Evaluation of the Recrudescence of *Actinobacillus pleuropneumonia* in Growing Pigs Following Pulmotil Treatments in Nursery and Grower

L.K. Clark, K.E. Knox, and C.C. Wu
Departments of Veterinary Clinical Sciences and Veterinary Pathobiology

Introduction

Pulmotil is effective at preventing clinical outbreaks of respiratory disease caused by *Actinobacillus pleuropneumoniae* (App) and *Pasteurella multocida* (Pm) when fed at 200-400 ppm. Dose determination studies with Pulmotil indicated that App could not be isolated from lungs of pigs following a 21-day treatment with 300 or 400 ppm Pulmotil. Studies examining the recrudescence of clinical disease in pigs following Pulmotil treatment have not been performed. We investigated the potential for recrudescence of App following Pulmotil treatment at 400 ppm in nursery or growing pigs.

Materials and Methods

Experimental design: A 2 x 2 x 2 factorial experimental design was used. Two Pulmotil treatments were tested (0 ppm and 400 ppm) in either nursery or growing pigs. Two dexamethasone treatments (10 mg/kg on 2 consecutive days, and 0 mg/kg) were used to enhance clinical expression of disease after Pulmotil treatment. Randomly selected pigs were necropsied at 3 and 5 weeks after cessation of Pulmotil feeding to monitor lung lesions and to determine infection with App and Pm.

Trial facility: Four disease isolation rooms were used for this experiment to minimize horizontal disease transmission among test groups. Each room contained four 4'x4' pens with solid dividers on raised decks. Each pen contained a feeder and nipple waterer.

Feed preparation and assay: All test diets were prepared at a commercial feed mill and assayed for Pulmotil concentration prior to shipment to the test facility.

Selection of test animals: Experimental pigs were selected from a commercial herd of swine that was endemically infected with App serotype 1. Serum was collected from ten 2-week-old nursing pigs for analysis for App by ELISA to verify herd infection with App serotype 1. Also, App isolation was attempted from tonsils of 4 clinically ill or dead grower pigs.

Trial animal procedures: Approximately 120 test pigs were selected and identified with ear tags on entry into the nursery. Although the management usually vaccinated all pigs for App, the test pigs were not vaccinated. Each treatment group of pigs (Pulmotil-nursery; Control-nursery; Pulmotil-grower; Control-grower) was identified with different colored tags. Each diet containing 400 ppm Pulmotil was fed for 21 days to 3 pens (30 head) of nursery pigs, and later to 3 pens (30 head) of growing pigs. The medicated feeds were fed to the growing pigs at least 7 days prior to an anticipated outbreak of App. At the end of the Pulmotil medication of pigs in the nursery, 24 Pulmotil-medicated and 24 control pigs were selected and transported to the test facility where they were weighed, and randomly allocated to treatment. At the end of the

Pulmotil medication of pigs in the grower, 24 Pulmotil-medicated and 24 control pigs were selected and transported to the test facility where they were weighed, and randomly allocated to treatment. Serum samples were collected from each pig at allocation and at necropsy.

Each of the 4 rooms housed 4 pens of pigs (6 pigs/pen). Both dexamethasone-treated and control pigs of each Pulmotil treatment group were placed in the same room (i.e., 24 pigs that received 400 ppm Pulmotil were in the same room; 2 pens of pigs received dexamethsone). The procedure was repeated for nursery and grower pigs.

All pigs received non-medicated diets after the initial, on-farm medication period.

In order to stimulate clinical disease, all pigs in half the pens in each room received dexamethsone IM 10 mg/kg for 2 consecutive days beginning 14 days after Pulmotil feed was withdrawn. Pens were randomly selected within each room for injection.

Half of the pigs from each treatment were weighed, euthanatized, necropsied and cultured at 21 and at 35 days after Pulmotil withdrawal from feed. Lung lesions were recorded for all pigs.

Measurements and Analyses

Clinical measurements: All pigs were weighed as they entered the disease isolation facilities at Purdue and at necropsy. Daily gains were calculated for all pigs. All pigs were monitored daily for clinical illness. Each pig was assigned a clinical impression score (0=normal to 3=moribund) daily. Any severely ill pig was euthanatized, necropsied, and culturally examined. Clinical impression scores were compiled daily for all pigs.

Disease serology: Serum samples collected from each pig as they entered the disease isolation facilities and at necropsy were assayed for antibodies to App. Differences of mean optical densities (reported as S/P ratios) and number of pigs seropositive and negative between the treatment groups were determined.

Isolation of respiratory pathogens: Nasal swabs, tonsil swabs and lung parenchyma from each pig were culturally examined for App and Pm. Differences in number of pigs positive or negative for the different organisms (determined by cultural examination) between the treatment groups were determined.

Lung Lesions: Differences in lung lesion scores as determined by digitization of lung lesion drawings (our standard method) were determined.

Results and Discussion

Growth: We hypothesize that the reason the treated and untreated groups of pigs removed from the nursery when 6 weeks old weighed the same (P>.90) was that some of the untreated pigs that were small or died were not moved to Purdue University (Table 1). Thus, only the best pigs of each group were moved and had not been exposed to the infectious agents long enough for the Pulmotil treatment to have an unequal effect on the pigs. During the 3 or 5 week period these pigs were in the isolation rooms, the Pulmotil group of pigs gained 0.84

pounds per day whereas the untreated pigs only gained 0.57 pounds per day (P<.0007). An effect of dexamethasone on growth rate in the pigs was not observed. We attribute the difference in ADG between the treated and untreated pigs to the removal of pathogens from the treated pigs during the 21 days they were treated in the feed prior to entry to isolation. We hypothesize that the lower pathogen load at entry enhanced the growth rate of these pigs.

The pigs treated with Pulmotil from 11 to 14 weeks of age were heavier than their untreated contemporaries (P<.0001) at entry to the isolation facilities (Table 2). We suspect that the difference was due to the presence of a high load of infectious agents in the grower pigs during this period of growth which, without Pulmotil treatment, would have reduced the treated pigs' growth rate as it did for the untreated pigs. The Pulmotil-treated grower pigs had a higher ADG than the untreated grower pigs (0.62 vs. 0.50, P<.004). These results indicated that Pulmotil not only increased growth during the period of treatment, but maintained that growth differential 3 and 5 weeks after it was removed from the feed if pigs were moved to clean facilities after treatment.

Clinical scores and coughing: The clinical scores (a measure of health) were significantly different between the Pulmotil-treated and untreated nursery pigs (P<.0001; Table 1) and grower pigs (P<.002; Table 2). These results further support the benefit of Pulmotil use in young pigs to enhance the health of pigs during later growth. Coughing scores between the Pulmotil-treated and untreated groups of nursery pigs (Table 1) were not different (P=.50), nor were they different (P=.17) between respective groups of grower pigs (Table 2). These results were expected, as Pulmotil is most effective against respiratory bacteria which do not induce coughing.

Serology: Upon entry to the isolation unit, all Pulmotil-treated 6-week-old nursery pigs were seronegative to App 1 and remained seronegative until they were euthanatized (Table 1). Two untreated pigs were seropositive to App at entry, and 2 different pigs were seropositive at the time of euthanasia. These results indicated that the pigs' dams were seropositive to App1 and passed colostral antibodies to their pigs. These titers decayed in most pigs by 6 weeks of age, such that only the 2 pigs remained seropositive at entry to the isolation facilities. In most endemic herds, App 1 does not clinically present until pigs are 14 to 16 weeks old, and even this presentation time is variable dependent on herd management (i.e., earlier in poorly managed herds). However, the 2 control pigs that were seropositive at 77 days of age when euthanatized were seronegative when tested at entry. This indicated evidence of subclinical infection in these untreated pigs, as no clinical evidence of App infection was observed. These 2 pigs could have served as carriers and under certain stressful conditions spread App to their penmates.

Interestingly, the 2 groups of contemporary pigs that remained on the farm were treated with Pulmotil in the grower from 11 to 14 weeks of age before they were placed in isolation facilities. Eighty-eight percent of the untreated pigs were seropositive for App, while none of the treated pigs had seroconverted to App. These results indicated that untreated pigs on this farm were exposed to App between 11 and 14 weeks of age.

In the pigs that were treated in the grower, those treated with Pulmotil were seronegative to App 1 at entry when 14 weeks old, whereas most (21/24) were seropositive if untreated (Table 2). These results were highly significant (P<.0001), indicating a very significant effect of the use

of Pulmotil on the colonization and immune system recognition of App 1 when used in the grower. A similar effect of Pulmotil was also observed in these pigs at the time of necropsy when the pigs were either 17 or 19 weeks old (Table 2). However, some of the Pulmotil-treated pigs had seroconverted to App 1, which indicated that these pigs had carried the organism, probably in the tonsils, throughout the treatment period and that the organisms had colonized these pigs in sufficient numbers to stimulate an immune response. Thus, we hypothesize that the Pulmotil-treated pigs that had seroconverted could serve as carriers of the disease and under stressful conditions initiate an outbreak of the disease at this age.

Respiratory pathogen isolation: The pattern of bacterial isolation of the pigs treated in the nursery with Pulmotil is difficult to explain (Table 1). By design, the pigs' tissues were culturally examined 3 or 5 weeks after the Pulmotil was discontinued, which allowed ample time for colonization or re-colonization of the lungs by App 1 and Pm. As for App, we were unable to isolate this agent from the tissues of any of the nursery-treated pigs; however, we were able to isolate App 1 from tissues of 2 pigs in the untreated nursery group. The pigs that we cultured App from were not the same pigs that were seropositive at this time; however, these results indicate further that subclinical infection in the untreated group of pigs was occurring. The lack of statistical significance between the treated and untreated pigs may have been due to the sensitivity of our cultural tests. We were able to isolate Pm from fewer pigs in the treated group than the untreated group (8 versus 14), but again no statistical significance was observed.

The pattern of bacterial isolation in the tissues of grower pigs (Table 2) indicated that Pulmotil had no effect ($P>.08$) on colonization of App 1 and Pm. We were unable to isolate App 1 in any tissues of any pigs at necropsy, even though many pigs were seropositive to this organism in both groups. Obviously, the organism was there, as seroconversion indicated that the immune system had recognized the presence of the organisms. We assumed that our cultural method for isolation of the organisms was not as sensitive as the pigs' immune system was for this organism. We were able to detect Pm in about 1/2 to 3/4 of the pigs whether they were treated with Pulmotil or not. These results suggested that had Pulmotil had an effect on the ability to isolate these organisms, that effect had been lost by the time the pigs were necropsied.

Lung scores: The mean lung lesion scores (7.3 and 10.0) of the untreated and treated pigs was not different ($P>.50$) in the portion of the study in which nursery pigs were treated with Pulmotil. In the portion of the study in which Pulmotil was used in grower pigs (pigs treated when 11-14 weeks of age), we observed a reduction in mean lung lesion scores from 14.7 to 8.7 ($P<.07$) in the Pulmotil treated pigs versus the untreated pigs (Table 2).

Summary

1. Pulmotil used for the first 3 weeks after weaning at 21 days improves the performance of pigs through the first 9-11 weeks of life.

2. In this study, we were unable to isolate App from pig tissues at 3 and 5 weeks after Pulmotil was withdrawn when Pulmotil was used for the first 3 weeks after weaning. These pigs also remained serologically negative to App.

3. Pulmotil when used from 11 to 14 weeks in the grower improved the performance of pigs through the 5 weeks subsequent to the treatment.

4. The serological results for App 1 indicated a reduction in organism exposure due to the use of Pulmotil in nursery and particularly in grower pigs.

5. No difference in cultural isolation of respiratory agents was observed in pigs treated with Pulmotil in the grower.

6. Lung lesions were reduced in pigs euthanatized at 17-19 weeks of age when pigs were treated with Pulmotil in the grower.

No matter whether pigs were treated with Pulmotil in the nursery or grower, treated pigs were clinically healthier than untreated pigs.

Table 1. The effect of Pulmotil treatment in early stage nursery pigs.

			Control	(P<)	Pulmotil
Weight	Entry		20.03	(.90)	20.2
	ADG		.57	(.0007)	.84
Clinical Scores	(+/all)		127/1288	(.0001)	17/1288
Coughing	(+/all)		72/591	(.17)	87/561
Serology	App1 ELISA	Entry	.16	(.56)	.17
		Terminal	.15	(.56)	.11
	App (+/all)	Entry	2/25	(.50)	0/24
		Terminal	2/25	(.50)	0/24
Bacteria	APP (+/all)		2/25	(1.0)	1/24
	PM (+/all)		14/25	(.15)	8/24
Lung scores			7.3	(.50)	10.0

Table 2. The effect of Pulmotil treatment in early stage grower pigs.

			Control	(P<)	Pulmotil
Weight	Entry		49.3 ± .74	(.0001)	65.9 ± 1.32
	ADG		.50 ± .02	(.004)	.62 ± .02
Clinical scores	(+/all)		33/1008	(.0014)	11/1008
Coughing			105/533	(.50)	115/533
Serology	App1 ELISA	Entry	.49 ± .03	(.0001)	.15 ± .01
		Terminal	.48 ± .04	(.0001)	.26 ± .03
	App (+/all)	Entry	21/24	(.0001)	0/24
		Terminal	18/23	(.0001)	5/24
Bacteria	APP (+/all)		0/24	NA	0/24
	PM (+/all)		17/24	(.08)	10/24
Lung scores			14.7 ± 2.4	(.07)	8.7 ± 2.2