Effects of oral administration of tilmicosin on pulmonary inflammation in piglets experimentally infected with Actinobacillus pleuropneumoniae

Erin M. Nerland, MSc; Justin M. LeBlanc, BSc; Jason P. Fedwick, BSc; Douglas W. Morck, DVM, PhD; John K. Merrill, PhD; Paul Dick, DVM, MSc; Marie-Anne Paradis, DVM; Andre G. Buret, PhD

Colonization of the lower respiratory tract with Actinobacillus pleuropneumoniae is responsible for pleuropneumonia in pigs. This highly contagious porcine disease is found worldwide and may be fatal to piglets within 24 to 48 hours of infection. Lungs taken from pigs with A. pleuropneumoniae infection reveal fibrotic necrotic lesions distributed on the cardiac and apical lobes and on the dorsal portion of the diaphragmatic lobe. Lesioned areas of the lungs contain high numbers of viable or necrotic neutrophils, which are major contributors to the tissue damage associated with pleuropneumonia. The primary role of these neutrophils is to destroy and eliminate invading bacteria. However, neutrophils also release cytokines and lipid mediators, such as leukotriene B₄, which are potent inducers and amplifiers of the local inflammatory response. Moreover, in the inflamed lung, neutrophils may die via necrosis or apoptosis. Apoptotic cell death is less damaging to host tissue than necrosis. In contrast with necrotic cells, apoptotic cells maintain their integrity and typically have condensation and fragmentation of nuclear chromatin, the formation of mononucleosomes and oligonucleosomes, and the subsequent cytoplasmic budding of extruded apoptotic bodies. Apoptotic cells also express phosphatidylserine on the surface of their plasma membrane, which is one of the receptor-mediated recognition systems that permit macrophages to phagocytose these cells. The nonphlogistic nature of this disposal mechanism is a major contributor to the resolution of inflammation.

Tilmicosin is used as an SC formulation to treat and prevent respiratory infections in cattle or as a feed formulation to control bacterial pneumonia in swine. The treatment success of tilmicosin has been attributed to its accumulation in appropriate tissues and low inhibitory concentrations. Tilmicosin has a high affinity for uptake within neutrophils, in which intracellular concentrations 40 times greater than those achievable in the serum have been reported. Results of recent studies indicate that injectable tilmicosin induces apoptosis in bovine neutrophils, regardless of the presence or absence of live bacteria. The proapoptotic effect of tilmicosin is in part drug and cell specific, and the antimicrobial decreases the synthesis of proinflammatory LTB₄. Consistent with these findings, a recent report revealed that tilmicosin, but not ceftiofur, increases the apoptotic indices of neutrophils in bronchoalveolar lavage (BAL) fluid of steers with signs of chronic respiratory disease. Taken together,

Objectives—To determine the effects of oral administration of tilmicosin in piglets experimentally infected with Actinobacillus pleuropneumoniae.

Animals—Forty 3-week-old specific-pathogen free piglets.

Procedures—Piglets were assigned to 1 of 4 groups as follows: 1) uninfected sham-treated control piglets; 2) infected untreated piglets that were intratracheally inoculated with 10⁷ CFUs of A. pleuropneumoniae; 3) infected treated piglets that were intratracheally inoculated with A. pleuropneumoniae and received tilmicosin in feed (400 ppm [µg/g]) for 7 days prior to inoculation; or 4) infected treated piglets that were intratracheally inoculated with A. pleuropneumoniae and received chlortetracycline (CTC) in feed (1,100 ppm [µg/g]) for 7 days prior to inoculation. Bronchoalveolar lavage (BAL) fluid and lung tissue specimens of piglets for each group were evaluated at 3 or 24 hours after inoculation. For each time point, 4 to 6 piglets/group were studied.

Results—Feeding of CTC and tilmicosin decreased bacterial load in lungs of infected piglets. Tilmicosin delivered in feed, but not CTC, enhanced apoptosis in porcine BAL fluid leukocytes. This was associated with a decrease in LTB₄ concentrations in BAL fluid of tilmicosin-treated piglets, compared with untreated and CTC-treated piglets, and also with a significant decrease in the number of pulmonary lesions. Tilmicosin inhibited infection-induced increases in rectal temperatures, as measured in untreated and CTC-treated piglets. Pulmonary neutrophil infiltration and prostaglandin E₂ concentrations in the BAL fluid were not significantly different among groups at any time.

these observations indicate that the proapoptotic properties of injectable tilmicosin on neutrophils may confer anti-inflammatory benefits to this antimicrobial. Whether tilmicosin may have similar effects in piglets, when administered in feed, remains unknown. By use of a model of piglets experimentally infected with *A. pleuropneumoniae*, purposes of the study reported here were to determine whether oral administration of tilmicosin induces apoptosis in porcine BAL fluid leukocytes and identify changes in LTB₄ synthesis and other parameters associated with pulmonary inflammation.

**Materials and Methods**

**Animals and clinical observations**—Weaned piglets that were 3 weeks old and weighed 4.5 to 9 kg were obtained from a *Mycoplasma* specific-pathogen free herd and used in all experiments. After 7 days of acclimation, piglets were weight-ranked and randomly assigned to 1 of 4 groups as follows: 1) uninfected sham-treated control piglets were used for parts of the study; piglets received antimicrobial-free starter rations ad libitum for 7 days and were intratracheally inoculated with 2 mL of endotoxin-free saline solution (0.9% NaCl) solution (vehicle) on day 7; 2) infected untreated piglets were fed endotoxin-free saline solution (vehicle) on day 7; 3) infected tilmicosin-treated control piglets were given feed with tilmicosin (400 ppm endotoxin-free saline solution; 3) infected tilmicosin-treated piglets were given feed with tilmicosin (400 ppm endotoxin-free saline solution; and 4) infected CTC allowed for delivery in feed worldwide. Feed analyses were performed at each time point) were used throughout this study. Drug doses were based on maximal concentrations of tilmicosin or CTC allowed for delivery in feed worldwide. Feed analyses confirmed that the feed preparations with antimicrobials contained tilmicosin (370 µg/g and CTC at < 9.1 µg/g) or CTC (1,020 µg/g and tilmicosin at < 10 µg/g) for the tilmicosin and chlorotetracycline groups, respectively. Piglets were housed in groups with each group separated in negative pressure level 2 biohazard rooms at the University of Calgary Life and Environmental Sciences Animal Resource Centre, in a controlled environment (20°C, 40% humidity, photoperiod of 12 hours light and 12 hours dark). Feed intake per pen was calculated daily. Piglets were given free access to water at all times. Rectal temperatures were taken from piglets at the time of infection (0 hour) and again at 3 and 24 hours after inoculation. Care and experimental practices were conducted under the standards of the Canadian Council on Animal Care and approved by the University of Calgary Life and Environmental Sciences Animal Care Committee.

**Bacteria and experimental infection**—The *A. pleuropneumoniae* serotype 1 strain used in this study was originally isolated from a pig that died of pleuropneumonia. Inoculum was prepared by growing 10-µL aliquots (stored at –70°C) on *Brucella* agar supplemented with 0.2% β-nicotinamide adenine dinucleotide and 5% horse serum as previously described. Plates were incubated at 37°C and 5% CO₂ for 24 hours. Ten colonies selected from the plate were suspended in 25 mL of *Brucella* broth supplemented with 0.2% β-nicotinamide adenine dinucleotide and 5% horse serum. Inoculated broth was incubated for 12 hours at 37°C and 5% CO₂ to generate late-log-phase bacterial suspensions. Next inoculum was diluted in endotoxin-free PBS solution (pH, 7.2; 0.15M NaCl) to a final concentration of 5 × 10⁶ CFUs/mL (as assessed by spectrophotometric analysis). Bacterial counts in each inoculum were confirmed by CFU enumeration on supplemented *Brucella* agar.

Anesthesia was induced by giving each piglet an IM injection of ketamine hydrochloride (35 mg/kg), followed by halothane inhalation. A sterilized 20-gauge aluminum wire was inserted into a 2.7-mm-diameter sterile all-purpose catheter to ease its guidance into the trachea. By use of a stainless steel laryngoscope, the catheter was introduced into the trachea through the mouth to the bifurcation of the trachea, and the wire was withdrawn. Two milliliters of an *A. pleuropneumoniae* suspension or endotoxin-free saline solution (uninfected sham-treated control piglets) was injected through the catheter with a 10-µL syringe. Piglets were returned to their rooms and left to recover under observation.

**Bronchoalveolar lavage**—At 3 or 24 hours after intratracheal inoculation, 4 to 6 piglets in each group were euthanatized with an overdose of sodium pentobarbital. A sterile all-purpose catheter was aseptically introduced into the exposed trachea and advanced to the level of the bifurcation and then toward the primary lobes. Bronchoalveolar lavage fluid samples were collected by 3 sequential washings with 10 mL of endotoxin-free sterile PBS solution. Leukocytes were counted by use of a hemocytometer. Bronchoalveolar lavage samples were serially diluted and plated onto *A. pleuropneumoniae* selective *Brucella* media containing 0.2% β-nicotinamide adenine dinucleotide, 5% horse serum, crystal violet (2 µg/mL), lincomycin (1 µg/mL), and bacitracin (128 µg/mL) and incubated overnight at 35°C for enumeration of live *A. pleuropneumoniae*. The identity of bacteria retrieved from the BAL fluid sample was verified by use of sugar fermentation profile analysis, as well as oxidase and urease activities. The BAL fluid (100 µL) was centrifuged onto a microscope slide by use of a cytopsin for 10 minutes at 20 × g. Slides were fixed and stained. Neutrophil infiltration (percent and total neutrophil numbers/mL of BAL fluid) and macrophage phagocytic activity were calculated from each slide. Macrophage phagocytic activity was determined as the percentage of macrophages in the process of ingesting ≥ 1 neutrophil. The remainder of the BAL fluid was centrifuged for 10 minutes at 10°C to 250 × g and the supernatant placed in aliquots and stored at −70°C for later analyses. The pellet was suspended in 10 mL of 1/2 Hank's balanced salt solution (HBSS) for leukocyte purification.

**Leukocyte purification**—Because porcine BAL fluid neutrophils and macrophages were found to have similar specific gravities (data not shown), experiments were performed on leukocyte populations instead of purified neutrophils. All infected piglets had similar neutrophil numbers in BAL fluid samples. The cell pellet was resuspended in 10 mL of HBSS and centrifuged for 10 minutes at 10°C and 250 × g. The supernatant was discarded, and erythrocytes were lysed by osmotic shock in 1 mL of double-distilled H₂O for 30 seconds, after which 1 mL of 2X HBSS was added to restore osmolality. After an additional wash in HBSS, purified cells were counted by use of a hemocytometer and the population purity was calculated from a cytosin slide. Both BAL fluid and enriched leukocytes were assessed for cell viability in trypan blue.

**Pulmonary lesions**—After euthanasia, BAL fluid collection, the lungs and heart were removed from the piglets and washed in a water bath. Photographs were taken of the dorsal side of the lungs by use of a digital camera. Planimetric analysis of digital photographs was used to quantify the lesional surface area of the lung. Briefly, by use of dig-
nal imaging, a grid overlay was placed on the photographs and the surface area covering the lesions was compared with the total photographic surface area of the entire lung. Lesional surface area was defined as the percentage of total lung that had lesions.

Myeloperoxidase activity and histologic examination—Nonlesional and lesional (when lesions were present) lung tissue specimens were snap frozen in liquid nitrogen. Within a week of tissue specimen collection, myeloperoxidase (MPO) activity, a reliable marker of leukocyte infiltration, was measured by use of an MPO assay kit. Briefly, tissue homogenates were diluted 1:10 (wt/vol) in hexa-decyltrimethylammonium bromide buffer, sonicated, and spun at maximum speed in a microcentrifuge for 2 minutes. The supernatant was incubated in ELISA plates with 2 substrates, 1% H2O2 and O-dianisidine dihydrochloride, and the enzymatic activity of MPO was measured in an ELISA plate reader at 450 nm.

Additional nonlesional and lesional lung tissue specimens were fixed in fresh 4% paraformaldehyde for 24 hours at 4°C. Tissues were dehydrated, cleared, and embedded in paraffin, and 5-μm-thick sections were stained with H&E. Qualitative observations of alveolar integrity and cellular inclusions were made.

Leukocyte apoptosis—Bronchoalveolar lavage leukocyte apoptosis was quantified by use of a cell death ELISA kit as previously described. This sandwich enzyme immunoassay measures histone regions (H1, H2A, H2B, H3, and H4) of mononucleosomes and oligonucleosomes that are produced during apoptosis. Photometric development was measured kinetically by reading the plate at 405 nm at 5, 10, 15, and 20 minutes. Apoptosis was calculated from triplicate samples containing 10^6 BAL fluid leukocytes and expressed as the absorbance ratio for cell lysates from infected CTC- or tilmicosin-treated piglets versus absorbance for cell lysates from untreated piglets, which was arbitrarily set at 1 (ie, 100%). The detection limit for this ELISA was 10^2 apoptotic cells.

**LTB4 and prostaglandin E2 assay**—Leukotriene B4 and prostaglandin E2 (PGE2) are lipidic immune mediators that are conserved across species and hence can be readily detected by use of standard immunoassay kits in any mammalian samples. Concentration of LTB4 in BAL fluid supernatants was measured as a marker of inflammation by use of a competitive enzyme immunometric assay kit. Plates were read at 405 nm. Specificity for the assay is 100% for LTB4 and <0.01% for arachidonic acid, 5(S),12(S)- and 15(S)-hydroxyeicosatetraenoic acid, as well as LTC4, LTD4, LTE4, and LTB4. This assay had a limit of quantitation of 4 pg/mL. To further characterize the production of arachidonic acid metabolites in the BAL fluid of experimental piglets, PGE2 concentration in BAL fluid supernatants was measured by use of a competitive enzyme immunometric assay kit. Specificity for the assay is 100% for PGE2, 43% for PGE1, 18.7% for PGE3, 1% for 6-keto PGFI2α, 0.25% for 8-iso PGF2α, and <0.01% for arachidonic acid; conjugated linoleic acid; misoprostol; thromboxane B2; and prostaglandins A1, A2, B1, B2, D1, F1α, F2α, and F3α. The assay had a limit of quantitation of 51 pg/mL.

**Statistical analysis**—Results were expressed as mean (± SEM) values. Software was used to distinguish parametric from nonparametric data. An ANOVA and a Tukey post hoc test were used to compare parametric data, which were expressed as mean (± SEM) values. The Mann-Whitney rank sum test and Dunn post hoc test were used for nonparametric data, which were expressed as median values. Results expressed as percentages underwent an arcsine transformation before analysis and were transformed back to percentages for the expression of mean (± SEM) values. Values of P < 0.05 were considered significant.

**Results**

Clinical observations—Feed intake by groups was consistent throughout the 7-day treatment and was not significantly different among groups at any time (data not shown). Immediately following inoculation, (ie, between 0 and 24 hours) infected piglets were lethargic with a decrease in appetite. At 24 hours, rectal temperatures were significantly increased in untreated and CTC-treated piglets, compared with baseline values, but not in tilmicosin-treated piglets (Figure 1). Rectal temperatures measured in piglets at 3 hours after inoculation were not significantly increased in any group (data not shown).

**Table 1**—Mean (± SEM) pulmonary neutrophil infiltration, bronchoalveolar (BAL) concentration of prostaglandin E2 (PGE2), and bacterial colonization of the lungs of piglets experimentally infected with Actinobacillus pleuropneumoniae.

![Figure 1](image-url)
Bacterial recovery—At 3 hours after inoculation, both CTC and tilmicosin significantly decreased *A. pleuropneumoniae* colonization in the bronchoalveolar space (by 23.2% and 45.4%, respectively), compared with untreated piglets (Table 1). At 24 hours, bacterial numbers measured in the BAL fluid were not significantly different among groups. Bacteria recovered from the BAL fluid, as well as *A. pleuropneumoniae* isolated from the inoculum, were able to ferment glucose, maltose, sucrose, and trehalose and had positive results for urease and oxidase (data not shown).

Neutrophil infiltration and MPO activity—At 3 hours after inoculation, in all infected groups, more than 50% of the BAL fluid leukocytes were neutrophils (Table 1). At 3 or 24 hours, pulmonary neutrophil infiltration was not significantly different among groups. At 3 hours, MPO activity of nonlesional tissue was low and not significantly different among any groups (data not shown). At this time point, no lesional lung tissue had formed in any of the piglets. At 24 hours, infection induced severe tissue lesions, which were characterized by an increase in MPO activity, compared with nonlesional tissues (Figure 2). Histologic examination confirmed the severe infiltration of neutrophils as well as the loss of alveolar structures at lesion sites (Figure 3). At 24 hours after inoculation, all untreated and CTC-treated infected piglets developed characteristic pulmonary lesions, whereas only 1 of 6 tilmicosin-treated piglets had lesions in the lungs.

Leukocyte apoptosis—Additional experiments assessed and compared the proapoptotic effects of each treatment on BAL fluid leukocytes. At 3 hours after inoculation, BAL fluid leukocytes from tilmicosin-treated piglets had significantly higher amounts of apoptosis, compared with BAL fluid cells from untreated or CTC-treated piglets, which were not significantly different from each other (Figure 4). At 24 hours...
after inoculation, BAL fluid leukocyte apoptosis returned to baseline and was not significantly different among groups (data not shown).

Macrophage phagocytosis of BAL fluid neutrophil—Elimination of apoptotic neutrophils by alveolar macrophages is an important contributor to the resolution of pulmonary inflammation. At 3 hours after inoculation, 6.0 ± 2.8% and 6.4 ± 2.6% BAL fluid neutrophils were phagocytosed by alveolar macrophages in untreated and CTC-treated piglets, respectively (Figure 5). In tilmicosin-treated piglets, this number reached 17.0 ± 4.5%, but this value was not significantly ($P = 0.083$) different from that of the other groups. Similar observations were made 24 hours after inoculation.
LTB₄ and PGE₂ synthesis—At 3 hours after inoculation, BAL fluid concentrations of LTB₄, a potent proinflammatory mediator, were low in all groups (Figure 6). At 24 hours, infection caused a significant increase in BAL fluid concentrations of LTB₄ in untreated and CTC-treated piglets. Leukotriene B₄ concentrations in tilmicosin-treated piglets were significantly lower than in untreated or CTC-treated piglets. In piglets given tilmicosin, BAL fluid LTB₄ concentrations at 3 and 24 hours were not significantly different from those measured in uninfected sham-treated control piglets. At 3 or 24 hours, BAL fluid concentrations of PGE₂ were not significantly different among groups.

Pulmonary lesions—Experimental infection with *A pleuropneumoniae* induced the formation of characteristic necrotic hemorrhagic pulmonary lesions at 24 hours (Figure 4). No lesions were observed in uninfected sham-treated piglets 3 hours after inoculation (data not shown). Compared with untreated piglets, lesional surface area was significantly decreased in lungs of piglets treated with tilmicosin, but not in piglets treated with CTC. Only 1 of 6 tilmicosin-treated piglets had pulmonary lesions, whereas all piglets of the untreated and CTC-treated group had lesions 24 hours after inoculation.

Discussion

Results from our study indicate that oral administration of tilmicosin activates bronchoalveolar leukocyte apoptosis in piglets. Infection with *A pleuropneumoniae* caused a significant increase of proinflammatory LTB₄ concentrations in the BAL fluid of untreated and CTC-treated piglets. Concurrent with its proapoptotic effects, oral administration of tilmicosin abolished infection-induced LTB₄ synthesis. These effects were associated with inhibition of the fever and prevention of the pulmonary necrotic hemorrhagic lesions caused by the *A pleuropneumoniae* infection. This was observed only in tilmicosin-treated piglets despite the concurrent presence of live bacteria in the lungs of all piglets from each infected group. These changes were observed in the absence of any effect on BAL fluid neutrophil infiltration or tissue MPO activity. The findings support the view that in addition to its antimicrobial properties, tilmicosin may be proapoptotic and anti-inflammatory in pig lungs infected with the lung without affecting local interleukin-8 mRNA production. Consistent with these findings, other investigators have demonstrated that administration of tilmicosin, but not cefotiofur, stimulates apoptosis in bronchoalveolar neutrophils of calves with subacute chronic airway disease. Results of our study, as well as other studies using peripheral bovine neutrophil in vitro, indicate that this effect has some degree of drug and cell specificity. In keeping with these observations, results from our study indicate that oral administration of tilmicosin in pigs also stimulates leukocyte apoptosis in the *A pleuropneumoniae*-infected lung. This effect coincides with inhibition of LTB₄ synthesis and with the decrease in inflammatory lesions and fever. Serum tilmicosin concentrations do not cor-
relate with efficacy in cattle or in swine, and in the bovine lung tissues, tilmicosin exceeds mean inhibitory concentrations for Mannheimia haemolytica for 3 to 4 days following a single SC injection (10 mg/kg); in contrast, in porcine pulmonary tissues, at tilmicosin treatment concentrations of 400 µg/g in the feed, tilmicosin remains below the mean inhibitory concentrations for most of the A pleuropneumoniae strains.24 These observations indicate that mean inhibitory concentrations cannot predict the clinical efficacy of this antimicrobial, which has been linked in part to its uptake and later release by phagocytes at the site of infection.20,22 In view of these findings, the data from our study lend further support to the hypothesis that induction of neutrophil apoptosis may confer anti-inflammatory properties and clinical efficacy to an antimicrobial.

Following neutrophil infiltration of inflamed tissues, apoptotic neutrophils and their fragments are removed by macrophages and other nonphagocytic cells, including neighboring epithelial cells.10,11,25 This phenomenon implicates a variety of mechanisms, including the binding to externalized phosphatidylserine on apoptotic cells, the deployment by phagocytes of surface αβ3 vitronectin receptors and CD36 to present bridging adhesive thrombospondin to apoptotic cells, and the binding to apoptotic cells of the opsonizing collectins surfactant protein-A and surfactant protein-D, as well as other mechanisms.10,11,25 This allows for the nonphlogistic phagocytic clearance of apoptotic neutrophil before they are given the opportunity to undergo secondary necrosis and cause tissue injury. Indeed, macrophages exposed to apoptotic cells increase their secretion of PGE2, which in turn decreases the ability of lipopolysaccharides-stimulated macrophages to release proinflammatory cytokines such as interleukin-1β.20 Moreover, these macrophages release more anti-inflammatory mediators, including interleukin-10.25 Tilmicosin-induced apoptosis of circulating bovine neutrophils is associated with increased translocation of phosphatidylserine on the outer leaflet of the cell membrane.20 In the context of our study, although the increased phagocytic uptake of neutrophil by alveolar macrophages in tilmicosin-treated piglets would be consistent with the observed proapoptotic properties of the antimicrobial, values failed to reach significant differences among groups. Results of a recent study20 indicate that injectable tilmicosin, given to calves at doses higher than those typically used in field practices, decreases lipopolysaccharides-stimulated PGE2 production by bovine alveolar macrophages. In our study, oral administration of tilmicosin did not decrease the high BAL concentrations of PGE2 measured 3 or 24 hours after inoculation. Future studies need to investigate the effects of tilmicosin on neutrophil-macrophage interactions and to assess whether the resulting profiles of proinflammatory and anti-inflammatory mediator release may be dependent upon dose and route of administration.

In our study, consistent with previous observations, bacterial pneumonia was associated with increased concentrations of proinflammatory LTβ in the bronchoalveolar spaces of the lung. In untreated and CTC-treated piglets had a > 3.5-fold increase in bronchoalveolar LTβ concentrations, compared with the baseline concentrations detected in uninfected sham-treated control piglets 24 hours after inoculation. In contrast, this increase was abolished in piglets given tilmicosin in the feed, despite the concurrent presence of bacteria in the lung. Similar effects are described in a previous report21 for tilmicosin-treated calves infected with M haemolytica. Recent observations37 also indicate that tilmicosin may directly impair spontaneous LTβ release from circulating bovine neutrophils in vitro. The mechanisms responsible for the apparent effects of tilmicosin on arachidonic acid metabolism warrant further investigation.

In summary, the results of our study indicate that oral administration of tilmicosin induces leukocyte apoptosis without altering neutrophil infiltration or tissue MPO activity in the A pleuropneumoniae-infected porcine lung. Tilmicosin significantly decreases pulmonary accumulation of LTβ, whereas BAL fluid samples from untreated and CTC-treated piglets maintain increased LTβ concentrations concurrently with their baseline amounts of leukocyte apoptosis. Tilmicosin also appears to attenuate the formation of the necrotic hemorrhagic lesions characteristic of A pleuropneumoniae infection. Activation of leukocyte apoptosis, significant inhibition of LTβ synthesis, fever reduction, and inhibition of lesion formation occurred only in tilmicosin-treated piglets despite the concurrent presence of live bacteria in the lungs of all infected piglets. Future research, such as using experimental models with proinflammatory stimuli other than live bacteria, will assess whether this suggests that the anti-inflammatory effects of tilmicosin directly reflect the proapoptotic properties of the drug rather than its antibacterial effects. We hypothesize that the inhibition of inflammation, lesion formation, and decrease in fever associated with the proapoptotic effects of oral administration of tilmicosin during A pleuropneumoniae infection in swine may translate into the maintenance of normal respiratory function and better growth rate.

a. D. Hall, Airdrie, AB, Canada.
b. LESARC, University of Calgary; Calgary, AB, Canada.
c. Pulmotil, Elanco Animal Health Division, Eli Lilly Canada, Calgary, AB, Canada.
e. Woodson-Tent Laboratories Inc, Memphis, Tenn.
f. Courtesy of Dr. M. Gottschalk, St Hyacinthe, QC, Canada.
g. 2-bromo-2-chloro-1,1,1-trifluoroethane, MTC Pharmaceuticals, Cambridge, ON, Canada.
h. Rusch Inc, Oakville, ON, Canada.
i. Jorgensen Laboratories Inc, Loveland, Colo.
j. Sonnitol, Bimeda MTC, Cambridge, ON, Canada.
k. Bright-Line improved neubauer, American Optical Corp, Buffalo, NY.
l. Shandon Southern Products Ltd, Cheshire, UK.
m. Baxter Healthcare Corp, Saint Paul, Minn.
n. Gibco HRL, Life Technologies Inc, Grand Island, NY.
p. Nikon Coolpix 995, Nikon Corp, Tokyo, Japan.
q. Cytostore/UTI, Calgary, AB, Canada.
r. Oxford Labware, St Louis, Mo.
s. Cell death detection ELISA kit, Boehringer-Mannheim, Laval, QC, Canada.


References