I. Abstract: Porcine circovirus -2 (PCV-2) infection and its associated diseases, postweaning multisystemic wasting syndrome (PMWS) and porcine dermatitis and nephropathy syndrome (PDNS) is a recently identified and newly emergent viral infectious disease threat to world pork production. In the USA, the incidence of disease is rising dramatically, particularly in the midwest. The mortality rate and loss of production associated with clinical manifestations of disease may be dramatic. There is no known treatment and a vaccine is not yet available. The agent is a small single stranded circular DNA virus which is a common subclinical infection in virtually every swine production unit. The virus has been isolated, cloned and sequenced. Using purified virus or cloned DNA, the disease (PMWS but not PDNS) has been reproduced in gnotobiotic, CD/CD, snatch-farrowed and conventional SPF swine. Macrophages and dendritic cells appear to support the bulk of infectious virus within pigs and there is now no dispute that PCV-2 alone is responsible for PMWS. It is not yet known how subclinical infection is converted into fatal disease in infected swine. Clearly, co-infection with other swine viral pathogens, notably porcine parvovirus (PPV) and porcine reproductive and respiratory syndrome (PRRS) potentiate PCV-2 replication and result in PMWS. Importantly, however, neither of these viruses is mandatory for production of disease as PMWS occurs in both PPV- and PRRS-negative herds. With the financial support of the NPPC, we have previously shown that PPV potentiates PCV-2 disease and that immunostimulation with an unrelated antigen contained within a strong macrophage-stimulating adjuvant, in the absence of any other commensal or potentially pathogenic infectious agent routinely potentiates PCV-2 infection and results in PMWS. At least one commercial vaccine product marketed for use against Mycoplasma sp. pneumonia may have the same effects as well.

In this funded NPPC project (FY 2001), we tested the hypothesis that profound immuno-suppression, the opposite of immunostimulation, may promote PCV-2 replication and result in PMWS. Separately-housed groups (4 piglets per group) of one day old gnotobiotic swine were infected with PCV-2 and then immunosuppressed with cyclosporine (cys), steroid and the combination of cys and steroid. The effects of these chemical immunosuppressive agents upon the replication of PCV-2 and expression of
this enhanced replication as PMWS were determined. Although 1 of 8 piglets treated with cys developed fatal liver disease, in general, immuno-suppression with either or both drugs did not promote development of PMWS even though the amount of infectious virus in tissues of treated pigs was increased compared to PCV-2-alone control piglets. Using a newly developed procedure to determine the cellular sites of viral replication in these piglets however, we demonstrated that productive viral infection occurred within hepatocytes and other epithelial cell types and is not restricted exclusively to monocytic lineage cell populations as has been previously thought. Collectively, these data indicate that immuno-suppression associated with concurrent infections or possibly drug therapies does not routinely potentiate PMWS in PCV-2-infected swine.

II. Introduction

In 1991, a new disease syndrome of weaned (6-12 week old) piglets was identified in several “high health” herds in western Canada and reported to the swine community in 1997-8. The syndrome, identified by the acronym, PMWS (porcine postweaning multisystemic wasting syndrome), is characterized by a constellation of clinical signs including progressive weight loss, jaundice and mortality rates of 10-40%. The incidence of subclinical PCV-2 infection in swine is high, a finding which suggests that most operations are at risk for development of PMWS under the right environmental/management conditions. PMWS has also been identified in Europe, Canada and the midwest. A novel circovirus (PCV-2) was recovered from PMWS cases and shown to be similar but distinct from avirulent PCV-1. Both PCV-2 antigen and viral DNA are present in PMWS tissues. Gross expression of PMWS such as generalized lymphadenopathy, hepatitis, nephritis and pneumonia are most severe in weanling pigs. The histologic features of PMWS are systemic angiocentric granulomatous inflammation, syncytial giant cells and intracytoplasmic basophilic viral inclusion bodies in phagocytes. Tissue damage and organ failure results from extensive inflammation and secondary parenchymal cell necrosis.

Reproduction of PMWS in conventional swine initially proved difficult but more recently has been reported as successful, even with cloned viral DNA. Inoculation of conventional or gnotobiotic piglets with PMWS tissue extracts or PCV-2 alone frequently resulted in subclinical infection; PMWS, when produced, was less severe than field disease or occurs in some but not all animals within a group. In the first NPPC-supported project, we showed that gnotobiotic swine, co-infected with PCV-2 (propagated in PCV-2-free PK cells) and PPV, the latter a virus of minimal virulence in neonates, develop disseminated granulomatous inflammation and fatal PMWS. In a second project supported by NPPC, we reported that immunostimulation by vaccination with a strong antigen emulsified in adjuvant (KLH/ICFA) upregulates PCV-2 infectious virus production in lymphoid tissues and produced PMWS in 100% (7/7) of immunized piglets. Immunization increased the number of virus-susceptible cells in lymphoid tissues such that incipient viral production in them outstripped developing antiviral immunity. The infectious virus burden in infected swine early (< 3 weeks) in the course of infection has emerged as one potential mechanism of disease in the pathogenesis of PMWS.

There is another general mechanism whereby infectious virus can be up-regulated in PCV-2-infected swine. If developing antiviral immunity is transiently or profoundly inhibited during the early stages of infection, the virus burden in tissues may increase beyond the level of containment by body defenses. During the convalescent stages of immunosuppression, lymphoid tissues regenerate and provide actively dividing cells for
infection by PCV-2 which ultimately results in PMWS. Many porcine viruses are thought to be immunosuppressive, most likely due to viral replication in cells of the immune system and subsequent functional derangements in them. The degree and duration of this effect is not known with confidence and attempts to design experiments using infectious agents as immunosuppressive co-factors for PMWS are cost prohibitive and complicated by the pleiotropic unrelated effects of these agents in swine. A more logical approach is to construct a “worse case scenario” immunosuppression in gnotobiotic swine with nonliving chemical agents known to produce the desired degree of immunosuppressive effect. This approach avoids confounding and uncontrollable variables inherent when using viral pathogens (PPV, PRRS, etc) which may have both conflicting (immunostimulatory and immunosuppressive) effects in swine, depending upon the stage of infection. In the germfree environment, there is minimal risk that the animals will develop opportunistic infection complications which often accompanies immunosuppression. Thus, the effects of immunosuppression on two key variables of PCV-2 disease (viral burden in lymphoid tissues and development of PMWS) can be determined under ideal environmental conditions. In these experiments, we will simulate the effects of immunosuppressive agents like PPV and PRRS in PCV-2-infected piglets by suppressing the immune response to PCV-2 in infected gnotobiotics with a parenterally administered steroid (Vetalog\textsuperscript{R}), cyclosporine A (Neoral\textsuperscript{R}), an anti-metabolite immunosuppressant of fungal origin used in solid organ transplants, and the combination of steroid and cyclosporine (cys). This strategy should provide optimal conditions for PCV-2 replication and spread to nonlymphoid tissues in swine without the interference of developing antiviral immunity.

In pilot studies\textsuperscript{17} in another system (H pylori infection in gnotobiotic swine) we have shown that cyclosporine (15.0 mg/kg/day, IP) completely suppressed the gastric inflammatory response to this bacterium; sera were antibody-ELISA negative through PID 14 and contained only trace amounts of IgG on PID 21. In contrast, 3 piglets treated with steroids (2.0 mg/kg, IM, SID) prior to and throughout infection demonstrated lymphopenia, lymphoid depletion and thymic atrophy, consistent with the concept of swine as a “steroid sensitive” species. However at necropsy, prominent gastric inflammatory infiltrates in response to the gastric infection were present and convalescent sera contained high levels of antibodies to H pylori of all 3 Ig isotypes (Krakowka, unpublished, 1994). In a pilot study more germane to this application, several PCV-2 infected piglets were treated with prednisolone (5.0 mg/kg, IM every third day until termination on PID 21). This regimen produced lymphoid depletion and increased the amount of PCV-2 antigen in lymphoid tissues. Because cellular stimulation and activation as well as lymphoid depletion and immunosuppression occurs with many different infections and manipulations including prophylactic vaccination, we have focussed our effort in this proposal to defining the in vivo effects of moderate (steroid) and severe (cyclosporine) drug-mediated immunosuppression upon manifestations (infectious virus titers, histologic lesions and production of PMWS) of PCV-2 infection in gnotobiotic swine. These data will either support or deny the hypothesis that immunosuppression is another key host factor for the production of PMWS under field conditions and will provide the experimental framework for assessment of suspect immunosuppressive insults such as vaccination with modified live viral vaccines\textsuperscript{5,25} which may occur in the field.

Research work on PMWS and PCV-2 at OSU has been generously supported by the NPPC for the last three years. With NPPC support, we defined the virulence of plaque-
purified PCV-1, PCV-2 and PPV, alone or in combination in neonatal gnotobiotic swine. Gnotobiotic piglets were inoculated with each virus, alone or in combination (PCV-1/PCV-2, PCV-1/PPV and PCV-2/PPV). Single agent, PCV-1/PCV-2 and PCV-1/PPV infected piglets were clinically asymptomatic, transiently viremic and seroconverted to homologous virus(es) only. All (4/4) PCV-2/PPV-infected piglets developed classic PMWS characterized by icterus, submucosal edema and death. Hepatocyte necrosis and liver failure was the cause of death and was related to the presence of PCV-2-positive infiltrating macrophages in the liver. In a second series of experiments, local and systemic immune activation as a risk factor was assessed in PCV-2-infected gnotobiotic swine by immunizations with a large foreign protein antigen emulsified in incomplete Freund’s adjuvant (KLH/ICFA). The parameters of up-regulation of in vivo virus production and induction of PMWS were assessed. Immunization increased the number of virus antigen-positive cells in draining lymph nodes and increased the amount of infectious virus recovered by 1-4 log10. In a second experiment, all (7/7) immunized gnotobiotic piglets developed moderate to severe PMWS whereas no piglets infected with PCV-2 alone developed PMWS. In PMWS-affected piglets, extensive replication of PCV-2 was documented by both immunocytochemistry and quantitative viral titrations. Thus, immune activation is one key component of the pathogenesis of PCV-2-associated PMWS in swine. The following conclusions were drawn from these two NPPC-supported studies: 1) In PCV-2-infected gnotobiotic piglets, co-factors are required to produce PMWS; 2) PCV-2 is a persistent infection characterized by continuous viremia, virus shedding in excretions and high serum antibody titers; 3) The major replication site of PCV-2 appears to be in monocytic lineage cells and the characteristic lesion of PMWS is disseminated angiocentric granulomatous inflammation and; 4) Immune dysregulation appears to be one critical component of the pathogenesis of PMWS.

III. Objectives The overall objective of this (FY 2001) NPPC-supported project was to simulate immunosuppressive effects of suspect co-factors (PPV, PRRS, physiologic stress) in single agent (PCV-2)-infected gnotobiotic swine to test the following experimental hypotheses:

Hypothesis 1: Immunosuppression promotes replication of PCV-2 in lymphoid tissues;

Hypothesis 2: Immunosuppression promotes systemic replication of PCV-2 and clinical expression as PMWS.

IV. Procedures Two litters of gnotobiotic piglets were derived and distributed into treatment and infection groups as indicated in Table 1 below. Piglets were terminated when signs of PMWS became apparent (one cys-treated piglet, PID 30) or on PID 35.

<table>
<thead>
<tr>
<th>Group ID</th>
<th>No. of piglets</th>
<th>CyS Tmt</th>
<th>Veta Tmt</th>
<th>CyS &amp; Veta Tmt</th>
<th>KLH/ICFA Immunizations</th>
<th>Infect with PCV2</th>
<th>Terminate on PID 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
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</tr>
<tr>
<td>C</td>
<td>4</td>
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<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>D</td>
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<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>E</td>
<td>4</td>
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<td>-</td>
<td>Yes</td>
<td>-</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>F</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
**Infection with PCV-2** Piglets of Groups A-D and F were oronasally infected with PCV-2, 4.0 x10^6 TCID_{50}/ml at one day of age as described^{24}.

**Immunosuppression** Virus-infected piglets were immunized with KLH/ICFA (Group F)^{24} or immunosuppressed with a single or dual drug therapy regimens (Groups A through D) as follows:

1. Piglets of Groups A and C received daily oral cys (Neoral^R), 50.0 mg/kg/day for the first week of age. Two days into treatment, watery afebrile diarrhea developed in these piglets. The dose of cys was reduced to 25.0 mg/kg and continued until PID 35.

2. Piglets of Groups B and C received twice weekly parenteral injections of steroid (Vetalog^R), 0.2 mg/kg for the first week of treatment. Thereafter, the dose was reduced to 0.1 mg/kg for the rest of the experiment.

**Controls for Immunosuppression** Piglets of Group F (positive controls) were immunized with KLH/ICFA, both hips, axilla at 1 and 7 days of age and infected piglets of Group E (negative controls) were not treated.

**Interim Evaluation and Clinical Signs** Piglets were examined three times per day during/after feeding for evidence of disease. Aside from the afebrile diarrhea seen in the cys-treated piglet groups, all piglets except one in the cys-PCV-2 alone group remained asymptomatic. Weekly clotted and unclotted blood samples were collected and hematologic determinations were made and serum samples were tested for total protein and for major protein fractions by serum electrophoresis.

**V. Results**

**Gross Pathology and Clinical Data** Our expectations for these studies were met in that it appears that the mechanism of clinical disease induction in PCV-2-infected piglets does not involve immunosuppression. Thus, one of the explanations for the observed potentiation of disease by co-infection with parvovirus and/or PRRS viruses is their supposed immunosuppressive effects in swine; this supposition is incorrect. A summary of the data collected is presented below.

1. All 4 KLH/ICFA pigs (positive controls) developed moderate but subclinical PMWS with bronchial/generalized lymphadenopathy (4/4) and pale, tan small livers (3/4).

2. One of 4 cys-treated piglets developed liver disease (edema, and icterus) and was terminated on PID 30. Except for mild bronchial lymphadenopathy (1/4 to 2/4 per group respectively), there were no significant gross lesions in any of the other immunosuppressed piglets from all other treatment groups.
3. Thymic atrophy was the only gross lesion associated with immunosuppression. This lesion was mild and the severity varied between and within groups of piglets.

4. Immunosuppression in PCV-2 infected piglets was achieved with cys at 25 mg/kg with or without co-treatment with steroid but not with steroid treatment alone. Absolute counts roughly 1/5 of normal (PCV-2-infected alone) were achieved. This effect seemed to be restricted to the lymphocyte subset in that counts of neutrophils, monocytes and platelets remained unchanged from pre-inoculation levels. Immunosuppressed piglets were severely hypoproteinemic, due chiefly to the lack of beta and gamma fractions in terminal sera.

5. Only piglets immunostimulated with KLH/ICFA (positive controls) and infected with PCV-2 had elevated group mean serum protein levels (4.5 g/l versus 3.3 to 3.7 g/l), a finding suggesting that these pigs were responding serologically to PCV-2 with antibodies and possibly acute phase serum reactants as occurs in conventional PCV-2-infected swine (Allan, personal communication, 2002) at the time of termination.

**Histopathology**

1. All 4 KLH/ICFA pigs (positive controls) developed active PMWS with variable moderate to severe granulomatous hepatitis, interstitial nephritis and myocarditis typical of PMWS in gnotobiotes reported previously \(^{22,24}\).

2. Piglets of Groups B and D (PCV-2 alone, and PCV-2 plus steroid) had minimal to mild lymphoplasmacytic infiltrates in the liver, (8/8), myocardium (6/8) and kidney 4/8) compatible with subclinical PCV-2 infection. In lymph nodes, B cell-dependent germinal center formation was prominent and T cell-dependent areas were cellular and well represented.

3. Piglets treated with cys (with or without steroid) had several different lesions associated with PCV-2 and cys treatment:

   a. One Cys-alone piglet developed progressive liver disease and was terminated when moribund on PID 30. In this animal, infiltrating macrophages and histiocytes and resident proliferating Kupffer cells contained numerous spherical viral inclusion bodies. Infiltrating lymphocytes and plasma cells were notably absent. Hepatocytes were necrotic or missing and lobules were collapsed around developing multifocal to diffuse granulomas.

   b. The seven remaining asymptomatic cys-treated piglets did not have histologic evidence of liver disease. Hepatocytes were normal in shape, size, orientation to hepatic cords and were indistinguishable from examined hepatic tissues from uninfected control piglets. Unlike gnotobiotes infected with PCV-2 alone, lymphocytic inflammatory cell infiltrates were notably absent from the interstitium and portal triad areas. Lymphoid tissues were inactive and did not contain germinal centers. Grossly apparent variable thymic atrophy was present and was accounted for by moderate depletion of cortical thymocytes.
c. Subclinical granulomatous pneumonia was seen in all cys-treated piglets. These granulomas as well as the surrounding pulmonary parenchyma were PCV-2-negative by immunohistochemistry (IHC). The granulomas were attributed to subclinical pulmonary colonization with a "mold" monocontaminant recovered from the isolators at the end of the experiment. As agryophilic fungal structures were seen in many of the granulomas. This finding is another indication that the level of immunosuppression in cys-treated piglets was severe.

**Virus Tissue Distribution by Immunohistochemistry**

1. Tissue section replicates were stained for PCV-2 structural (capsid) protein using a monoclonal antibody specific for this protein and the indirect immunoperoxidase (Vectastain®) method as described previously. Viral antigen was widely distributed in lymphoid tissues and liver sections of the positive controls (KLH/ICFA) and in the one of fatally affected cys-treated piglet. As described previously, antigen was predominately restricted to monocytic lineage cells of phagocytic (macrophages lining the sinusoids and Kupffer cells of the liver) and to dendritic follicular cells in lymphoid tissues. In the PCV-2-infected alone and steroid alone treatment groups, viral antigen was sparse and restricted to lymphoid tissues predominately in cells of dendritic/histiocytic and macrophage lineages.

2. Surprisingly the liver parenchyma of the cys-treated asymptomatic PCV-2 infected piglets (7/7) were strongly virus antigen-positive. Numerous hepatocytes and Kupffer cells contained both intranuclear and intracytoplasmic accumulations of PCV-2 nucleocapsid. Moreover, unlike PMWS-affected gnotobiotes many of the morphologically recognizable hepatocytes in the one fatally affected cys-treated piglet, were PCV-2 structural protein positive. These findings were unexpected in light of the absence of inflammatory cell infiltrates and any other histologic indices of cellular damage in hepatocytes in the liver sections by conventional light microscopy of hematoxylin and eosin-stained sections. Moreover, these findings suggest that it is very likely that the remaining asymptomatic cys-treated PCV-2-infected piglets would have developed fatal wasting disease had the observation interval exceeded 35 days of age.

3. Tissue demonstration of the ORF1 viral gene product (the DNA replicase) has, to date, been unsuccessful using standard IHC methods (Allan, personal communication, 2000-2002, Krakowka, unpublished, 2001, 2002) except in frozen sections where the morphologic preservation of virus-positive cell type(s) is poor. In light of the hepatocyte findings above, a modified IHC procedure involving brief ethanol fixation, subsequent saponification and then proteolytic digestion of section replicates was successfully developed for the demonstration of the cellular and tissue distribution of the nonstructural viral replicase protein using a monoclonal antibody specific for the DNA replicase. Translation of the ORF1 product and accumulation within cells is a de facto marker of the cellular site(s) of active virion synthesis versus the ORF2 (nucleocapsid protein) which stains not only sites of encapsidation but also accumulations of produced virions contained within phagocytic cells which may have been produced elsewhere. The latter (cytoplasmic aggregates) largely accounts for the strong
antigen-positive signal seen in macrophages. In situ hybridization for viral DNA has the same limitations for distinguishing between sites of replication and site(s) of accumulation of viral materials. Using this new ICH method, ORF1 product and hence active viral replication was confirmed to occur within hepatocytes, Kupffer cells, dendritic cells and unidentified "interstitial cells" in lymphoid tissues and livers.

**Quantitative Virus Re-isolation** Quantitative viral titrations (infectious virus/gm of tissue) were performed as described with tissue homogenates from all piglets and the results are presented in Table 2 below.

As expected from previously published work, (21,24) immunizations with KLH/ICFA increased the amount of infectious virus in tissues by roughly 1-2 log10. As anticipated from the ICH data, cys, with or without steroid, increased viral titers (vs the PCV-2 alone control group) 2-3 log10. Treatment with steroid at the dose and regimen used did not appreciably affect levels of infectious virus in tissues, a finding consistent with the lack of histologic lesions of PMWS in these animals.

### Table 2. A summary of the quantitative virus titrations in 4 tissue compartments of gnotobiotic piglets infected with PCV-2 and treated with immunosuppressive drugs.

<table>
<thead>
<tr>
<th>Group Treatment</th>
<th>Bronchial Lymph Nodes</th>
<th>Mesenteric Lymph Nodes</th>
<th>Liver</th>
<th>Thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV-2, Cyclosporine &amp;</td>
<td>1.5 x 10⁸ᵃ</td>
<td>3.7 x 10⁷</td>
<td>1.9 x 10⁸</td>
<td>4.3 x 10⁷</td>
</tr>
<tr>
<td>Cyclosporine/steroid (n=8/group)</td>
<td>(n=5)ᵇ</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>PCV-2, Steroid</td>
<td>2.7 x 10⁷</td>
<td>3.5 x 10⁶</td>
<td>2.2 x 10⁷</td>
<td>4.6 x 10⁷</td>
</tr>
<tr>
<td>(n=4/group)</td>
<td>(n=4)</td>
<td>(n=4)</td>
<td>(n=4)</td>
<td>(n=4)</td>
</tr>
<tr>
<td>PCV-2, KLH/ICFA</td>
<td>1.9 x 10⁷</td>
<td>1.4 x 10⁷</td>
<td>1.7 x 10⁶</td>
<td>6.3 x 10⁵</td>
</tr>
<tr>
<td>(n=4/group)</td>
<td>(n=4)</td>
<td>(n=4)</td>
<td>(n=4)</td>
<td>(n=4)</td>
</tr>
<tr>
<td>PCV-2 alone</td>
<td>2.7 x 10⁶</td>
<td>2.1 x 10⁶</td>
<td>2.6 x 10⁵</td>
<td>6.5 x 10⁵</td>
</tr>
<tr>
<td>(n=4/group)</td>
<td>(n=4)</td>
<td>(n=4)</td>
<td>(n=4)</td>
<td>(n=4)</td>
</tr>
</tbody>
</table>

ᵃ data expressed as the mean titer per gram of tissue
ᵇ number in parentheses is the number of pigs titrated per group. For the cyclosporine and cyclosporine plus steroid-treated piglets, endpoints were not reached in two piglets and samples were lost as a result of broken vials in another.

**Summary and Value of this Research to the North American Swine Industry** PCV-2 infection and its most severe clinical manifestation, PMWS, has recently emerged as a potentially serious economic threat to the hog industry. Affected herds may experience up to 40% recurrent mortality. Abortions, stillbirths and related disorders such as sow abortion and mortality syndrome (SAMS) have been recognized and the incidence of subclinical infection in swine is high, approaching 100%³¹. There is a critical need to understand the pathogenesis of PCV-2 infection and the role of both living (PPV and PRRS viruses) and nonliving (stress and vaccinations) co-factors which may contribute to the development of PMWS so that control measures based upon scientific information can be developed for producers at risk for development of PMWS. The means whereby subclinical infection is converted to PMWS are not known although concurrent viral infections and immunostimulation are known to potentiate PMWS. Disrupted immune homeostasis (immunostimulation, immunosuppression or both) is emerging as an important co-factor for development of PMWS. Other factors such as post-weaning social stress, suboptimal nutrition and genetic susceptibility in certain genetic lines of swine are also now receiving attention for their contributions to the emergence of this “new” infectious disease.
future work is to be able to separate the effects of PCV-2 from the effects of PPV, PRRS (or other viral agents) in the genesis of PMWS. Immunosuppression, mediated by infectious disease, stress associated with SEW and even some drug therapies all have the potential to potentiate PMWS in PCV-2-infected swine.

The completed experiments described above have provided additional valuable insight into the dynamics of PMWS in swine which are of benefit to both producers and research workers in swine infectious diseases. The specific findings are summarized in points outlined below.

1. Moderate immunosuppression (steroids) associated with infectious diseases or drug therapies appear to be unlikely to potentiate PMWS in PCV-2-infected swine.

2. The inflammatory lesions characteristic of PMWS are immune mediated. This was shown by noting the absence of lymphoplasmacytic cellular infiltrates into tissues known to contain infectious virus in cys-treated animals.

3. Cellular site(s) of active viral synthesis can be distinguished from sites of accumulation of infectious virus using a newly developed method for the IHC demonstration of the viral DNA replicase (ORF1 gene product). This development will allow us to determine the cellular pathogenesis of infection and spread within experimentally infected piglets so that a more precise understanding of the pattern(s) of virus production, spread and infection can be determined. In spite of all of the published work on this disease syndrome, the mechanisms of infection and the cell(s) involved in producing infectious virions are still undetermined.

4. This virus can persist and actively replicate in infected cells without visible viral cytopathic effects and in the absence of any detectible histopathologic changes in infected tissues (hepatocytes of the liver). This suggests that the absence of gross or histologic lesions ordinarily associated with this virus infection is not indicative of a virus-free status in that animal.

5. The use of cys to inhibit the protective immune response in PCV-2-infected piglets has demonstrated that hepatocytes (and likely other epithelial cells of the respiratory and gastrointestinal tracts) can be productively infected with virus in the absence of overt histologic lesions in these cells. Future exploitation of this finding, combined with point 3 above, will ultimately provide both the producers and research workers with this crucially important information, necessary for rational development of protective vaccine formulations and for management recommendations based upon scientific knowledge and not unsubstantiated opinion.

References
VI. Publications The following is a list of publications which have been supported in part or exclusively by the NPPC in 2001-2002. At least two additional papers will be prepared from the data generated above. While these are not yet completed, tentative titles are provided to the NPPC as a part of this report. Copies of the submitted papers, as they are generated will be sent to the NPPC office.


   Note: This paper was submitted to VII last fall and was not accepted for publication chiefly because the referees were concerned that this paper had a pathologic focus not immunology, and that the audience would not find it useful. We are re-writing this paper in light of these criticisms and will include additional studies of apoptosis in the cys-treated piglets above. Even in these piglets with extensive hepatocyte infection as described above, apoptosis does not appear to be a feature of disease. These findings conflict with recent observations in conventional PMWS swine (Segales, personal communication, 2002). There will be some controversy here.


   Note: This letter to Vet Record was submitted for publication in November, 2001. I have not received word that it will be (or has been) published yet.

