Intradermal vaccination against pseudorabies virus and swine influenza in growing/finishing pigs

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Summary

Objective—*To investigate the effect of vaccination against pseudorabies virus (PRV) and swine influenza virus (SIV) in growing/finishing pigs on growth performance parameters.*

Methods—*In a herd free of porcine reproductive and respiratory syndrome virus (PRRSV), 300 growing/finishing pigs were vaccinated according to one of five protocols: 1) a single PRV vaccination, 2) a double SIV vaccination, 3) a single PRV and a double SIV vaccination, 4) a double PRV and a double SIV vaccination, and 5) no vaccination (control). Average daily gain (ADG), average total feed consumption (ATFC), feed conversion ratio (F:G), and pathological and bacteriological findings were compared among treatment groups.*

Results—*Pigs vaccinated twice against PRV and SIV had significantly better ADG, ATFC, and F:G than all other treatment groups. Twice-vaccinated pigs also had significantly lower lung lesion scores. Bacteriological examination revealed the presence of secondary bacterial pathogens, such as* Pasteurella multocida, Staphylococcus *spp and* Proteus *spp, in lungs of all treatment groups except those twice vaccinated against PRV and SIV.*

Implications—*Double intradermal vaccination of pigs against both PRV and SIV may improve growth performance and may reduce the impact of secondary bacterial pathogens on lungs.*

Pseudorabies virus (PRV) (Aujeszky's disease) and swine influenza are viral respiratory diseases of major economic importance for the swine industry worldwide.[1–6] Both PRV and SIV predispose swine to secondary bacterial and viral infections, although the severity of the disease depends on the health status of the infected farm.[1,3–5,7] Severe respiratory disease and increased production losses have been reported, for example, when viral respiratory infections, especially swine influenza virus (SIV) infection, are combined with porcine reproductive and respiratory syndrome virus (PRRSV).[8,9]

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Growing/finishing pigs that received intranasal and parenteral vaccination prior to being challenged with PRV had reduced weight loss and significantly shorter periods of arrested growth compared to unvaccinated animals.[10–13] Moreover, animals twice vaccinated against PRV had higher antibody titers at slaughter and better weight gain performance than pigs that were vaccinated once.[14] Vaccines that contain the main serotypes H1N1 and human H3N2 have been shown to confer clinical protection against SIV.[2,15–17]

The objective of this study was to evaluate the impact of vaccination against pseudorabies and swine influenza with intradermally administered vaccines under field conditions on growth parameters and on microbiologic and pathological findings.

Materials and methods

Pretrial period

A serological survey was performed in six industrial pig units with a total of 9700 sows. Blood samples were collected from 14 growing pigs (total of 84 pigs) at 90 and 110 days of age. Serological analysis was performed by serum neutralization (SN) test to detect antibodies against PRV and by hemagglutination inhibition (HI) test to detect antibodies against SIV. On the basis of the laboratory results, we selected the herd that had the highest antibody titers against PRV and SIV to serve as the study herd in this trial.

Trial herd

We performed this trial in 1992 in a modern, 2500-sow farrow-to-finish pig unit that produces 48,000 finishing pigs per year. This unit has a fully automated feed mill and a private slaughterhouse. The farm is located in the most densely pig-populated area in Greece, where PRV is endemic. Swine influence virus infection had also been diagnosed in the herd within the 3 years previous to the trial.

Herd health status

A routine vaccination program against PRV with inactivated vaccine (breeding animals) and attenuated vaccine (finishing pigs) had been carried out in the study herd for the 3 years previous to the start of this trial. Vaccination against SIV with inactivated vaccines containing both SIV serotypes (H1N1 and H3N2) had also been performed since the first outbreak of the disease, also 3 years previous to the start of the trial. Furthermore, the manager of this herd was routinely vaccinating boars, breeding sows, and replacement stock against the following

diseases:

- porcine parvovirus,
- colibacillosis,
- swine erysipelas,
- *Clostridium perfringens* infection (types A and C),
- leptospirosis, and
- atrophic rhinitis (AR).

During the experimental period, clinical and laboratory tests failed to find evidence of PRRSV infection. Until the end of 1992, Greece was free of PRRSV (i.e., no cases had been reported and breeding stock imports had been intentionally restricted during the onset of the disease in other European countries).

Antibacterials in feed were administered to:

- the breeding herd (700 ppm chlortetracycline for 8 consecutive days 3 times per year);
- the growing herd (100 ppm tylosin for 8 days); and
- the finishing herd (400 ppm oxytetracycline plus 200 ppm penicillin-V and 100 ppm of monensin sodium for 10 consecutive days).

Also, animals in this herd were routinely treated for parasites, both internal (275 mg per kg bodyweight piperazine citrate as 1-day medication at the beginning of the growing and finishing stages) and external (animals, pens, and surroundings sprayed with 0.05% phoxim solution [Sebacil®, Bayer] once per month).

Trial animals and treatments

Three hundred 11-week-old pigs were selected, ear-tagged, and placed in 15 identical pens with 18–22 animals per pen.

Five different trial groups were allocated in the above pens, representing the following treatment groups (3 pens per treatment group):

- "Controls": not vaccinated against either PRV or SIV,
- "Single PRV": vaccinated against PRV at 100 days of age,
- "Double SIV": vaccinated against SIV at 100 and 121 days of age,
- "Single PRV and Double SIV": vaccinated against PRV at 100 days of age and against SIV at 100 and 121 days of age, and
- "Double PRV and Double SIV": vaccinated against PRV and SIV at 100 and 121 days of age

for a total of 60 pigs per treatment group.

Each block of five pens was one experimental block where the above five treatments were randomly assigned. Male and female pigs were evenly represented in each treatment group, and the average weight was not significantly different ($P > .05$) among treatments at the beginning of the trial.

Treatment groups were placed in adjacent pens of the same building. Feeding conditions were the same for all trial animals during the study. The offered feed was a balanced grower diet (up to 50 kg liveweight with 13.54 mJ per kg digestible energy, 18.76% crude protein, 1.22% lysine, 0.44% methionine, and 60 ppm salinomycin as performance enhancer) and finishing diet (50 kg liveweight to slaughter age with

13.42 mJ per kg digestible energy, 16.57% crude protein, 1.00% lysine, 0.35% methionine, and 30 ppm salinomycin as performance enhancer), delivered by hand during the study.

An attenuated vaccine against PRV (Alfort-26 strain, Dergeskalone®, Rhone Merieux, France) and an inactivated vaccine against SIV that contained H1N1 and H3N2 serotypes (Derflu®, Rhone Merieux) were used to vaccinate the study pigs. Both PRV and SIV vaccines were intradermally administered using a high-pressure needleless device (Pigjet®, Rhone Merieux).

Serological examinations

From nine pigs per treatment group (n = 45), we collected blood samples at 80, 100, 120, 140 days of age, and at slaughter (180 days of age). Serum samples were analyzed to detect antibodies against PRV (using the serum neutralization [SN] test) and the serotypes H1N1 and H3N2 of SIV (hemagglutination inhibition [HI] test). Titers > 1:2 for PRV, > 1:16 for the H1N1 serotype, and > 1:32 for the H3N2 serotype were considered as positives for control group pigs.

Growth performance data

For all treatments, we calculated average daily gain (ADG) for the following experimental subperiods:

- days 80–119 of age,
- days 120–139 of age,
- days 140–180 of age, and
- days 80–180 (overall period).

Feed:gain ratio (F:G) and average total feed consumption (ATFC) values were calculated for the overall period on a per-pen basis.

Health monitoring

For each trial animal, we assessed general health status, noting respiratory problems (coughing, sneezing, abdominal breathing, etc.) for a period of 12 consecutive hours on a daily basis. Medications via feed or injectables were also recorded for the whole experimental period. To all animals exhibiting respiratory signs, we administered 10 mg per kg body weight of injectable oxytetracycline daily for 3 consecutive days. Animals removed from the study were examined according to appropriate clinical and laboratory procedures to determine the cause of removal.

Snout morphology

Snouts of all trial animals were examined at slaughter for morphological lesions due to AR by coronal sectioning at the level of the second premolar. Morphological lesions were scored according to the Weybridge snout grading system (i.e., snout scores range from 0 (unaffected) to 5 (totally devoid of turbinate bones).[18]

Statistical analysis

Data were subjected to one-way analysis of variance, with the pen/replicate as the experimental unit, using the general linear models (GLM) procedure of the Statistical Analysis System (SAS).[19] The analysis of variance

(ANOVA) model included terms for the treatment effect on least squares means and error. Duncan's multiple range test was used as the comparison test to distinguish the statistical difference among the five treatment groups. The level of significance was set at $\alpha = .05$.

Lung lesions

Lungs of all trial animals were examined at the slaughterhouse. For the purposes of this trial, we attempted to discriminate between chronic lesions typical of enzootic pneumonia (i.e., cranioventral consolidation) and lesions resulting from acute infection.

We scored lesions we attributed to chronic enzootic pneumonia using the methods of Walton.[17] Briefly, for each lobe of the lung we assigned a value corresponding to the approximate proportion of the total lung damage that lobe represents. Degree of consolidation in each of these lobes was assessed, and each was given a score of either 0–5 (for apical and intermediate lobes) or 0–10 (for cardiac and diaphragmatic lobes). The maximum possible total score was 55.[18] A mean value for each treatment was calculated for comparison among groups.

Lesions we attributed to viral infection were scored on a scale of 0 to 4, according to the following criteria:

  0 = absence of lesions,
  1 = small foci with congestion,
  2 = extended foci with red and grey hepatization,
  3 = extended foci with red and grey hepatization and presence of adhesions, and
  4 = greatly enlarged lung tissue and "fish flesh" consistency.

A Chi-square analysis was performed on the association between treatment and the proportions of pigs in each lesion category for lesions attributed to acute pneumonia.

Bacteriological examinations

We randomly collected two lung samples per pen at the slaughterhouse (six for each trial group), which we cultured to detect *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Streptococcus suis*, *Staphylococcus* spp, and *Proteus* spp.

Results

Serology

Pigs in both the control group and in the "double SIV" group seroconverted to PRV during the trial period (Figure 1). Antibodies against both serotypes of SIV were consistently present in the control pigs during the trial (Figure 2).

Growth performance data

All three growth parameters measured (ADG, F:G, ATFC) were significantly improved in pigs in all vaccinated treatment groups compared to pigs in the control group (Figure 3).

Pigs in the "double PRV and double SIV" treatment group had significantly higher ADG compared to pigs in all other groups, except during
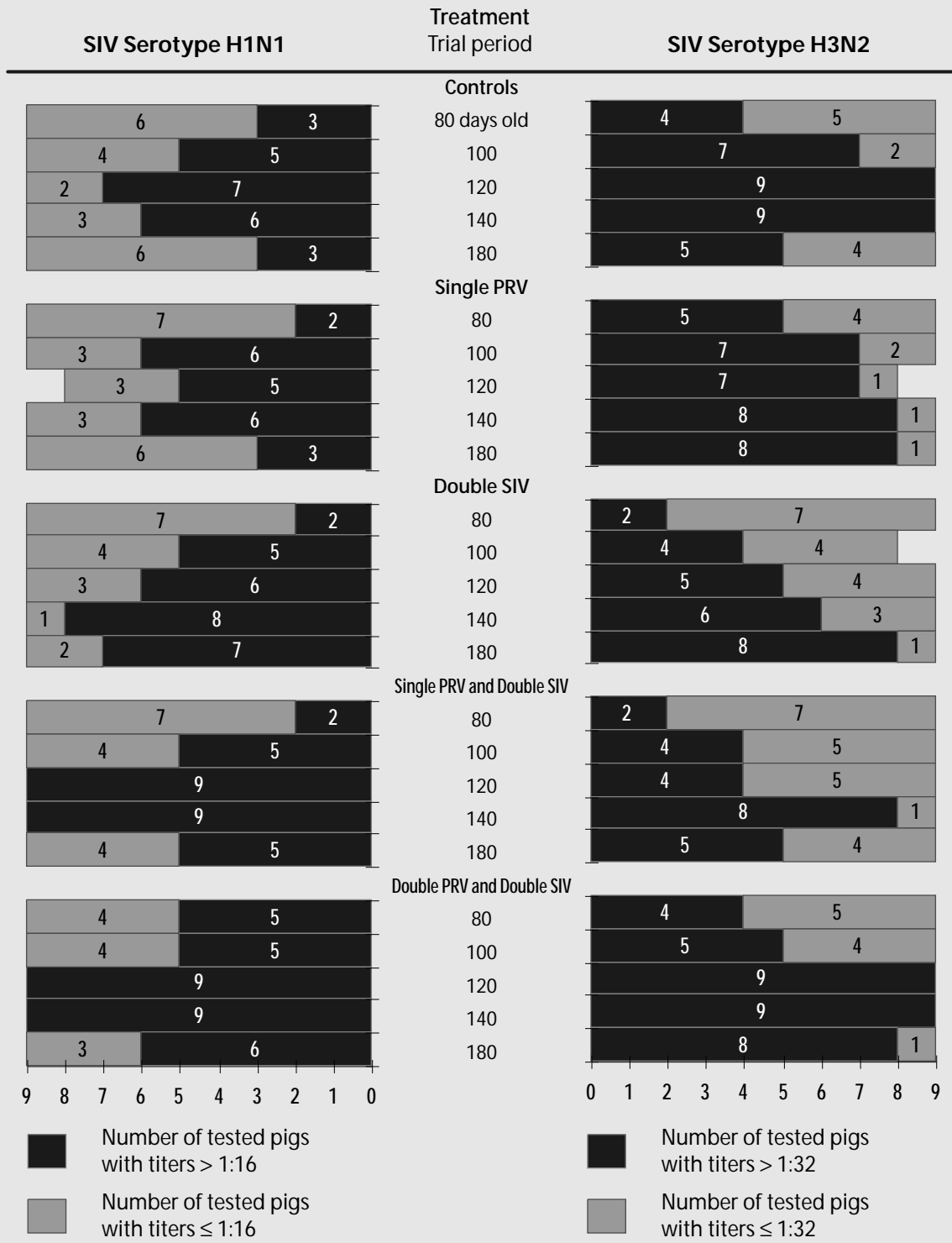

Figure 1

Antibody response to PRV
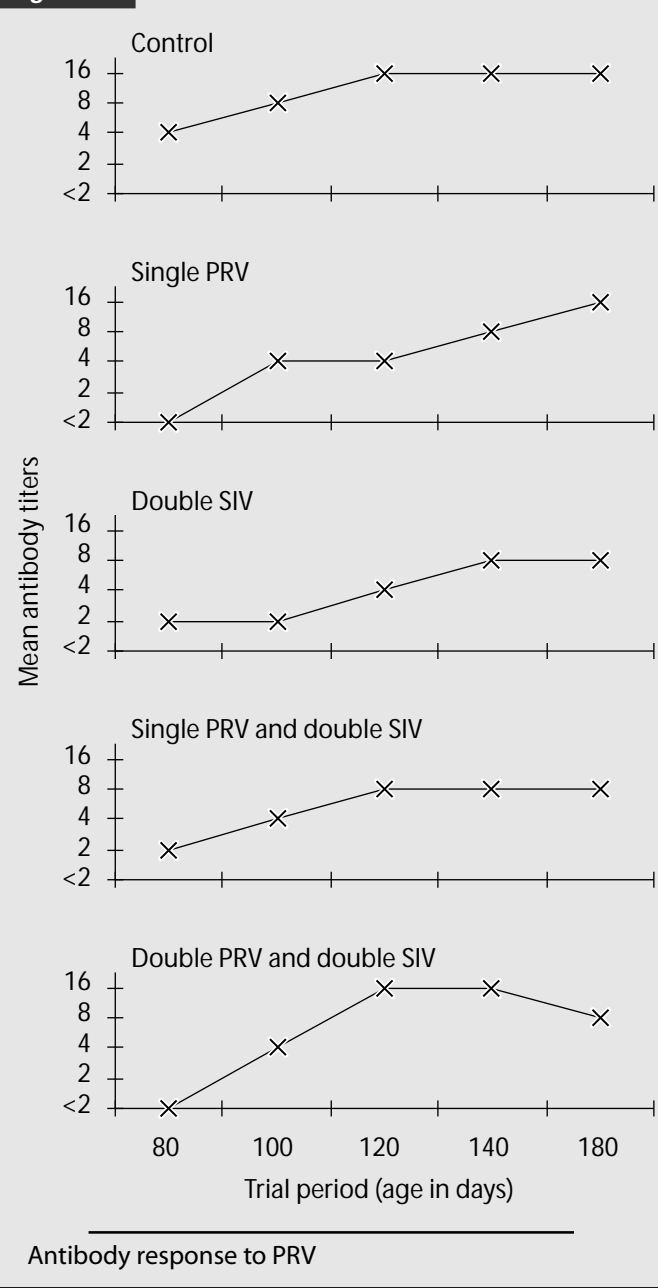
the first trial subperiod, when there was no significant difference between ADG in the "single PRV and double SIV" treatment group and the "double PRV and double SIV" treatment group.

All vaccinated groups had significantly lower F:G values than the control group pigs. Pigs in the "double PRV and double SIV" group had significantly better F:G and ATFC ($P < .05$) compared to the other vaccinated trial groups.
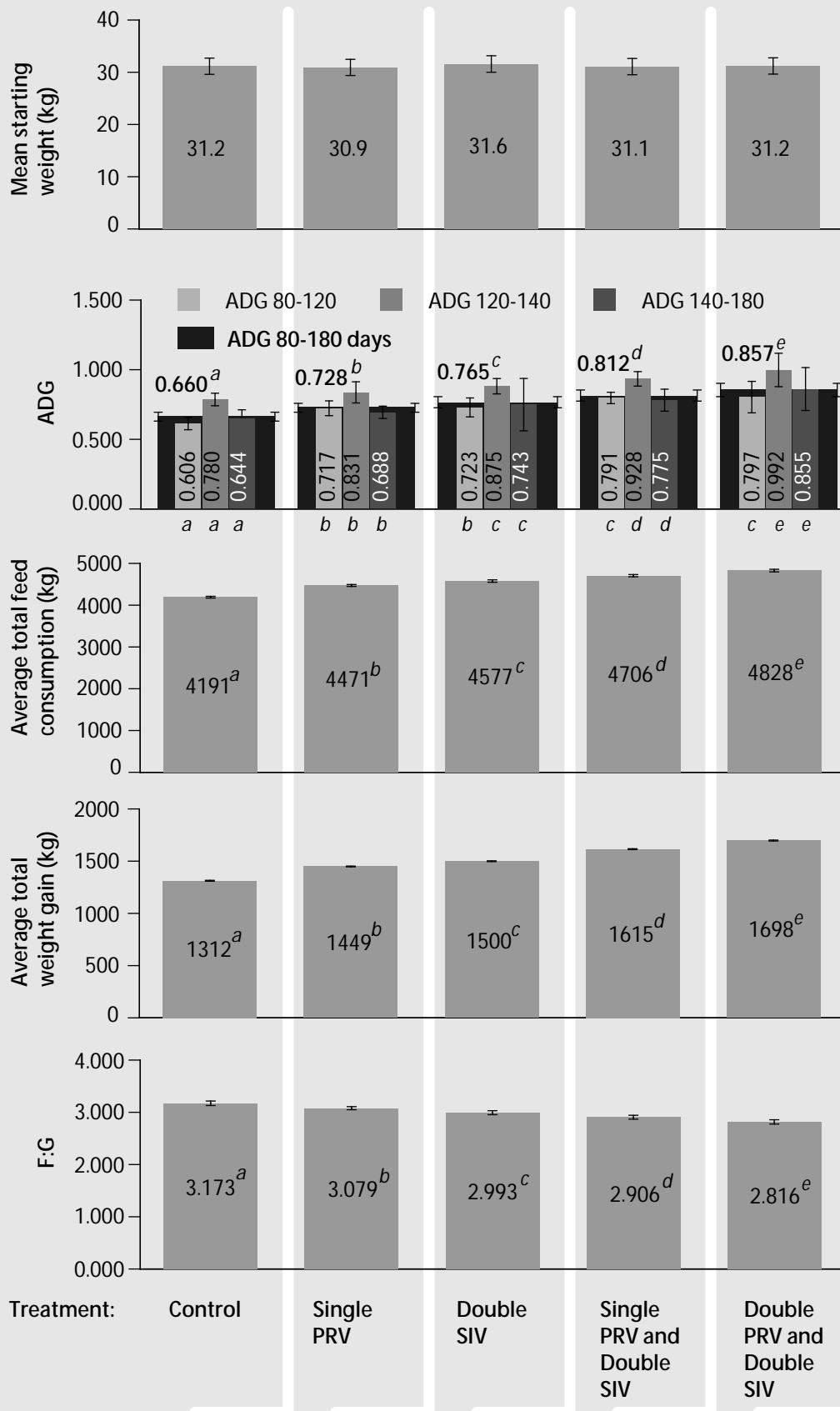
Health monitoring

Mortality during the trial was 0% for all experimental groups. However, four trial animals (two from "single PRV" group, one from the "double SIV" group, and one from the "single PRV and double SIV" group) were removed and sent to the slaughterhouse before the end of the experimental period due to severe streptococcal arthritis

Figure 2

SIV Serotype H1N1

Treatment
Trial period

SIV Serotype H3N2

Controls

H1N1	Days	H3N2
6 / 3	80 days old	4 / 5
4 / 5	100	7 / 2
2 / 7	120	9
3 / 6	140	9
6 / 3	180	5 / 4

Single PRV

H1N1	Days	H3N2
7 / 2	80	5 / 4
3 / 6	100	7 / 2
3 / 5	120	7 / 1
3 / 6	140	8 / 1
6 / 3	180	8 / 1

Double SIV

H1N1	Days	H3N2
7 / 2	80	2 / 7
4 / 5	100	4 / 4
3 / 6	120	5 / 4
1 / 8	140	6 / 3
2 / 7	180	8 / 1

Single PRV and Double SIV

H1N1	Days	H3N2
7 / 2	80	2 / 7
4 / 5	100	4 / 5
9	120	4 / 5
9	140	8 / 1
4 / 5	180	5 / 4

Double PRV and Double SIV

H1N1	Days	H3N2
4 / 5	80	4 / 5
4 / 5	100	5 / 4
9	120	9
9	140	9
3 / 6	180	8 / 1

H1N1 axis: 9 8 7 6 5 4 3 2 1 0
H3N2 axis: 0 1 2 3 4 5 6 7 8 9

■ Number of tested pigs with titers > 1:16
▨ Number of tested pigs with titers ≤ 1:16

■ Number of tested pigs with titers > 1:32
▨ Number of tested pigs with titers ≤ 1:32
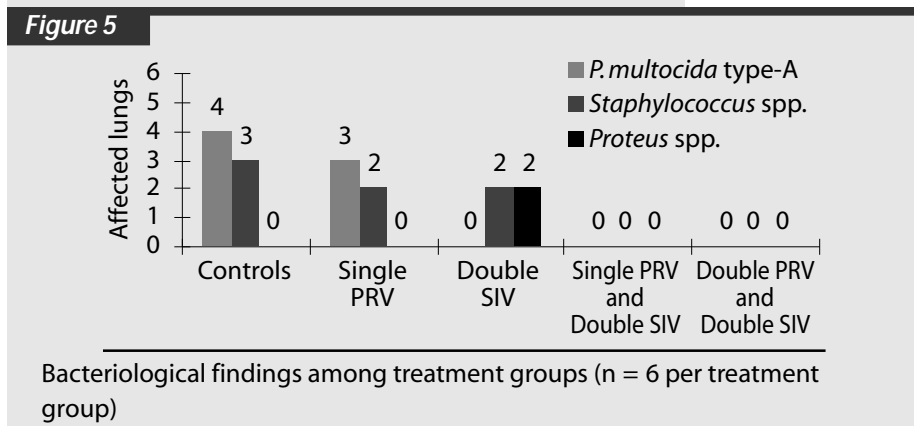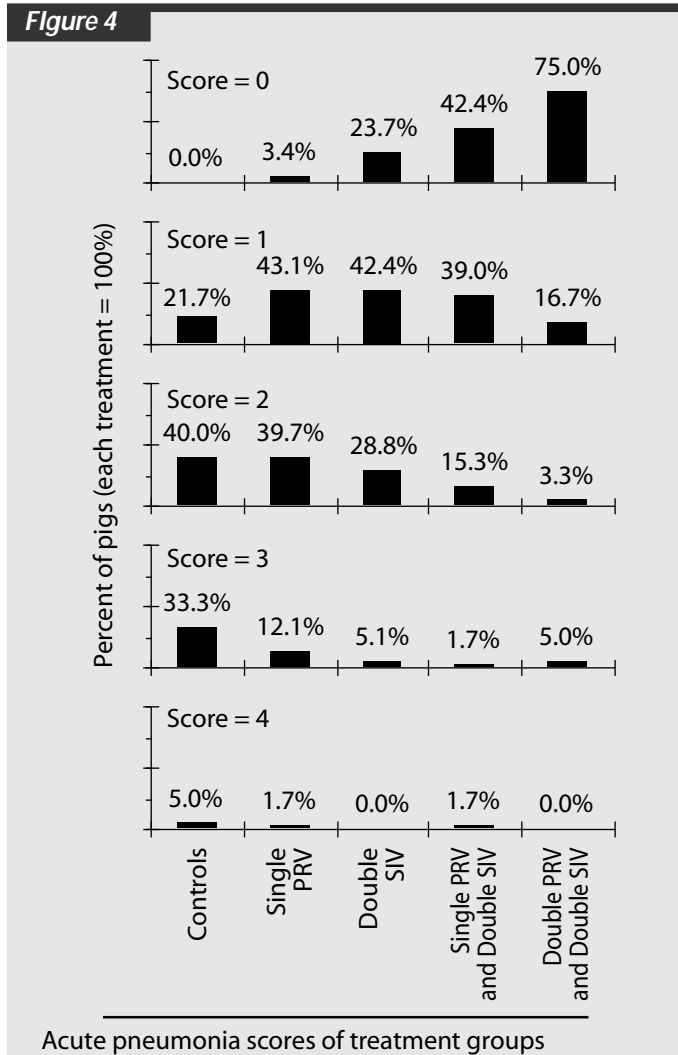
Antibody response to SIV

Figure 3

Growth performance of treatment groups

a…e Within each performance measure, values with different superscripts differ significantly, *P* < .05

characterized by swelling of the tarsal joints, lameness, and unwillingness to move.

Respiratory distress with coughing and sneezing, varying in intensity, appeared in:

- 12 of 60 pigs in the control group,
- 10 of 60 pigs in the "single PRV" group,
- 10 of 60 pigs in the "double SIV" group,
- six of 60 pigs in "single PRV and double SIV" group, and
- three of 60 pigs in "double PRV and double SIV" group.

Figure 4

Acute pneumonia scores of treatment groups

Figure 5

Bacteriological findings among treatment groups (n = 6 per treatment group)

The duration of the symptoms varied from 5–8 days.

Snout morphology

No significant differences in snout morphology were found among treatment groups.

Lung lesions

There was no significant difference in chronic lung lesion scores among all treatment groups.

For acute lung lesions, the distribution of pigs among lesion categories varied significantly among treatments ($P < .001$, Chi square = 153.6) (Figure 4). There was a significantly higher proportion (75%) of pigs in the "double SIV and double PRV" treatment group that had lesion scores of 0 compared with all other groups.

Bacteriology

Pasteurella multocida was isolated from four of six lung samples of the control group and from three of six samples in the "single PRV" group (Figure 5). Nonhemolytic *Staphylococcus* spp were isolated from three of six samples of the control group and from two of six in the "single PRV" and the "double SIV" groups. *Proteus* spp were isolated from two of six lung samples of the "double SIV" group. No pathogens were isolated from the samples of the "single PRV and double SIV" and the "double PRV and double SIV" groups.

Discussion

Pseudorabies virus and SIV antibodies in the control group pigs indicated the presence of these viruses in the study herd during the trial period. The relatively low HI titers observed in the groups of animals vaccinated against SIV is remarkable and, in association with the results obtained for growth performance, may indicate that intradermal vaccination provides sufficient protection for finishing pigs even when the level of antibodies is lower than that provided by intramuscular vaccination.[13]

Our failure to detect pathogenic bacteria in the lung samples of pigs in the "double PRV and double SIV" group suggest that double vaccinating against PRV and SIV may eliminate the presence of pathogens in the lungs as secondary complicating factors.

The similar ADG of pigs from 80–120 days of age in the "single PRV and double SIV" and the "double PRV and double SIV" groups is the only exception to our observation that the double-vaccinated pigs had superior growth performance compared with pigs in all other treatment groups for all parameters measured. The superiority of double-vaccinated animals against both PRV and SIV in growth performance may indicate that double vaccination against PRV and SIV can protect pigs from the clinical manifestation of a mixed infection with the simultaneous presence of PRV and SIV.

The increased lung lesion scores in control pigs, in association with the bacteriologic findings, suggest that vaccination against PRV and SIV may reduce secondary bacterial complications.

Our administration of oxytetracycline to animals found coughing and sneezing during the study probably confounded the lung lesion findings, helping many of the lesions to resolve before slaughter. It seems likely that the lung lesions would have been more severe in those animals that received antibiotics than the slaughter lung lesion scores they actually received.

The complete absence of tissue irritation at the site of injection and the reduced labor associated with intradermal injection make this the preferred vaccination strategy for PRV and SIV vaccines.

Since this trial was conducted, this herd has become infected with PRRSV. The best vaccination protocol must be reconsidered in light of the availability of live attenuated vaccines against PRRSV.[20]

Implications

- Double vaccination of finishing pigs against PRV and SIV can improve growth performance and eliminate the negative effects of secondary pathogens.
- Double vaccination appears to be a valuable measure and is recommended for preventing viral respiratory diseases of growing/finishing pigs.
- Intradermal vaccination with a needleless device appears to be an effective means of administering vaccine.

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Practice tips

Fly control

Each week, write the date on some 3" × 5" notecards and pin them up in various areas of the swine barn. After 7 days, count the number of flyspecks on each card. If the cards exceed 50 flyspecks, take preemptive action to control the fly population. The fly population is getting ready to escalate dramatically because of warmer conditions. This tip was "stolen" from the poultry industry.

— Butch Baker, DVM