RESEARCH REPORT
SWINE HEALTH

Title: The Effect of Strain Recombination on the Virulence of Porcine Reproductive and Respiratory Syndrome Virus **NPB# 01-046**

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I. **Stated Objective:** To determine whether genetic recombination among strains of porcine reproductive and respiratory syndrome virus can result in the emergence of "new" strains with enhanced ability to cause clinical disease.

II. **Progress toward meeting objective:** The study has been successfully completed.

III. **Status of project in regard to stated timeline:** The study was completed within the stated timeline (i.e., the study was completed by the stated term date of October 1, 2001)

IV. **Modifications of project from original proposal:** There were no changes in the project direction and objectives.

V. **Results: (A brief review of Materials and Methods is included with each of the 2 studies described)**

Study 1

Materials and Methods. – Fifteen specific-pathogen-free pigs 2-3 weeks of age were allocated to 3 experimental groups (groups A, B, and C, 5 pigs/group). Group A pigs were kept as nonexposed controls, i.e., they were not exposed to porcine reproductive and respiratory syndrome virus (PRRSV). Group B pigs were exposed oronasally to 2 ml (infectivity titer = 5 X 10^5 cell culture infectious units/ml) of a known recombinant of PRRSV (strain 154). *The 154 recombinant was obtained (in a previous experiment) from a pig 4 weeks after it had been exposed to a mixture of attenuated strains NADC 8-251, NADC 9-251, NVSL-14-*

251, RespPRRS®, and JA-142-251. By sequencing the entire genome of the 154 recombinant it was found that it comprised parts of strains NADC-9, NVSL-14, RespPRRS, and JA-142-251, i.e., it is a 4-way recombinant. Group C pigs were exposed oronasally to 2 ml (1 X 10^5 cell culture infectious units/ml) of a highly virulent field strain (strain JA-142-3) of PRRSV. *Strain JA-142-3, which had been isolated from an epidemic of atypical PRRS, was passaged 3 times in cell culture before it was used in this study. Several previous studies confirmed that at this passage level it was still highly virulent for pigs*. All pigs were observed for clinical signs throughout the study, temperatured daily, and weighed and bled at postexposure days (PED) 0, 7, and 14. On PED 14 all pigs were euthanized and necropsied. Lungs were observed for macroscopic lesions and then lavaged. Selected lymph nodes were dissected from surrounding tissues and weighed to provide an objective measurement of lymph nodes enlargement.

Results. – All measurements suggested that the recombinant PRRSV was somewhat more virulent than the attenuated strains of PRRSV from which it had arisen *(on the basis of previous observations with the parent strains).* However, it was much less virulent than the field strain of PRRSV with which it was compared. On average, pigs exposed to the recombinant strain had larger lymph nodes, higher body temperatures, and gained slightly less weight than did nonexposed pigs (i.e., controls). Conversely, they (group B) had lower titers of virus in their blood and lungs, fewer lung lesions, lower body temperatures, less lymph node enlargement, and better weight gains than did pigs exposed to virulent field virus. Weight gains for groups A, B, and C are presented in Table 1.The values are the collective weights (in pounds) of the 5 pigs in each group.

Table 1

Group	Day 0	Day 7	Day 14	Exposure strain
A	55.2	75.3	95.9	None/Controls
B	55.9	73.5	90.0	154/ Recombinant strain
C	57.3	67.5	79.5	JA-142-3/ Virulent field strain

Conclusions. – The recombinant appeared to have some slight degree of virulence above that typically seen with attenuated PRRSV, but it was much less virulent than the virulent field virus to which it was compared.

Study 2

Materials and Methods. – Forty specific-pathogen-free pigs 3-4 weeks of age were allocated to 8 experimental groups (groups A, B, C, D, E, F, G, and H, 5 pigs/group). The groups and strains to which they were exposed are presented in Table 2.

Table 2

Group	Exposure strain
A	None Control
B	NADC 8-251/Attenuated parent strain
C	NADC 9-251/Attenuated parent strain
D	NVSL 14-251/Attenuated parent strain
E	RespPRRS® /Attenuated parent strain
F	JA-142-251/ Attenuated parent strain
G	154/Recombinant strain
H	JA-142 -3/Virulent field strain

With the exception of RespPRRS®, all of the attenuated parent strains to which the recombinant donor was exposed, and all of the parent strains used in this study, had been serially passaged 251 times in cell culture to cause attenuation. The passage level of RespPRRS® (i.e., the commercial vaccine used for the control of PRRS) is thought to be about 80.

Each pig of groups B-H was exposed oronasally to 2 ml (1 X 10^6 cell culture infectious units of virus/ml) of the indicated virus preparation. Thereafter all pigs were observed and treated as described for experiment 1.

Results. – None of the pigs of groups B, C, and D became infected following exposure to strains NADC-8-251, NADC-9-251, and NVSL-14-251, respectively. Additionally, only 3 of the 5 pigs in group E and 1 of the 5 pigs in group F became infected (These seemingly unlikely results following exposure of pigs to a large amount of virus, namely, 2 million cell culture infectious units/pig, will be discussed later). In contrast, all of the 5 pigs of group G (exposed to the recombinant) and all of the 5 pigs of group H (exposed to virulent field virus) became infected. Infection, or lack thereof, was confirmed by consistency of test results (either positive or negative) when samples and specimens were tested by virus isolation, polymerase chain reaction, and ELISA (i.e., antibody detection). None of the pigs of groups A-D had any clinical signs (to be expected in that group A pigs were noninfected controls and none of the pigs of groups B-D became infected despite exposure to virus). Body temperatures were essentially normal throughout the study for pigs of groups A-F. They were somewhat elevated for pigs of group G (exposed to recombinant virus) and more elevated for pigs of group H (exposed to virulent field virus). There were macroscopic lung lesions only for pigs (4 of the 5 pigs) of group H. They ranged from relatively mild (less than 10%) to severe (more than 50%). In general, the clinical response of pigs paralleled weight gains. Group H, which was the only group obviously affected by exposure to virus, also had the poorest rate of gain (Table 3). There was no obvious reason for the relatively poor rate of gain for group D. As mentioned above, none of the pigs of this group became infected after exposure and none had any signs of

illness during the 14-day interval of the study. Notice that in contrast to study 1, there was no indication that infection with recombinant virus affected the rate of gain.

Table 3

Group	Day 0	Day 7	Day 14	Treatment
A	109.9	138.4	176.0	Controls
B	110.1	143.8	183.2	Attenuated parent
C	109.5	142.0	178.2	Attenuated parent
D	110.0	140.3	166.1	Attenuated parent
E	110.2	135.0	183.0	Attenuated parent
F	110.1	145.7	184.8	Attenuated parent
G	110.5	148.6	186.9	Recombinant
H	109.7	125.5	151.6	Virulent

Conclusions and Comments. – The attenuation of PRRSV by 251 passages in cell culture resulted in virus than either failed to infect pigs (strains NADC-8-251, NADC-9-251, and NVSL-14-251) or infected only 1of the exposed pigs (strain JA-142-251). The commercial vaccine (RespPRRS®) also infected only some (3 of 5) of the exposed pigs. This lack of infectivity is associated with ***oronasal exposure*** and has been seen in other studies in our laboratory. Our assumption is that because attenuation was performed in monkey kidney cells (the only cell line that is known to support the replication of PRRSV in the laboratory) the virus is changed to the point that it initially replicates poorly or not at all in pigs when administered oronasally. The result is different (i.e., a greater percentage of pigs are infected) when the virus is administered intramuscularly (shown in other studies), but in this study we chose to use what is believed to be the natural route of exposure. What was clear from the study was that the recombinant was much more infectious than any of the parent strains from which it was derived. On the other hand, it appeared to be of relatively low virulence when compared with a virulent field strain (JA-142-3) of PRRSV.

The recombinant story

The 2 studies described above (which were in large part made possible by funding from the National Pork Board) are just 2 pieces of the PRRSV recombinant puzzle. The results viewed alone may seem rather trivial, but they are important to a full understanding of the clinical and financial impact that recombination among strains of PRRSV may have on the swine industry. The following – presented in a question and answer format – summarizes our current state of knowledge in this regard.

Question 1– Do existing strains of PRRSV recombine (this can be thought of as a mix and match procedure) to create “new” strains; and if so, how frequent is

recombination? First we need to emphasize that for 2 or more existing strains to recombine they have to infect the same cell or cells and replicate at the same time in that cell or those cells. That is, recombination only takes place during active replication. From a broader perspective that means that a pig has to be infected with 2 or more strains at the same time. But it isn't quite so straightforward. For example, we set the stage for dual infection whenever we vaccinate during an epidemic of PRRSV. Is that a problem? Probably not -- because we know that if a pig is infected with attenuated vaccine virus and virulent field virus, even at the same moment, there is little chance for recombination. The reason is that virulent virus very quickly predominates. Because of this there is soon only 1 strain replicating in the pig, namely, the virulent strain, and so from that time on there is no possibility of recombination. We know this from a previous study in which pigs were simultaneously exposed to both virulent and attenuated PRRSV. Even when attenuated virus was given in a great excess (400 million infectious units of attenuated virus and 40 infectious units of virulent virus) virulent virus was soon the only strain identified in the pig's circulation. Moreover, even if there was recombination there is no reason to suspect that a recombinant comprised of both a virulent strain and an attenuated strain would be more virulent (i.e., cause a more severe disease) than the virulent parent. Nor is it likely that the recombinant would be able to compete, replication wise, with the virulent parent. As a consequence it would soon disappear from the pig – just as if it never existed. The potential danger is when a pig is infected with 2 or more strains that replicate at about the same rate, i.e., 2 or more attenuated strains, or 2 or more virulent strains. When this happens there is a chance for recombination and a chance that at least 1 of the recombinants will replicate at a greater rate than either or all of the parents. If so the recombinant will likely predominate in the pig with the possibility of being shed to other pigs. Through this mechanism a new strain can become established. From a previous study (funded in part by NPPC) we know that when a pig is infected with multiple strains of PRRSV that replicate at about the same rate there can be recombination followed by predominance of the recombinant so that it becomes the only strain in the pig's circulation. Keep in mind, however, that the study was designed to provide the best conditions for recombination, namely, pigs were simultaneously infected with 5 attenuated strains that were believed to replicate at about the same rate (so none would predominate and exclude the others) over a long period of time. The use of attenuated strains was also important in that a recombinant that replicated even slightly better could quickly predominate.

From the results of the aforementioned study we now know that recombination among strains of PRRSV can be a common event under idealized conditions. And it probably also happens in the field under typical field conditions. But in the latter case it is probably a relatively rare event for the reasons already mentioned.

Question 2 – How do recombinants predominate? Without being able to predominate recombinants are – from a practical perspective – a non-issue. That is, they may briefly be a minor component of the overall virus population in a pig's tissues and fluids, but their existence is ephemeral. This limited longevity has been recognized in vitro by infecting cell cultures with 2 strains of the same virus and then testing for recombinants with methods that will identify them even if they comprise only a small part of the total virus population. What has been observed is that recombinants are detected soon after infection of cell cultures with 2 strains, but they soon disappear and only the parent strains persist. On the other hand, we know from our studies using pigs that recombinants of strains of PRRSV can predominate and we would like to know how. The 2 most likely scenarios are the following. First, as a result of genetic changes recombinants may predominate simply by out replicating their parents. The rapid replication rate of PRRSV would allow 1 strain to quickly predominate even if it had only a slight rate advantage. Second, a recombinant may be less affected by antibody raised to its parent strains and thus predominate by immune selection. We are currently investigating both of these possibilities but as yet we have no definitive answer of which is more important. Perhaps they work in concert.

Question 3 – Does recombination among strains of PRRSV result in the emergence of "new" strains with enhanced ability to cause disease. On the basis of several recent studies, including the subject study of this report, it appears that recombinants (i.e., those that are recognized by virtue of their predominance) can be of greater virulence than their parent strains. However, there is no evidence that recombination among attenuated strains results in a "new" strain with a degree of virulence approaching that of a fully virulent field strain. On the other hand, recombination among highly virulent field strains (fortunately this is probably a rare event) and subsequent predominance and shedding of the recombinant could explain the emergence in 1996 of strains of PRRSV that caused a particularly severe form of PRRS often referred to as "atypical" or "acute" PRRS.