ORIGINAL RESEARCH

Efficacy of tilmicosin, and a combination of tylosin and sulfamethazine, for control of swine atrophic rhinitis involving infection with toxigenic *Pasteurella multocida* type D

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Summary: *Tilmicosin, 20-deoxo-20-(3,5-dimethylpiperidin-l-yl) desmycosin, is a new semi-synthetic macrolide antibiotic that has been reported to have excellent in vivo and in vitro effect against both Gram-positive and Gram-negative bacteria, including* Pasteurella multocida. *In its injectible form, under the trademark of Micotil®, it is approved for control of shipping fever/pasteurellosis in cattle. In swine, parenterally administered tilmicosin is toxic even at low dosage levels. However, when orally administered, tilmicosin does not cause toxic reactions. Two trials were conducted to test the ability of tilmicosin as a feed additive to prevent transmission of progressive atrophic rhinitis (AR), involving* P. multocida, *from endemically affected pigs to AR-free pigs. The test pigs were 3 weeks old at the start of the trial. Tilmicosin fed continuously over 6 weeks at concentrations of 200 g per ton of feed controlled transmission of AR both in Trials 1 and 2. Weight gains were positively affected, and there were fewer nasal swabs positive for* P. multocida *at the end of the study period.*

Tilmicosin, 20-deoxo-20-(3.5-dimethylpiperidin-l-yl) desmycosin, is a semi-synthetic macrolide antibiotic developed by Lilly Research Laboratories.[1] Excellent in vitro activity is reported for Gram-positive bacteria and mycoplasma, and also for certain Gram-negative microbes such as *Actinobacillus pleuropneumoniae, Pasteurella hemolytica,* and *Pasteurella multocida.*[1,2] Under the trade mark of Micotil®, it is approved for treatment of bovine respiratory disease (shipping fever associated with *P. hemolytica* and *P. multocida*) at a dosage level of 10 mg per kg injected subcutaneously (sc).[3,4,5,6] Cattle tolerate dosages as high as 50 mg per kg when the drug is administered sc but show toxic reactions that indicate cardiovascular damage when injected intravenously at doses as low as 5 mg per kg. Pigs show toxic reactions (increased respiration, emesis, convulsions) at 10 mg per kg administered intramuscularly.[7]

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If tilmicosin is administered orally, it greatly reduces the risk of toxic reactions in pigs. No adverse reactions were seen when tilmicosin was administered at a concentration of 300 g per ton of feed, (which equals 15–18 mg tilmicosin per kg bodyweight at an expected feed consumption of 0.04–0.06 kg feed per kg bodyweight). The concentration of tilmicosin in the lung was 2.16 µg per g of tissue, equaling 12%–14% of the oral dosage of the drug.[8] This was low in comparison to the 9.8 µg per g concentration of tilmicosin found in the lungs of cattle 24 hours post sc injection of 10 mg tilmicosin per kg bodyweight,[8] but still high enough to justify tests of the drug's ability to prevent transmission of swine diseases with bacteria sensitive to tilmicosin.

This article describes two efficacy studies of tilmicosin against transmission of atrophic rhinitis involving toxigenic *P. multocida*, orally administered at the levels of 100 and 200 g per ton of feed for 6 weeks (3–9 weeks of age) to AR-free pigs housed in nose-to-nose contact with pigs with endemic AR involving toxigenic *P. multocida* type D. The results were compared to nontreated controls and oral treatment with tylosin and sulfamethazine at 100 g + 100 g per ton of feed.

Materials and methods

Pigs

Trial 1

We used 40 crossbred AR-free barrows as recipient pigs and 24 pigs from a herd with endemic AR as seeder pigs. The AR-free pigs (Herd A) originated from the University of Wisconsin (UW) Specific-Pathogen-Free (SPF) Swine Center, Arlington, Wisconsin. Since its origin in 1984, the Wisconsin SPF Swine Center herd has always been free of AR, as determined by slaughter checks and cultures of nasal swabs. The AR-positive herd (Herd B) had a long history of clinical AR problems, and slaughter checks of market hogs revealed moderate to severe turbinate atrophy in 80% of the hogs. Nasal swabs of 6-week-old pigs in Herd B showed 25%–42% positive for combinations

of *Bordetella bronchiseptica*, and nontoxigenic *P. multocida* type A (PmA-) and toxigenic and nontoxigenic *P. multocida* type D (PmD+ and PmD-). The pigs were offspring of sows not being vaccinated for *B. bronchiseptica* and *P. multocida*, nor had they received medicated feed prior to the trial. At the start of the trial the recipient pigs were 3 weeks old and the seeder pigs were 6 weeks old. The seeder pigs already showed clinical signs of AR at the start of the trial.

Trial 2

Seventy healthy crossbred barrows and gilts, weaned at 3 weeks of age, were obtained from the University of Wisconsin SPF Swine Center. Cultures of nasal swabs were all negative for *P. multocida* and *B. bronchiseptica*. Forty-eight seeder pigs were purchased from another herd (Herd C) with endemic AR, documented by clinical history, necropsies, slaughter checks, and positive culture swabs for *B. bronchiseptica* and toxigenic *P. multocida* type D. The prevalence of AR lesions and positive nasal cultures was similar to Herd B.

Pig allotment

All pigs were carefully examined at each farm before and at the start of the trial. Upon arrival, all pigs were weighed, individually identified with ear tags, and randomly allocated to pens/rooms and treatments on the basis of weight (Table 1).

Trial facilities and herd management procedures

We conducted the study in January, February, and March. We kept all pigs in a total of 13 identical isolation rooms at the Animal Resource Center, School of Veterinary Medicine, University of Wisconsin-Madison. Each room was equipped with one 40.5 sq ft (3.76 m^2)pen and one 37 sq ft (3.4 m^2) pen, allowing nose-to-nose contact between the pens. Thus, the AR-free pigs received continuous challenge with AR infection from the seeder pigs by aerosol and nose-to-nose contact throughout the 6 weeks of the study. The AR seeder pigs and the control pigs received no medication.

The rooms had self-feeders and automatic waterers and were bedded with wheat bran. We maintained the room temperature at 24°C with an optimal rate of air exchange. After we gave pigs 3 days to adjust to the pens, we gradually reduced the amount of bedding and lowered the room temperature to 16°C to mimic common environmental winter conditions. Each room was equipped with separate equipment for feeding, cleaning, and handling. A foot bath was set outside each room to minimize carry-over of infectious agents between rooms.

Experimental design

Trial 1

Trial 1 ran for 6 weeks (3-9 weeks of age for the test pigs). All seeder pigs were fed nonmedicated feed. Three groups of recipient pigs were medicated with tilmicosin at a level of 200 g per ton of feed. Three groups of recipient pigs served as nonmedicated exposed controls and two groups as nonmedicated, non-exposed controls.

Trial 2 (conducted 1 year after Trial 1)

Trial 2 also ran for 6 weeks. Again, all seeder pigs were fed nonmedicated feed. Of the recipient pigs:

- three groups were medicated with tilmicosin at a level of 200 g per ton of feed;

Table 1

Allotment of pigs into treatment groups

Treatment	AR-free (recipient) pigs			AR challenge (seeder) pigs		
	#pigs per pen	#pens	Total #pigs	#pigs per pen	#pens	Total #pigs
Trial 1:						
Tilmicosin 200 g/ton feed	5	3	15	4	3	12
Nonmedicated, exposed	5	3	14*	4	3	12
Nonmedicated, unexposed	5	2	10			
Trial 2:						
Tilmicosin 100 g/ton feed	5	3	15	4	3	12
Tilmicosin 200 g/ton feed	5	3	15	4	3	12
Tylosin and Sulfamethazine	5	3	15	4	3	12
Nonmedicated, exposed	5	3	15	4	3	12
Nonmedicated, unexposed	5	2	10			

*One pig died at the start of the trial.

- three groups were medicated with tilmicosin at a level of 100 g per ton of feed; and
- three groups were medicated with 100 g tylosin + 100 g sulfamethazine per ton of feed.

Three other groups served as nonmedicated exposed controls, and two other groups as nonmedicated, non-exposed controls.

Ration formulation and feed assays

The UW-Arlington feed mill provided the feed, which met the standard nutritional requirements of the test pigs. The sponsor provided tilmicosin and tylosin + sulfamethazine, which were mixed with the feed at the UW Arlington feed mill.

We took two samples of all batches of feed and mailed them to the sponsor for drug concentration assays. The assays showed that the concentrations of the drugs in the feed were within the approved range.

Efficacy measurements and records

Pigs were weighed at the start and end of the trial. For any trial drop-outs, we recorded the identity of the pig, reason for elimination (e.g., mortality and apparent cause), date, and drop-out weight. We recorded pig weights, snout scores, and nasal cultures for each pig and recorded feed consumption for each group of pigs to determine average daily gain (ADG) and feed : gain (F : G) ratio. Pigs were checked daily for clinical signs of AR.

We used a visual scoring method to assess the degree of turbinate atrophy and a semi-automatic digitizing tablet method we previously developed at our lab to measure turbinate perimeter ratio.[9]

We obtained nasal cultures for all pigs at the start and end of trial and evaluated them for the presence of toxigenic and nontoxigenic *P. multocida* types A and D and *B. bronchiseptica*, according to standard culture and testing techniques previously described.[10,11]

We used analysis of variance to evaluate overall differences among groups for AR visual scores, turbinate perimeter ratios, ADG, and F : G. Fisher's least significant difference test was used to determine differences between means when significant F values were found.

Results

Throughout the study, none of the tilmicosin-treated pigs showed signs of adverse reaction to the drug.

Within the first 3-4 days of exposure to the seeder pigs, several of the recipient pigs became ill with signs of *Hemophilus parasuis* infection. One pig in each trial died. In Trial 1 all the other pigs recovered clinically within several days.

In Trial 2, chronic lesions of mild pleuritis and pneumonia were found in some of the pigs at the time of slaughter. We found mild pleuritis in:

- two of the 15 pigs in the nontreated exposed group;
- two of the 14 pigs fed tilmicosin at 100 g per ton;
- one of the 15 pigs in the group fed tilmicosin at 200 g per ton; and
- one of the 15 pigs fed tylosin-sulfamethazine.

We found pneumonia in:

- five of the 15 pigs in the nontreated exposed group;
- three of the 14 pigs in the group fed tilmicosin at 100 g per ton;
- two of the 15 pigs in the group fed tilmicosin at 200 g per ton; and
- no pneumonia in the 15 pigs in the group fed tylosin-sulfamethazine.

The seeder pigs showed clinical signs of AR with sneezing, tearing of eyes, and some twisting and shortening of snouts. Some of the recipient pigs developed similar clinical signs by the end of the study. However, most of them showed few or no clinical signs during the trial period. The nonmedicated nonexposed pigs showed the best results for weight gain and nasal cultures in both trials (Table 2). Among the treatments groups, tilmicosin at the concentration of 200 g per ton of feed was superior for AR score, while ADG was similar for both dosage levels of tilmicosin and for tylosin-sulfamethazine (Table 2). Feed efficiency did not differ from the nonmedicated exposed pigs. Tilmicosin at 100 g per ton only showed marginally positive effects, similar to the tylosin-sulfamethazine treatment. There was no explanation for the slower growth of pigs in Trial 1.

B. bronchiseptica was isolated from 33%–83% of nasal swabs in all exposed groups. Apparently, neither of the treatments we used had any effect on *B. bronchiseptica*. *Pasteurella multocida*, in particular toxigenic *P. multocida*, appeared to be less prevalent in the nasal swabs of tilmicosin-treated pigs (Table 2).

Discussion

As in previous studies, the transmission model we used in this project was effective.[9,10,11] When housed together with fence line (nose-to-nose) contact for several weeks, AR-affected pigs transmitted the disease to healthy test pigs, thus mimicking natural transmission under field conditions. Unfortunately, the seeder pigs can also transmit other diseases, such as *H. parasuis*, as in this study. None of the feed additives used in the study appeared to protect against *H. parasuis*. There are no data available regarding sensitivity of tilmicosin to *H. parasuis*. Except for the pig that died, the disease was mild, and weight gain and performance did not differ from the other test pigs.

The results of the study indicated that tilmicosin at 100 g per ton had only a marginally prophylactic effect compared to 200 g per ton of feed. To achieve a therapeutic effect, higher dosages might be needed, in particular since lung tissue concentrations appear to be relatively low when the product is

Table 2

Results among 3- to 9-week old pigs of turbinate atrophy scores, nasal cultures, weight gain, and feed intake during the 6-week study period in Trials 1 and 2

Treatment	N	ADG	F : G	AR scores* visual	AR scores* TPR	% nasal cultures positive for ‡ Bb	PmA-	PmD-	PmD+
Trial 1									
Tilmicosin 200 g/ton feed	15	339 (a)	2.63 (a)	0.52 (a)	1.25 (a)	83	20	0	0
Nonmedicated exposed	14†	199 (b)	3.79 (b)	1.57 (b)	0.73 (b)	57	21	20	9
Nonmedicated unexposed	10	393 (c)	2.54 (c)	0	1.32	0	0	0	0
Trial 2									
Tilmicosin 200 g/ton feed	15	426 (a)	1.94 (a)	0.37 (a)	1.26 (a)	53	7	7	0
Tilmicosin 100 g/ton feed	14†	430 (a)	2.01 (a)	0.82 (b)	1.10 (bc)	64	7	0	0
Sulfameth 100 g and Tylosin 100 g	15	411 (ab)	2.19 (b)	0.77 (b)	1.16 (cd)	47	20	7	7
Nonmedicated exposed	15	373 (b)	2.04 (a)	1.13 (b)	1.02 (b)	33	20	13	7
Nonmedicated unexposed	10	462 (a)	2.13 (a)	0	1.45	0	0	0	0

Bb: *Bordetella bronchiseptica;* **PmA-:** *Pasteurella multocida* type A–; **PmD-:** *Pasteurella multocida* type D–; **PmD+:** *Pasteurella multocida* type D+.

*Visual AR score (0–3) according to Bäckström, et al.[10] and turbinate perimeter ration (TPR) score according to Collins, et al.[9]

† One pig died at the start of each trial.

‡ The nasal swabs were examined at the end of the study. We considered the number of positive nasal swabs too small to warrant statistical analysis, particularly in light of the high number of false positives we commonly note for this pathogen.

Different letters in parentheses between means in the vertical columns indicate $P \leq .05$ differences when using Fisher's least significant difference method for statistical analysis.

administered orally to swine.[8] Reduced appetite in sick pigs might also reduce the intake of the drug. Because toxicity is reported when tilmicosin is administered by injection, high dosages of the drug will have to be tested with care.[7]

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