tissues were collected from all remaining pigs. All contact control pigs were euthanatized on day 42-post inoculation. Tissues collected at necropsy for histologic examination and *in situ* hybridization were tonsil, thymus, bronchial lymph nodes, mesenteric lymph nodes, superficial inguinal lymph nodes, bone marrow, lung, liver, kidney, spleen, stomach, ileum (with Peyer's patch), and colon. Lung, thymus, bronchial lymph node, mesenteric lymph node, inguinal lymph node, spleen, and tonsil were collected for virus isolation.

Indirect fluorescent antibody titers were determined against PCV2-infected PK-15 cells on 96-well plates and titers were expressed as the reciprocal of at the last dilution where fluorescence was observed. Serum virus neutralization (SVN) tests were performed using constant virus and varying serum concentrations. An IFA was used to detect non-neutralized virus. Titers were expressed as a reciprocal of the dilution where the 50% fluorescent antibody infectious dose ($FAID_{50}$) was determined.

Results:

No differences in clinical signs, packed-cell-volume or gross lesions were observed among treatment groups. Microscopic lesions consistent with what is seen in natural cases of PMWS were seen in a lymphoid tissues of pigs from THV, THV-CC, CCV, and CCV-CC as well as in the strict control pigs. Multinucleated giant cells were present in germinal centers of tonsil, lung, lymph nodes, and Peyer's patches and corticomedullary junction of thymuses (Tables 1 & 2). The majority of the multinucleated giant cells were observed in thymuses from pigs in the CCV and THV groups. No lesions suggestive of PCV infection were seen in inguinal lymph nodes. Pigs within the strict control group inadvertently became infected with PCV and four pigs had microscopic lesions of PMWS.

Multifocal histiocytic pneumonia, typical of PCV infection was seen in several pigs (Table 3). Lesions consisted of discrete aggregates of histiocytes in alveolar septae and in peribronchiolar areas.

No PCV2 was isolated from the collected serum samples. Results of tissue sample virus isolation and *in situ* hybridization are summarized in Table 4. Tissues that yielded the highest number of positive samples by virus isolation were spleen and mesenteric lymph node. Tissues that appeared to yield the highest number of positive samples by *in situ* hybridization were inguinal lymph node, spleen, and mesenteric lymph node, respectively.

Serologic response to PCV2 infection was determined by IFA and SVN methods (Figures 1 and 2). All inoculated and contact control pigs seroconverted. The remaining strict control pig present at the end of the study seroconverted as well. Seroconversion of the remaining strict control pig was considered to be a result of accidental infection. Humoral responses were detected between 12 and 14 days after inoculation in THV and CCV groups with a lag time of approximately two weeks for the contact control pigs to seroconvert.

These results indicate PCV is readily transmitted horizontally from pig to pig. Experimentally infected pigs develop a serologic response similar to naturally infected pigs. Infection by experimental inoculation closely resembles natural infection.

Table 1. Number of pigs with multinucleated giant cells

Treatment	Pigs/group
CCV	15/20
THV	15/20
CCV-CC	3/4
THV-CC	2/2
Strict Control	4/6
Preinoculation Controls	0/4

Table 2. Number of pigs with multinucleated giant cells per tissue

Treatment	Tonsil	Thymus	Lung	BrLN[1]	Spleen	MLN[2]	Peyer's patch
CCV	0/20	14/20	0/20	0/20	0/20	2/20	1/18*
THV	1/20	13/20	1/20	1/20	1/20	0/19*	0/17*
CCV-CC	0/4	3/4	0/4	0/4	0/4	0/4	0/3*
THV-CC	0/2	2/2	0/2	1/2	1/2	1/2	0/2
Strict Control	0/6	3/6	0/6	0/6	0/6	1/6	0/6
Preinoculation Controls	0/4	0/4	0/4	0/4	0/4	0/4	0/4

1. Bronchial Lymph node
2. Mesenteric Lymph Node
* Not all specimens available

Table 3. Multifocal histiocytic pneumonia

Treatment	Pigs/group
CCV	6/20
THV	2/20
CCV-CC	1/4
THV-CC	1/2
Strict Control	1/6
Preinoculation Controls	0/4

Table 4. Percentage of tissues positive

	***In situ* hybridization**	**Virus Isolation**
Tonsil	40.0%	7.7%
Thymus	12.9%	5.8%
Lung	3.1%	9.6%
BrLN	4.6%	5.8%
Spleen	57.1%	11.5%
MLN	54.5%	13.5%
ILN	65.7%	5.8%

Figrue 1. Serum Neutralizing Response Following Intranasal Inoculation

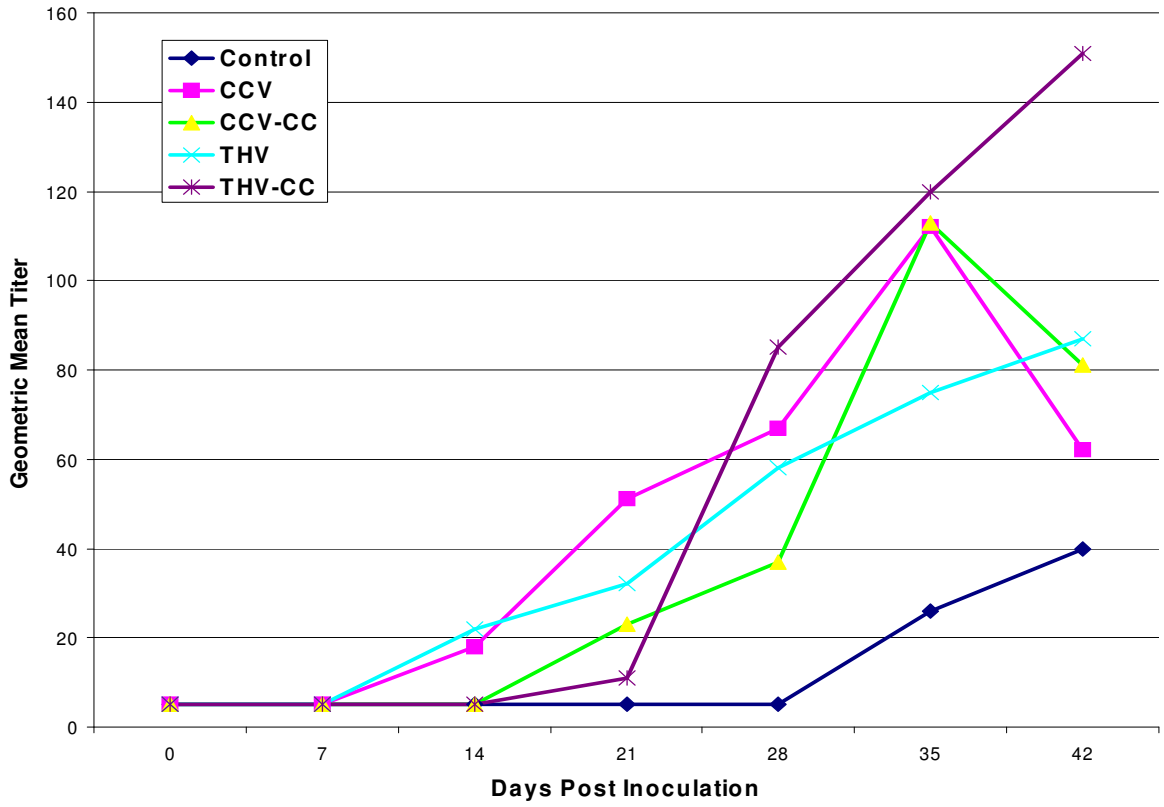
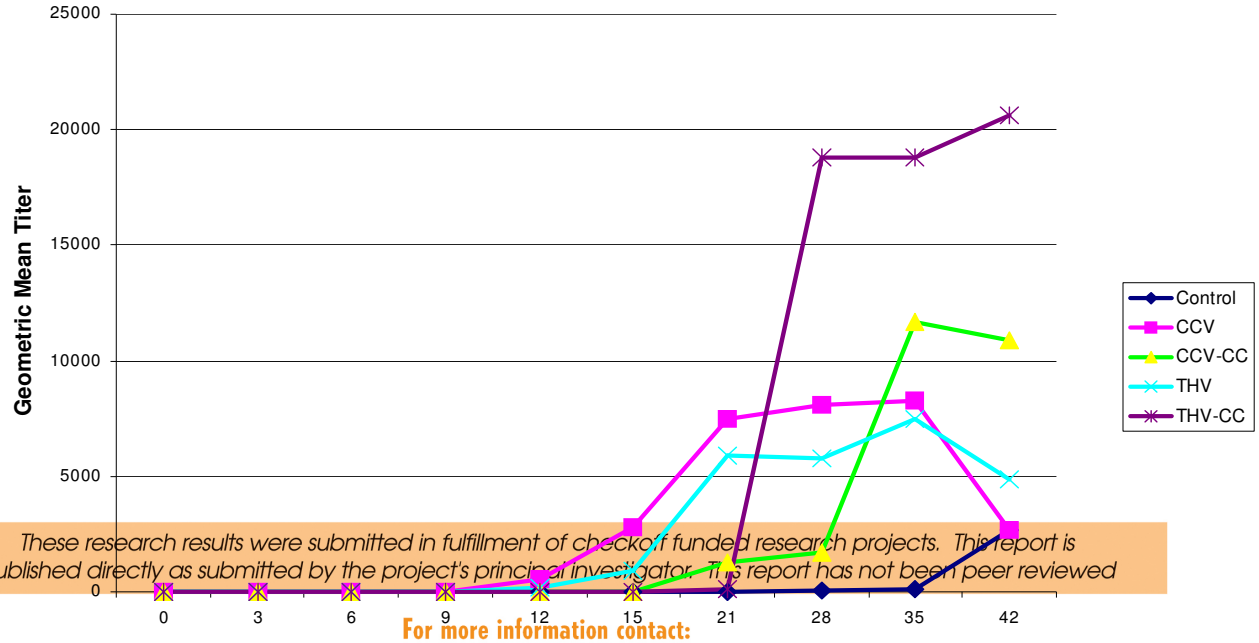


Figure 2. Indirect Fluorescent Antibody Response Following Intranasal Inoculation



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