

Serological Investigation of Three Australian Herds in Which SEW Failed to Control Respiratory Disease

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Segregated early weaning (SEW) is a term used to describe a management technology for producing high health status pigs from sow herds endemically infected with a multitude of disease agents with minimal use of vaccines and antibiotics. The theories behind SEW technology are not new, but have only recently been used together in such a manner that respiratory disease has been reduced and growth performance has been enhanced. This technology is being adopted across the world pig industry because of the improved performance of high health status pigs, which increases the producer's competitive advantage. The theories behind SEW technology include the following. **1) Weaning age** – Pigs weaned from their dams at less than 21 days benefit from high concentrations of colostral antibodies and are usually protected from colonization of infectious agents carried by their dams. Weaning ages vary for the control of transmission of specific diseases. **2) Biosecurity** – Cleaning, disinfection, and other biosecurity measures are required to prevent disease agents present in the environment from being transmitted to growing pigs. **3) Segregation** – Rearing batches of pigs (usually no more than 7 days age variation) all-in, all-out by room, building, or site is required. Facilities with the best segregation usually result in pigs with the highest health status. These theories are used in combination (SEW), such that producing high health status pigs is consistent and predictable.

In controlled university experiments (Wiseman et al., 1992; Clark et al., 1994; Schinckel et al., 1995; Dritz et al., 1996), *Mycoplasma hyopneumoniae* (Mhp) and *Actinobacillus pleuropneumoniae* (App) have been controlled and even eliminated by use of SEW. The use of SEW technology in commercial situations has not always met with successful control of these diseases. In actuality, Mhp and App have become major etiological agents in the porcine respiratory disease complex (PRDC) observed all too frequently in 16 to 20-week-old pigs when commercial producers implement SEW. The U.S. experience with PRDC includes both viral and bacterial respiratory infections. In Australia, pigs are free of the viral components of PRDC; none-the-less, the early adopters of SEW technology have had considerable difficulty with respiratory disease in 16 to 20-week-old pigs. Thus, the opportunity to investigate less complicated PRDC exists in Australia. We report the results of both cross-sectional and longitudinal serological investigations of 3 herds that either failed or succeeded to minimize the effect of Mhp or App in their herds. Additionally, we surveyed the production systems of these herds to study the utility of the serosurveys to aid the determination of the cause of success or failure of SEW. Thus, future adopters of SEW will have this information to guide their implementation efforts.

Materials and Methods

Herd Surveys

A survey was used to determine whether SEW had been implemented under the accepted guidelines of the technology. Five major categories were assessed on each farm. The first included the disease and vaccination status of the breeding herd. The second was the method by which new genetics were entered into the herd. The third category related to the weaning procedures used by the farm. The fourth included all the biosecurity measures used in the growing finishing herd, and finally the methods of age segregation were assessed and recorded.

Herds Selected

All herds were selected because they were using or had recently used SEW technology. Some herds were currently having clinical respiratory disease in finishing pigs and others were not. All three herds were selected from the State of Victoria. The Victorian herds were bled in a cross-sectional and longitudinal manner.

Experimental Design

Mycoplasma hyopneumoniae and App were the most common respiratory disease agents reported in the pigs of the selected herds. Therefore, these diseases were serologically investigated. The cross-sectional sampling of each herd consisted of bleeding 30 pigs of five age groups (4, 8, 12, 16, and 20-week-old pigs) at one point in time. Thirty pigs of each age group were selected such that the investigators had an 80% chance of detecting at least one positive sample when 10% of the pigs had seroconverted. For the longitudinal serological investigation, 30 four-week-old pigs were sub-classified into 10-pig groups of small, medium and large pigs and ear tagged as they were bled. These sera were used in both the cross-sectional and longitudinal investigations, but these pigs were also bled at 4-week intervals as they aged towards market weight. Thus, each pig was bled 5 times during its growth period. At slaughter, all available tagged pigs were bled and examined for lung lesions; the lesions were scored using the Goodwin method (Goodwin et al., 1969).

In addition to these studies, the first herd was used to determine the serological status of 36 sows of parities zero through five or greater. When their pigs were 14 days old, one small, one medium, and one large pig of each of three sows from parities one through five were bled. Thus, nine samples from pigs of sows of each parity and 15 samples from pigs of each size were collected for analysis.

Serological Tests

For Mhp, an ELISA described by Djordjevic et al. (1994) was used to detect passive and active circulating antibodies. The response was measured as an ELISA test ratio (ETR). The whole cell ELISA developed and validated by the Animal Research Institute was used to detect antibody titers to App serovars 1 and 12 (Bowles et al., 1997). The cut-off titers – i.e., those that are considered negative, positive or suspect – vary according to age, and were determined at the Animal Research Institute.

Results and Discussion

While all herds had made improvements on previous production measures by implementing some aspect of SEW technology, none of these herds adhered rigidly to the accepted guidelines for SEW technology. The SEW guidelines that may have been broken as determined by the herd surveys are presented in Table 1. These would be the components of SEW that would be corrected for each herd if seroconversion to any of the diseases of the sow herd were identified in the serological investigation or if the pigs showed clinical signs of either disease.

Herd 1

Mhp serology (sows and pigs): The pregnant gilts had a higher concentration ($P<.002$) of antibodies to Mhp than older parity sows (Table 2). We have observed similar results in other studies, as has Holtcamp (Pfizer Alliance, 1996). A difference in concentration of Mhp antibodies among the sows of different parities was not observed. However, some sows in the older parities were seropositive to Mhp, which indicated the possibility of subpopulations of positive and negative sows in the herd or circulation of Mhp among these older sows. The association (effect) of the dams' antibody concentrations on their progeny was about 45% as determined by regression analysis. However, the concentration of the pigs' antibodies predicted by the concentration of the dam's antibodies was only about 20%. From these results, we expected the gilt pigs' sera to have a higher antibody concentration than sow pigs' sera, as was observed ($P<.01$). Size of pig did not have an effect ($P>.05$) on serum concentrations of Mhp antibodies. Because smaller pigs were not expected to suckle as much colostrum as the larger pigs, we expected the smaller pigs to have a lower concentration of Mhp antibodies than larger pigs. We hypothesized that the smaller pigs that did not get adequate colostrum died before they were 2 weeks old, and that those that survived had suckled adequate colostrum.

App serology (sows and pigs): An App serovar 1 vaccine that was under development was used on the sows in this herd. By the time the gilts had farrowed, they had received three doses of the App1 vaccine, while the sows were only vaccinated once. Thus the effect of App vaccination was reflected in the serological profiles for the gilts, sows and their offspring (Table 3), with the gilts and the offspring from the lower parity sows tending to have the highest titers. These results were similar to those observed in the serological investigation of Mhp in these sows. As with Mhp, exposure to App in the older sows and development of seropositive and negative subpopulations of sows were apparent in the data. A better association was observed between sow and pig titers for App 1 (80%) than for Mhp. The variation in sow titers for App1 predicted about 64% of the variation in pig titers. Part of this effect may have been due to the sow vaccination.

The App12 titers were high and were not different between gilts and sows nor among sows of different parities. An association between sow and piglet titers for App12 was not apparent in these data, although pig App12 titers were also high.

From the serological data of the sows and two-week-old pigs of this herd, we concluded that the sow herd has been exposed to Mhp, App1, and App12, even though the owners were unaware (not listed in the survey) of the App12 exposure. We also concluded that the young pigs were receiving

colostral antibodies to a disease agent in proportion to the concentration of antibodies present in their dam's sera.

Mhp serology (growing pigs): From the cross-sectional results (Table 2), the mean Mhp antibody concentrations for Herd 1 sera of 4-week-old pigs was higher ($P < .01$) than for any other age group of pigs. These results indicated that passive antibodies were present in some of the pigs at four weeks of age. These antibodies had decayed in the older pigs bled at this time, and none of the pigs of any age tested positive. Thus we concluded that the older pigs had not been exposed to Mhp. However, the ELISA test used requires 4 weeks from time of exposure to Mhp to indicate a positive reaction. In a previous study, half of the pigs (weaned in 28 days and segregated from the dam) were seropositive to a Mhp ELISA test at 18 weeks of age (Clark et al., 1991). Thus, we concluded from this cross-sectional study that vertical transmission of Mhp from dam to pig had not occurred in this herd.

From the longitudinal serological results (Table 2a), Mhp titer results were quite similar to the results of the cross-sectional serological results for Herd 1. However, the serological sample collected at slaughter indicated that the pigs had seroconverted to Mhp. Additionally, lesions typical of Mhp were observed in the lungs at this time. From these serological investigations, we concluded that Mhp was not transmitted from sows to pigs, but rather was transmitted from pig to pig during the late phase of growth. Thus, the weaning age used in this herd was satisfactory, but the biosecurity issues of work sequencing and distance between groups was suspect.

App serology (growing pigs): From the cross-sectional serological results (Table 3), App colostral antibody transfer was apparent, as the App1 titers were higher in sera of four-week-old pigs than eight-week-old pigs ($P < .05$). The App titer decay was complete at eight weeks, as further reduction in titer did not occur in older pigs. The 20-week-old pigs' sera had titers higher than any other age group ($P < .05$), and 12 of these 30 samples were considered seropositive. The herd manager had reported acute death loss due to App1 in pigs over 16 weeks of age in many weekly groups of pigs. Thus, App was probably transmitted from sows to pigs and presented clinically after 16 weeks of age, and may have been responsible for the late development of clinical Mhp in this herd.

From the longitudinal serological results (Table 3a), we observed that 16-week-old pigs seroconverted to App1. Thus this group of pigs had to have become infected four weeks earlier than the pigs represented in the cross-sectional results. Additionally, 24 of the pigs had lesions of pleurisy (an indication of resolving App lesions) at slaughter. Obviously, not all groups of pigs become infected at the same time in a herd; however, either method of serological investigation would have identified the infection in this herd.

Evidence was not present in these results that App12 was of consequence in this herd. In fact, the owners had not reported any evidence that App 12 was present in their herd. However, the serological tests carried out on sows and piglets in this herd indicated that the herd was subclinically infected. By 12 weeks of age, all pigs but one were seronegative and remained negative to 20 weeks in both sets of samples. At slaughter four animals that were in the longitudinal sample group had titers that were suspect (Table 3a).

Herd 2

Survey: In this herd, evidence of the presence of Mhp and App1 and 12 were reported (Table 1). The major concerns that might have resulted in failure of SEW in this herd were 1) maximum weaning age too high to prevent transfer of App, 2) cross-fostering after day one practiced, and 3) work with different aged pigs not sequenced.

Mhp serology: The mean ETRs for sera of all aged pigs in Herd 2 were low both in the cross-sectional and longitudinal samples (Tables 2 and 2a), except at slaughter in the longitudinal sample. The observation of low ETRs in the four-week-old pigs did not indicate that the sow herd was free of Mhp, as pigs from Mhp seropositive herds are commonly found seronegative to Mhp when pigs are four weeks old (Clark et al., 1991). Pigs were not found to be seropositive at any age in the cross-sectional serum samples, which indicated that vertical transmission of Mhp from sows to pigs had not occurred. However in the longitudinal study, two, three and six pigs in the 16-week-old, 20-week-old, and slaughter groups, respectively, were seropositive. This indicated that some of these pigs became infected during this time period. The finding of some pigs with lung lesions typical of Mhp at slaughter (mean lung lesion score was 3.7%) substantiated the serological results. Thus, we concluded that some pigs in some groups in this herd developed Mhp, but the source of the infection cannot be determined beyond what was presented in the survey.

App serology: In this herd, cross-sectional and longitudinal serological evidence indicated that colostral antibodies to App1 and App12 were transferred from sows to pigs, as the mean titers for four-week-old pig sera were higher ($P < .05$) than for eight and 12-week-old pig sera (Tables 3 and 3a). These results confirmed that both diseases were present in the sow herd. Evidence was not present that the 20-week-old pigs had seroconverted to App1, as their App1 titers were low and not different ($P > .05$) from titers of eight, 12 and 16-week-old pigs. The mean titer of the sera of pigs at slaughter in the longitudinal study was higher than that of the pigs when they were eight to 20 weeks old, and indicated that some (10) pigs had seroconverted. Only three of these pigs had pleurisy when examined. Additionally, the owner did not indicate that the pigs had expressed clinical signs of App. Thus we concluded that the seroconversion to App was likely due to the late expression of mycoplasmal pneumonia that enhanced colonization but not clinical expression of this disease.

The mean App12 titers of the cross-sectional sera of 16 and 20-week-old pigs on this farm were very high and significantly different ($P < .001$) from titers of 8 and 12-week-old pigs. Additionally, the App12 titers of the 16 and 20-week-old pigs in the cross-sectional study were substantially different from the App12 titers for the same age groups that were in the longitudinal study, which were seronegative. The pigs that were in the longitudinal study remained on the same site, and originated from two sources. However, for the cross-sectional study, the last three groups – i.e., 12, 16 and 20 weeks – were housed on another site, where pigs from 13 sources were commingled. We concluded that the seroconversion of the pigs to App12 in the cross-sectional study was due to horizontal exposure to carrier animals from the second site. Nevertheless, clinical evidence of respiratory disease was not present in these pigs during sample collection.

Herd 3

In the survey (Table 1), mycoplasmal pneumonia was reported to be present in this herd and clinically evident in pre-slaughter pigs. This herd was also reported to have had exposure to App, but the owner had not reported clinical evidence of the disease. Provided App doesn't become clinical, the major concerns that might cause failure of SEW were 1) cross-fostering after day one, 2) disinfection not used between batches, and 3) work with different aged pigs not sequenced.

Mhp serology: Little evidence of passive transfer of Mhp antibodies was observed in this herd, as was expected.

The mean ETR for the cross-sectional sera of 20-week-old pigs was higher ($P < .001$) than that of pigs of any other age group (Table 3), and 3 of these pigs were considered seropositive. Some of the pigs in the longitudinal study appeared to be in the process of seroconverting at 20 weeks of age and at slaughter (Table 3a). Unfortunately, data from only 6 animals were collected at slaughter, though their high average lung score of 17.6% reflects the cause of the higher Mhp ETRs observed. We concluded that Mhp exposure occurred in the last finisher that held the pre-slaughter pigs, because some of these pigs were observed to cough. We hypothesize that the workers are transferring Mhp from this building to younger pigs in the next building, as work is not sequenced.

App serology: In this herd, serological evidence of App1 or App12 was not observed in any aged pigs in either the cross-sectional or longitudinal samples, as titers were low although significantly different ($P < .05$) among the age groups (Tables 3 and 3a). However, none of the pigs had a titer considered to have been seropositive. Additionally, these pigs did not have clinical evidence of pleuropneumonia. However, to determine whether either disease is actually in the sow herd would require serological investigation of these animals.

Applications

1. Pregnant gilts have the highest titers to Mhp and App.
2. Subpopulations of Mhp and App seropositive and seronegative sows exist within infected sow herds.
3. The concentration of antibodies for Mhp and App that pigs receive in colostrum is related to the concentration of those antibodies in sow serum.
4. Although longitudinal and cross-sectional serum sampling is useful for research studies, these results indicate that cross-sectional sampling is satisfactory for Mhp and App infected herds.
5. For these two diseases, 30 samples per age group is probably too many; 10 should be satisfactory.
6. Cross-sectional sampling of pigs when two to four weeks old, 10 to 12 weeks old, and near slaughter would give satisfactory information on the epidemiology of these diseases.

7. Failure to follow all the rules for SEW will result in growing pigs developing diseases of the sow herd and possibly diseases of older growing pigs.

References

- Bowles, R., P. Blackhall, B. Smith, and B. Fenwick. 1997. Development, validation and application of ELISAs for *Actinobacillus pleuropneumoniae* serovars 1, 7 and 12. In: *Proceedings Australian Association Practicing Veterinarians*. p. 93-98.
- Clark, L.K., C.H. Armstrong, A.B. Scheidt, et al. 1991. Investigating the transmission of *Mycoplasma hyopneumoniae* in a swine herd with enzootic pneumonia. *Vet Med*. 86:543-550.
- Clark, L.K., M.A. Hill, T.S. Kniffen, et al. 1994. An evaluation of the components of medicated early weaning. *Swine Hlth and Prod*. 2(3):5-11.
- Djordjevic, S.P., G.J. Eamens, L.F. Romalis, and M.M. Saunders. 1994. An improved enzyme linked immunosorbent assay (ELISA) for the detection of porcine serum antibodies against *Mycoplasma hyopneumoniae*. *Veterinary Microbiology* 39:261-274.
- Dritz, S.S., M.M. Chengappa, J.L. Nelssen, M.D. Tokach, R.D. Goodband, J.C. Neitfeld, and J.J. Staats. 1996. Growth and microbial flora of nonmedicated, segregated, early weaned pigs from a commercial swine operation. *J Am Vet Med Assoc* 208:711-715.
- Goodwin, R.F.W., R.G. Hodgson, P. Whittlestone, and R.L. Woodhams. 1969. Some experiments relating to artificial immunity in enzootic pneumonia in pigs. *J Hyg Camb* 67:193-208.
- Schinckel, A.P., L.K. Clark, G. Stevenson, et al. 1995. Effects of antigenic challenge on growth and composition of segregated early weaned pigs. *Swine Hlth and Prod*. 3:227-234.
- Wiseman, B.S.A., R.B. Morrison, G.D. Dial, et al. 1992. Influence of weaning age on pathogen elimination and growth performance of commingled pigs derived by medicated early weaning (MEW). In: *Proceedings 12th Congress Intl Pig Vet Soc*. p. 500.

Table 1. Disease found in the 3 herds and components of SEW technology that may have resulted in failure to control respiratory disease in growing pigs.

Herd	1	2	3
Disease	Mhp, App1, 12	Mhp, App1, 12	Mhp
Prewaning mortality ^a	21%	11%	12%
Wean age ^b	14 to 16 days	14 to 17 days	14 to 18 days
Work sequenced ^c	No	No	No
Batch separation ^d	Too close	OK	OK
Cross-fostering ^e	Yes	Yes	Yes
Disinfection ^f	Yes	Yes	Yes

a) If pre-weaning mortality >12%, the pig quality at weaning is suspect.

b) Maximum wean age >14 days is not known to prevent sow to pig transfer or to eliminate either disease from pigs.

c) If workers don't work from youngest to oldest pigs, disease transfer could occur.

d) If buildings are too close together (<50 ft), disease transfer is likely.

e) Cross-fostering after 24 hours may result in sow to pig transfer of disease.

f) Failure to disinfect a building between groups may enhance disease transfer.

Table 2. *Mycoplasma hyopneumoniae* cross-sectional serology measured as ELISA test ratios for 3 SEW Victorian herds.

Age	N	Herd 1		Herd 2		Herd 3	
		Mhp	Pos	Mhp	Pos	Mhp	Pos
Gilts	8	.54 ^a		NT		NT	
Sows	28	.22 ^b		NT		NT	
2 weeks	45	.21		NT		NT	
4 weeks	30	.16 ^a	4	.10 ^a	0	.11 ^a	0
8 weeks	30	.10 ^b	0	.08 ^b	0	.10 ^a	0
12 weeks	30	.07 ^b	0	.09 ^{abc}	0	.12 ^a	0
16 weeks	30	.08 ^b	0	.10 ^{abc}	0	.12 ^a	0
20 weeks	30	.10 ^b	0	.11 ^{ac}	0	.23 ^b	3

NT = Not tested

^{abc}Means in columns with different superscripts differ (P<.05).

Table 2a. *Mycoplasma hyopneumoniae* longitudinal serology measured as ELISA rest ratios for 3 SEW Victorian herds.

Age	N	Herd 1		Herd 2		Herd 3	
		Mhp	Pos	Mhp	Pos	Mhp	Pos
4 weeks	30	.16 ^a	4	.10 ^a	0	.11 ^a	0
8 weeks	30	.09 ^b	0	.09 ^a	0	.10 ^a	0
12 weeks	30	.09 ^b	0	.10 ^a	0	.11 ^a	0
16 weeks	30	.12 ^{ab}	0	.12 ^a	2	.13 ^{ab}	2
20 weeks	30	.11 ^{ab}	0	.16 ^{ab}	3	.14 ^a	2
at slaughter ^d	()	.33 ^c	12 (28)	.20 ^b	6 (32)	.22 ^b	1 (6)

^{abc}Means in columns with different superscripts differ (P<.05).

^dNumber of pigs tested at slaughter is given in parentheses.

Table 3. *Actinobacillus pleuropneumoniae* cross-sectional serology for 3 SEW Victorian herds.

Age	No	Herd 1				Herd 2				Herd 3			
		App1	Pos	App12	Pos	App1	Pos	App12	Pos	App1	Pos	App12	Pos
Gilts	8	16416	6	12888	1	NT		NT		NT		NT	
Sows	28	11980	9	14836	12	NT		NT		NT		NT	
4 wks	30	4820 ^a	25	NT		10025 ^a	24	7440 ^a	21	2962	8	3064	15
8 wks	30	1593 ^b	0	NT		2362 ^b	8	2909 ^{ab}	10	1970	2	1804	2
12 wks	30	2565 ^b	1	1696	0	2713 ^b	0	1699 ^b	0	2636	0	1941	0
16 wks	30	2093 ^b	0	1968	0	3225 ^b	0	20708 ^c	24	3042	0	2242	0
20 wks	30	14867 ^c	22	2346	0	2908 ^b	0	20796 ^c	30	3693	1	2200	0

NT = Not tested

^{abc}Means in columns with different superscripts differ (P<.05).

Table 3a. *Actinobacillus pleuropneumoniae* longitudinal serology for 3 SEW Victorian herds.

Age	No	Herd 1				Herd 2				Herd 3			
		App1	Pos	App12	Pos	App1	Pos	App12	Pos	App1	Pos	App12	Pos
4 weeks	30	4820 ^a	23	NT		10025 ^a	24	7440 ^a	21	2962 ^a	8	3064	15
8 weeks	30	2111 ^a	5	1905 ^{ab}	1	3764 ^b	14	2437 ^b	11	2346 ^a	6	1771	0
12 weeks	30	1894 ^a	0	1774 ^a	0	2171 ^b	0	1700 ^b	0	2487 ^a	0	2104	0
16 weeks	30	15746 ^b	20	2619 ^a	1	3493 ^b	1	3072 ^{ab}	0	3555 ^b	0	3482	0
20 weeks	30	14037 ^b	20	3033 ^{ab}	1	4130 ^b	1	2728 ^{bc}	2	4128 ^b	0	2521	0
slaughter ^d	()	11512 ^b	12(28)	5193 ^b	4(28)	10419 ^{ac}	10(32)	3976 ^{ac}	2(32)	3344 ^{ab}	0(6)	2156	0(6)

^{abc}Means in columns with different superscripts differ ($P < .05$).

^dNumber of pigs tested at slaughter is given in parentheses.