Time and Concentration Relationships of Gentamicin in Serum and Bronchial Lavage Fluid of Horses Administered Gentamicin Intravenously and by Aerosol

by

Harold C. McKenzie III DVM

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Michael J. Murray, Chair
Martin O. Furr
Lydia L. Donaldson

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Chairman: Michael J. Murray
Veterinary Medical Sciences

(ABSTRACT)

This study was performed to compare the delivery of the antimicrobial gentamicin to the respiratory tract of adult horses following aerosol and intravenous administration. Nine adult horses were used in a crossover design. Aerosol administration of gentamicin was performed using a close fitting facemask and an ultrasonic nebulizer. Intravenous gentamicin was administered via a jugular venous catheter. Samples of pulmonary epithelial lining fluid were collected by bronchial lavage performed at 0.5, 4, 8 and 24 hours after gentamicin administration. All samples were analyzed for gentamicin concentration, and cytologic examination was performed on aliquots of bronchial lavage fluid from times 0.5, 8 and 24 hours. Comparisons were made using the Wilcoxon signed-rank test. The bronchial lavage fluid gentamicin concentration after aerosol administration was significantly greater ($p<0.05$) than after intravenous administration at 0.5, 4, and 8 hours. The bronchial lavage fluid total nucleated cell count increased significantly ($p<0.05$) from 0.5 to 24 hours following both routes of gentamicin

administration, with the increase observed following aerosol administration being significantly greater ($p<0.05$) than that observed following intravenous administration. A significant increase in neutrophil count was detected between bronchial lavage fluid samples taken at 0.5 hours and 24 hours, regardless of route of gentamicin administration. We conclude that aerosol administration of gentamicin to the equine respiratory tract achieves bronchial lavage fluid gentamicin levels that are significantly higher than levels obtained following intravenous administration for at least the first 8 hours after administration, while inciting a mild inflammatory response.

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DEDICATION

Dedicated with love and appreciation to my wife, Cindi McKenzie, for her tolerance of adversity, unflagging support and continued encouragement throughout this trying stage of our life together.

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Aerosolized antimicrobials in the horse: Theory and clinical application.

Introduction

One of the major limitations of systemic antimicrobial therapy for the treatment of lower respiratory infectious disease is the low activity or bioavailability of many of these medications in the lower respiratory tract.[1-6] Consequently, the administration of antimicrobials by the aerosol route has been of interest to clinicians since these compounds were first developed, with penicillin being administered by aerosolization as early as the 1940's.[7,8] Aerosolized antimicrobial therapy can achieve high concentrations of these agents at the site of infection,[9-14] while minimizing the development of systemic side effects.[15-17] Other potential advantages to delivering medications to the lower respiratory tract by aerosolization include a decrease in the total dose administered and a rapid onset of action.[15-17] The administration of antimicrobials by aerosolization does have limitations, however, including: 1) the potential for pulmonary tissue irritation or injury, 2) inability to deliver medication to areas of the lung which are not ventilated, 3) potential inactivation of the medication, 4) development of antimicrobial resistance, 5) atmospheric contamination, 6) potential contamination of the antimicrobial solution with micro-organisms, 7) time consuming administration, and 8) the expense of required equipment. [17-22]

The effect of an aerosolized medication is only achieved following deposition of the aerosolized particles on the mucosal surface of the respiratory tract, and the therapeutic efficacy of an aerosol medication derives from the dose deposited at the target site.[23] The deposition of particulate material in the respiratory tract follows a fundamental

pattern, which is determined by the interaction between the characteristics of the aerosol itself, and those of the individual inhaling the aerosol.[24] The interaction of these factors determines the site to which medication is delivered, the dose delivered, the efficiency of delivery and therapeutic efficacy of the treatment.

Aerosol deposition

An aerosol is a suspension in which the dispersing phase is a gas (usually air) and the dispersed phase is made up of solid or liquid particles.[18,25] The dispersed particles in a therapeutic aerosol contain the active constituent of the preparation.[25] The pattern of deposition of aerosol particles and the efficiency of aerosol delivery are influenced by the characteristics of the aerosol itself, including the size distribution of the aerosol particles and the aerosol density,[26,27] and these characteristics can vary significantly with different delivery systems and drug formulations.[28] Several patient dependent factors are also important in determining aerosol deposition within the respiratory tract, including ventilatory parameters, such as respiratory frequency, respiratory flow rate, duration of breath holding and tidal volume, as well as respiratory morphology, which may be influenced by the presence of pathologic changes. [18,24,29]

Mechanisms of particle deposition

The primary mechanisms of particle deposition functioning within the respiratory tract are inertial impaction, sedimentation and diffusion. Inertial impaction affects those particles whose mass is great enough that they are unable to rapidly alter course when there is a change in the direction of the airflow. This results in the particle being displaced away from its position within the airstream and toward the wall of the respiratory tract, where it can impact the mucosal surface. The displacement which particles undergo increases with airstream velocity, the angle of airstream deflection and the square of the particle diameter.[30] Inertial impaction is most effective at removing particles of greater than 10 μm diameter, which for this reason are considered non-

respirable.[31] However, particles of 2 μm diameter or greater are affected by inertial impaction as well.[30]

Another mechanism for deposition is sedimentation, which occurs when particles are greater in density than the surrounding air. Gravity causes the particle to impact on the mucosal surface. Due to the relatively weak force of gravity this effect only becomes significant when the relative velocity in the airway is low, and the resulting residence time in the airway is longer, allowing time for this effect to develop.[24] Sedimentation primarily affects particles 0.2 to 5 μm in size, and is of greatest importance in the peripheral airways.[32]

The mechanism of deposition that is most important in the terminal airways and alveoli is diffusion. This process is the result of the inherent motion of aerosol particles caused by their interactions with gas molecules, known as Brownian motion.[24,26] At very low airflow velocities and increased residence times this random motion leads to the collision of particles with the mucosal surface. As particle size decreases, the effects of inertia and sedimentation decrease in importance, and the effect of diffusion becomes more important.[30] Diffusion is the dominant mechanism determining the deposition of particles less than 0.1 μm in diameter, and sedimentation and diffusion are both important in the deposition of particles between 0.1 and 1 μm in diameter.[30] The net result of the process of aerodynamic filtration is that particles of 10 μm diameter or greater are deposited within the nasal passages and nasopharynx, particles of 10 to 2 μm diameter are deposited within the large airways, particles of 2 to 0.5 μm are deposited in the

terminal airways and alveoli, and particles less than 0.5 μm diameter are exhaled prior to deposition.[33]

Particle Characteristics

The most important of the aerosol particle characteristics are the diameter and mass of the individual particles. Therapeutic aerosols are typically polydisperse, with the particles varying in size. For this reason aerosols are characterized by the particle diameter distribution, typically expressed as the mass median aerodynamic diameter (MMAD).[25] This value represents the size of particle such that half of the mass of the aerosol is contained in larger particles and half in smaller particles.[34] The size of the particle determines the degree to which it will be able to penetrate the respiratory tract, with smaller particles, less than 5 μm in diameter, able to penetrate further into the periphery of the lung, and larger particles, greater than 5 μm in diameter, being subject to upper respiratory deposition.[33] The portion of the dose of aerosolized particles that are less than 5 μm in diameter is termed the respirable fraction (RF), and in humans there is often a good correlation between the RF and the bioavailability of inhaled drugs.[35]

Other characteristics are the particle density of the aerosol, the hygroscopicity of the material in the particle, and the charge and shape of the particle.[26] The particle density of the aerosol is of importance in determining the number of particles, containing the therapeutic agent, delivered to the patient per unit time.[36] Hygroscopicity can result in the increase in size of a hyperosmotic particle as it encounters the humid (99.5% relative humidity) environment of the lung, leading to a decrease in peripheral deposition, while hypoosmotic particles will lose water and decrease in size.[30,37] The effects of electric

charge on particle deposition are most significant with submicron particles,[30] and are likely of minimal importance in therapeutic aerosols. The relationship between shape and deposition is of importance when considering particles that differ from the typical spherical shape of liquid droplets, such as the elongated fibers of asbestos, and is not typically a concern when discussing therapeutic aerosols.[38]

Patient characteristics

There are important characteristics of the individual that affect particle deposition patterns, with the most important being the anatomy of the respiratory tract itself and the respiratory pattern.[26] The anatomy of the respiratory tract is such that most inhaled or aspirated material will be deposited in the conducting airways, an effect that is termed aerodynamic filtration.[32] The respiratory pattern determines the velocity of the airflow in the respiratory tract, the inspiratory volume and the duration of each respiratory cycle during which the particle may be deposited.[29,39]

The behavior of aerosolized particles within the upper respiratory tract is primarily the result of the structural geometry of the airways. Horses are obligate nasal breathers.[40] The inhalation of air through the nares results in very high velocity airflow, which combines with the large surface area and redirection of airflow provided by the nasal conchae leads to greatly increase inertial deposition of particulate matter in the upper respiratory tract when compared with mouth breathing.[25,41] After air passes through the constriction of the larynx it enters the trachea as a high velocity laryngeal jet.[24] This induces a large amount of turbulence within the trachea, enhancing the effects of inertia and diffusion on impaction.[42] Air velocity remains high in the trachea, and at the carina

and bronchial bifurcations, where the branching of the large airways causes relatively acute changes in the direction of airflow, leading to increased inertial deposition.[42] On entering the small airways the velocity of airflow greatly decreases, as a result of the significant increase in the total cross-sectional area of the airways.[43] This decrease in velocity leads to a decrease in inertial deposition. At this level the primary mechanism for deposition is sedimentation.[24]

The importance of respiratory pattern in particle deposition can be understood when these mechanisms of particle deposition are analyzed in terms of their relationship to airflow velocity, tidal volume and respiratory rate. Increasing the velocity of the inhaled air enhances inertial impaction of large particles in the conducting airways, while decreasing the deposition of particles in the small airways by sedimentation, which allows for increased penetration of small (2 to 0.5 μm) particles into the alveoli.[30] Increased tidal volume leads to increased peripheral deposition of small particles, due to the increased depth of penetration of inspired air into the lung periphery.[44] Deposition of small particles in the lung periphery is also enhanced by a decrease in respiratory rate, which increases the residence time of particles within the peripheral airways, and allows for increased deposition due to sedimentation and diffusion.[45] The respiratory pattern of the horse has been described as being well suited to inhalation therapy, because the large tidal volume and high flow rate enhance deep pulmonary deposition of aerosols.[40]

The presence of pulmonary disease is a major consideration in the administration of therapeutic aerosols, because the changes in pulmonary function and mechanics can impair the delivery of aerosolized medications to the affected region.[25,26,40] Inflammation

and airway sensitization is commonly associated with pulmonary disease, and can cause bronchoconstriction, mucus hypersecretion and mucosal edema, resulting in variable amounts of airway obstruction. Airway obstruction increases particle deposition at the site of obstruction, but diverts airflow to non-obstructed airways, resulting in increased delivery of therapeutic particles to the less affected portions of the lung.[30] The net result is a shift toward more central deposition of drug, and decreased peripheral distribution.[8] Total obstruction completely prevents deposition in the affected region peripheral to the site of obstruction, and as a result no medication will reach a consolidated region of the lung following aerosol administration.[8,18]

Clearance

Following deposition of aerosol particles the defense mechanisms of the respiratory tract act to remove the material, a process known as clearance. The difference between the deposition and clearance of foreign material is known as retention, and this factor is of primary importance in aerosol delivery of therapeutic agents to the respiratory tract, because it represents the amount of material that remains within the respiratory tract to exert a therapeutic effect.[26,46] Once a therapeutic agent has deposited on the mucosal surface, it undergoes pharmacokinetic processes, including absorption, distribution, metabolism and excretion, which determine the final amount of drug which reaches the site of action. [47,48]

The aerosol particle initially deposits in the mucus lining the airway lumen, where it will either dissolve or complex with compounds within the mucus.[47,49,50] Inactivation of therapeutic compounds can occur within the mucus layer due to nucleoprotein complex formation, the presence of acid pH in purulent secretions, high ionic concentrations, or by the action of enzymes within the mucus.[5,47,51,52] Dissolved material can diffuse within the mucus, and either exert an effect at this level or diffuse into the mucosal epithelium, thereby reaching epithelial and sub-epithelial sites of action.[47,49] Passive diffusion of a therapeutic substance will depend on the concentration gradient across the cell membrane, the lipid solubility of the substance and it's ionization and molecular size.[47,53] Disease conditions can alter the rate of diffusion by altering epithelial permeability.[47] Material that remains within the mucus layer will be transported to the pharynx by the mucociliary escalator, where it is swallowed.[35,49,54] If the therapeutic agent administered

by aerosolization is orally bioavailable some fraction of the inhaled dose will be absorbed from the gastrointestinal tract.[35] Phagocytosis of small particles (<3 μm) of therapeutic compounds by alveolar and airway macrophages may also occur, contributing to clearance of insoluble material.[35,55]

Administration

Aerosol delivery of therapeutic substances presents several challenges. The therapeutic agent should be non-irritating, non-allergenic, and lack local toxicity.[20] The therapeutic substance must be capable of being absorbed into the respiratory mucosa, or of exerting it's action on the mucosal surface.[47] The therapeutic substance should be poorly absorbed, in order to minimize systemic toxicity.[20] The substance to be delivered must be available in a formulation, liquid or powdered, which can be delivered directly to the respiratory tract as an aerosol, or it must be soluble in a liquid that can be safely aerosolized into the respiratory tract. The delivery method must be capable of producing particles that are of an appropriate size to deliver the medication to the desired portion of the respiratory tract,[56] and the delivery method should be able to deliver the total dose in a reasonable period of time with reasonable efficiency. Also, ideally, the cost of the equipment required for administration of the aerosol should not be excessive.

Devices

The generation of therapeutic aerosols is accomplished using several different inhalation drug delivery systems. None of these systems, however, is capable of delivering aerosolized medication with high efficiency, with less than 10% of the original dose being deposited in the lower respiratory tract regardless of delivery system.[21,27,34,57,58] Two of the most commonly used aerosol delivery devices in human medicine are metered dose inhalers (MDIs) and dry powder inhalers (DPIs), which generate small volume aerosols of liquids and powders, respectively. These devices have the advantages of being preformulated, prepackaged, and capable of delivering multiple

doses, however they do require some dexterity during administration to ensure that administration is correlated with inhalation.[59]

Aerosols are generated from liquids by nebulizers, either compressed-gas driven (jet) or ultrasonic. Nebulization is considered to be the optimal method of administration when the dose to be administered exceeds 200 μg,[59] and does not require coordination of administration and inhalation, depending only on normal tidal breathing.[28,60] Nebulisers and metered dose or dry powder inhalers must be used with a facemask in the conscious animal patient, with attendant aerosol wastage on the face, facemask and in the nasal passages and nasopharynx.[19,57] Nebulisers can be used within the circuit of positive pressure ventilators as well, thereby avoiding the aerosol losses associated with facemasks and the upper respiratory tract, although this has not been shown to have any definite therapeutic advantage in terms of aerosol delivery in human patients.[57]

Metered dose inhalers

Metered dose inhalers are self-contained devices, consisting of a canister with an integral metering valve. The canister contains the drug, one or more liquefied propellants and often a surfactant.[56] The drug can be dissolved within the propellant/surfactant fluid,[56] or it can be in the form of solid particles suspended within this fluid.[61] Solid particles must be of an appropriate size, because this determines the smallest particle size which can be produced upon actuation of the device, and this is accomplished by milling, or micronizing, the solid drug.[61] The propellant serves as a dispersion medium for the drug, and as an energy source to expel the formulation from the valve as large droplets that rapidly evaporate following exposure to the air, leaving a drug containing particle

ideally of respirable size.[56] Due to the high velocity of the aerosol as it leaves the actuation valve these devices have been associated with high levels of oropharyngeal deposition in human patients.[24] In an effort to avoid this effect, numerous spacer devices have been developed that allow the metered dose inhaler to be actuated into the device, where the particle velocity dissipates, the propellant evaporates further and the non-respirable particles impact onto the device, resulting in a decrease in MMAD and decreased oropharyngeal deposition.[58,59]

Dry powder inhalers

Dry powder inhalers are available as either unit dose or multiple dose devices.[59] These devices have the advantage of not requiring a propellant for dispersion of the drug, and they do not require coordination of inhalation and actuation, because they are breath actuated.[61] As with metered dose inhalers the particles of drug must be of an appropriate size (<5 μm) for inhalation, and this is accomplished by micronizing or spray drying the drug compound.[56] These small particles are very difficult to disperse, and many formulations include carriers, such as lactose particles, to improve particle dispersion.[56] Relatively high inspiratory airflows (30 to 60 l/min) are required to aerosolize the powder formulations, and may be difficult to achieve in the face of pulmonary disease or small patient size.[58,59]

The deposition of radioaerosol in the equine lung using a metered dose inhaler-spacer and facemask was examined by Viel and Tesarowski,[62] and it was demonstrated that significantly more of the dose of radioaerosol was deposited in the lung (6.12%) using this device combination than was observed with jet or ultrasonic nebulizers. The

MDI-spacer-facemask combination was tested on adult ponies, however, while the nebulizers were tested on 5 to 7 month old foals, [62] perhaps influencing the pattern of deposition of radioaerosol. The therapeutic administration of beta-2 agonist bronchodilators to horses by metered dose inhaler has been validated by Tesarowski *et al.*[63] and Derksen *et al*.,[64] and the administration of an anticholinergic bronchodilator by dry powder inhaler was validated by Duvivier *et al.*,[65] but these devices have not been used for antimicrobial administration in the equine. In general, the metered dose and dry powder devices are not capable of delivering large enough doses of antimicrobials to be clinically useful. However, one study investigated the viability of delivering micronized gentamicin powder to the human lower respiratory tract using a dry powder inhaler, and similar gentamicin concentrations (9.3 mg/L versus 8.0 mg/L) were obtained in the bronchial lavage fluid following dry powder (180 mg) or nebulizer (160 mg) administration.[66] While these devices hold some promise for antimicrobial administration, there are no commercially available metered dose or dry powder inhalers containing an antimicrobial formulation at this time.

Jet nebulizers

Jet nebulizers are more widely used than ultrasonic nebulizers in human medicine, because they are inexpensive and relatively easy to use. In a jet nebulizer compressed gas is forced through a nozzle, or 'jet', resulting in formation of a region of negative pressure, which draws the nebulization solution to the area of the nozzle.[56] The fluid enters the airstream and forms an unstable film that collapses due to surface tension, and forms droplets.[56] Baffles within the nebulizer are used to impact the larger (non-respirable)

particles, and prevent them from leaving the nebulizer.[22] Over the course of nebulization the temperature of the nebulization solution decreases, due to the latent heat of evaporation, and the solute concentration increases, due to the evaporation of water.[59]

The particle characteristics of aerosols produced by jet nebulizers are dependent on the formulation of the drug solution, the design characteristics of the nebulizer and the air pressure used to operate the nebulizer.[22] Jet nebulizers are limited, in most cases, to reservoir volumes of 1-3 milliliters. The time required to aerosolize this volume of fluid is anywhere from 10 to 15 minutes,[61] although this is variable, depending on the device itself, the flow rate of air through the device, the solution being aerosolized and the relative humidity in the environment.[22,59] The 3 to 4 milliliter fill volume of most jet nebulizers is adequate to allow administration of antimicrobials to most animals, but when administering these drugs to adult large animals the time required to deliver a single dose may become excessive. These devices have the additional disadvantage of producing significant noise, both from the compressor and the nebulizer itself. Votion *et al.*[19] demonstrated that with one type of jet nebulizer 7.4% of the initial dose of radioaerosol was delivered to the lungs of horses, with significant peripheral deposition. Viel and Tesarowski[62] examined the deposition of radioaerosol in horses using three different jet nebulizers and found that lung deposition ranged from 0.32 to 1.33% of the initial dose. Importantly, the horses used in the study by Votion *et al.* were sedated, while the horses in the study of Viel and Tesarowski were anesthetized and maintained in sternal recumbency, likely resulting in a difference in ventilatory parameters affecting deposition.

Ultrasonic nebulizers

Ultrasonic nebulizers utilize high-frequency sound waves, produced by a piezoelectric crystal, to form an aerosol from a liquid drug formulation.[58,59] Some devices allow the nebulization solution to come into direct contact with the transducer containing the piezoelectric crystal, while others utilize a liquid couplant between the transducer and a cup, which can be shaped to enhance the transfer and focusing of energy, containing the solution to be nebulized.[67] The ultrasonic vibrations pass through the nebulization solution and result in the creation of a 'fountain' of liquid at the surface.[22] Droplets of variable size are created by the collapse of cavities (cavitation) formed within the liquid fountain by the waves generated by the ultrasonic transducer.[22,68] The size distribution of the aerosol particles produced is influenced by the frequency of the transducer, with higher frequencies yielding smaller particles.[67] An airstream is passed through the nebulization chamber to carry the aerosolized particles to the patient, and a fan or the patient's inhalation can produce this airflow.[61,67]

Not all of the energy imparted to the nebulization solution contributes to the formation of aerosol particles, because some of the energy is dissipated and imparts heat to the solution, resulting in a gradual increase in solution temperature over time.[22] Also, there is a gradual increase in solute concentration over time, although this effect is less significant than that seen with jet nebulizers.[22,67] Ultrasonic nebulizers are generally capable of producing more aerosol per unit time than jet nebulizers,[69] resulting in shorter treatment times and greater ease of use. Larger fill volumes (up to 30 ml), or more with continuous feed systems, are possible, allowing the convenient administration of much

larger doses than are possible with jet nebulizers. Votion *et al*. demonstrated the efficacy of one type of ultrasonic nebulizer for delivery of radioaerosol particles to the equine lung, with 5.1% of the initial dose delivered to the lungs.[19] Viel and Tesarowski[62] examined the radioaerosol deposition from two types of ultrasonic nebulizers and found that lung deposition ranged from 0.27 to 0.33%. These devices are expensive and fragile, however, limiting their clinical application outside of referral institutions.[17]

Drug formulations for nebulization

The formulation of a therapeutic nebulization solution can have a major impact on the characteristics of the aerosol produced,[22] and on the patient response to aerosol administration. Nebulization solutions are ideally isotonic and neutral formulations, because hyper/hypotonic or acidic solutions have been shown to induce coughing and/or bronchoconstriction.[59,70-72] The concentration of solute, as well as the surface tension and viscosity of the solution are of great importance, because increases in one or all of these factors leads to a decrease in aerosol output and in particle size.[36,73] A recent study determined that the antimicrobial concentration in a nebulization solution, regardless of the specific antimicrobial, should ideally be 100 mg/ml or less, in a saline solution of 0.23% to 0.45% concentration.[36] Lower antimicrobial concentrations were recommended for ultrasonic nebulizers, with an ideal concentration of approximately 50 mg/ml.[36] Many antimicrobial solutions marketed for intravenous use, as well as some marketed for aerosol administration, contain preservatives such as sodium metabisulfite, benzalkonium chloride or EDTA, and these compounds have been shown to induce coughing and/or bronchoconstriction.[36,56,59,73] No long term adverse affects have been associated with the

aerosol administration of these compounds, but they should probably be avoided, given their potential interference with efficient and consistent aerosol administration.[36]

Clinical application

Aerosol administration of antimicrobials has been investigated extensively because many antimicrobials do not achieve high concentrations in the pulmonary epithelial lining fluid, or require very high systemic concentrations in order to achieve adequate concentrations in the pulmonary epithelial lining fluid.[1,3,19,21,74-79] Several studies in humans and animals have demonstrated the effectiveness of aerosol administration of antimicrobials in achieving high antimicrobial concentrations in the pulmonary epithelial lining fluid and/or respiratory mucosa of the lower respiratory tract.[9-14] Human and animal studies have also demonstrated the effectiveness of aerosol antimicrobial administration in decreasing the severity, or shortening the course of, lower respiratory bacterial infections.[14,80-84]

Bacterial Respiratory Disease

Human studies

Most studies of aerosol antimicrobial administration in human beings have focused on patients with cystic fibrosis, due to the frequent development of persistent lower respiratory infections that are poorly responsive to systemic antimicrobial therapy in these patients. [76,85] The administration of aerosolized antimicrobials to cystic fibrosis patients has been utilized both to suppress airway colonization with bacterial organisms and as an adjunct to intravenous therapy for treatment of acute exacerbations. There has also been investigation of aerosolized antimicrobial therapy for treatment of *Pneumocystis carinii* pneumonia in human patients with Acquired ImmunoDeficiency Syndrome (AIDS), the treatment of pneumonia in mechanically ventilated patients,

delivering antiviral compounds to children with respiratory syncytial virus, and prophylaxis and treatment of fungal pneumonia.

Cystic fibrosis

Most of the studies that have demonstrated a clinical benefit from aerosolized antimicrobial therapy have utilized an aminoglycoside, alone or combined with other drugs, as well as systemic therapy.[21] Hoff *et al.*[86] investigated the combined administration of tobramycin by the intramuscular (~2.5 mg/kg four times daily) and aerosol (100 mg twice daily) routes of administration in nine pediatric cystic fibrosis patients with chronic *Pseudomonas aeruginosa* pulmonary infections. Eradication of the organism from the respiratory tract was accomplished in five of the patients, while all patients demonstrated a clinical response to treatment. Hodson *et al.*[87] performed a 6 month, double blind, randomized, crossover study of twice daily aerosol carbenicillin (1 gram) and gentamicin (80 mg) in the treatment of young adult cystic fibrosis patients with chronic *Ps. aeruginosa* infections and documented significant improvement in pulmonary function and in patient symptoms.

A long-term prospective study of the efficacy of aerosolized tobramycin therapy (80 mg three times daily) in cystic fibrosis patients with chronic *Ps. aeruginosa* infections was performed by MacLusky *et al.*[88] They demonstrated that patients receiving aerosolized tobramycin therapy remained clinically stable over the study duration, while the patients receiving the control treatment experienced a decline in both pulmonary function and clinical status. Ramsey *et al.*[83] conducted a multicenter, double-blind, placebo-controlled, three-period crossover trial of aerosolized tobramycin (600 mg in 30

ml 0.45% saline three times daily) in cystic fibrosis patients for treatment of *Ps. aeruginosa* infections. They found significant improvement in pulmonary function, decreased bacterial density, decreased peripheral white blood cell counts and fewer pulmonary exacerbations during treatment periods as opposed to control periods. No evidence of toxicity was detected, and the emergence of tobramycin resistant bacterial strains was the same during control and treatment periods. Wiesemann *et al*.[89] performed a placebo-controlled, double-blind, randomized study of aerosolized tobramycin administration (80 mg twice daily for 12 months) for early treatment of *Ps. aeruginosa* colonization in cystic fibrosis patients, and they determined that early treatment with aerosolized tobramycin may prevent and/or delay *Ps. aeruginosa* pulmonary infection.

While the studies described above demonstrated some degree of benefit when utilizing aerosolized antimicrobials to suppress airway colonization with bacterial organisms, the results have been less encouraging when aerosol antimicrobials are used as an adjunct to intravenous therapy for treatment of acute pulmonary exacerbations in cystic fibrosis patients. Stephens *et al*.[90] investigated the use of inhaled tobramycin in the treatment of acute pulmonary exacerbations by comparing the response of patients to intravenous treatment (3.3 mg/kg tobramycin three times daily with 75 mg/kg ticarcillin four times daily) or intravenous treatment plus aerosolized tobramycin (80 mg three times daily). They found that although aerosolized tobramycin therapy resulted in an increase in eradication of *Ps. aeruginosa* from the respiratory tract, there was no improvement in clinical response or prevention of recolonization as compared to intravenous treatment alone. Schaad *et al*.[91] examined the effect of aerosolized amikacin (100 mg twice daily)

in conjunction with intravenous antimicrobial therapy (11 mg/kg three times daily with 62.5 mg/kg four times daily) as compared to intravenous therapy alone. They also reported an increase in eradication of *Ps. aeruginosa* from the respiratory tract, but no difference in clinical response or prevention of recolonization. The authors' conclusions in both of these studies were that the addition of aerosolized antimicrobial therapy to intravenous therapy for treatment of acute exacerbations in cystic fibrosis had no measurable clinical benefit.

Pneumocystis pneumonia

Pneumonia caused by *Pneumocystis carinii* is the most common cause of death in human AIDS patients.[21] Prophylaxis and treatment of this condition is usually accomplished with oral trimethoprim-sulfamethoxazole and/or intravenous pentamidine, but these treatments are associated with serious adverse effects.[21] Aerosol administration of pentamidine to the lower respiratory tract was investigated in a murine model,[92] and it was demonstrated that high, sustained levels of pentamidine were achieved within the lung, while negligible levels of the drug were found in extrapulmonary sites. Montgomery *et al*.[12] compared the aerosol administration of pentamidine to the lungs to intravenous pentamidine administration in human AIDS patients with suspected *P. carinii* pneumonia. They found that aerosol administration yielded significantly higher pulmonary concentrations of pentamidine, while serum levels of pentamidine following aerosol administration were low or undetectable. Prophylactic use of aerosol pentamidine has been examined in a large clinical trial by Leoung *et al*.,[93] in which pentamidine was administered by jet nebulizer at 30 mg or 150 mg once every two weeks, or 300 mg once

every four weeks. The recurrence rate for *P. carinii* pneumonia was lowest for patients receiving 300 mg once monthly, at 6.2%, compared to 13.3% for 150 mg bimonthly and 18.8% for 30 mg bimonthly. However, when aerosol pentamidine prophylaxis (300 mg monthly) was compared to trimethoprim/sulfamethoxazole prophylaxis, it was found that the recurrence rate of *P. carinii* pneumonia was 28% with aerosol pentamidine versus 11% with trimethoprim/sulfamethoxazole.[94] Recurrence of *P. carinii* pneumonia when patients are being treated with aerosol pentamidine has been primarily associated with the upper lung lobes, which receive lower concentrations of aerosolized pentamidine, and with extrathoracic disease.[21] As a result, the use of aerosol pentamidine prophylaxis is reserved as an alternative treatment for patients who are unable to receive trimethoprim/sulfamethoxazole.[5]

Ventilator associated pneumonia

Colonization of the lower respiratory tract with gram-negative bacteria is a common complication leading to pneumonia in hospitalized human patients receiving respiratory supportive therapy.[5,95] Palmer *et al*. investigated aerosol administration of antimicrobials to mechanically ventilated patients colonized with gram-negative organisms.[14] The efficiency of delivery and the clinical response to aerosolized gentamicin (80 mg three times daily) or amikacin (400 mg every eight hours) were assessed. They found that 15 to 29% of the initial dose placed in the nebulizer was deposited within the lungs. Trough sputum concentrations were determined for gentamicin (289 +/- 41.4 μg/ml) and amikacin (474 +/- 55.0 μg/ml), as were the post administration concentrations of gentamicin (1179 +/- 394.5 μg/ml) and amikacin (5353

+/- 496.0 μg/ml). Clinical response was determined based on bacterial growth, volume of respiratory secretions and the level of Interleukin-1β in the respiratory secretions. There was a significant decrease in gram-negative isolates from respiratory secretions, with eradication of all organisms in six of the nine trials. The volume of respiratory secretions decreased in all patients following aerosolized antimicrobial therapy. The concentration of Interleukin-1β decreased two-fold during and after aerosolized antimicrobial therapy, but the decrease was not statistically significant. This study demonstrated the effective delivery of antimicrobials to the lower respiratory tract of mechanically ventilated human patients, and that this therapy was associated with a significant clinical response.

Animal studies

The use of aerosolized antimicrobials in the treatment of infectious lower respiratory disease has not received much attention within the veterinary medical community, and much of the animal research has been directed towards potential human applications. This may be the result of the belief that aerosolized administration of antimicrobials is unlikely to deliver sufficient quantities of drug to the lower respiratory tract.[96] However, much has been learned about generating therapeutic aerosols, and quantifying the effectiveness of pulmonary delivery of therapeutic substances, in the 15 years since that statement was made.

Klebsiella pneumonia

Two studies have been performed by Berendt *et al*. examining the efficacy of aerosolized kanamycin in the treatment of experimentally induced *Klebsiella pneumoniae* infections. The first study utilized the murine model, and used an LD_{95} dose of

aerosolized *K. pneumoniae*.[80] A kanamycin aerosol was generated which had an MMAD of 3.5 μm. In the initial single treatment phase of the experiment the infected mice were treated with 5 mg/kg kanamycin intramuscularly or exposed to the kanamycin aerosol for 40 minutes at 6, 24 or 30 hours after infection. Aerosol administration of kanamycin was more effective than intramuscular injection at every time point. In the second phase of the experiment mice were treated once daily with either intramuscular (10 mg/kg) or aerosolized kanamycin, starting at 24 or 48 hours post infection, or by combined intramuscular (5 mg/kg) and aerosolized administration (20 minutes exposure) starting at 48 hours post infection. For treatment initiated at 24 hours the administration of kanamycin by aerosol was more effective in preventing death than intramuscular administration, with a 92% survival rate for aerosol administration, versus a 70% survival rate for intramuscular administration. When treatment was delayed until 48 hours after infection the survival rate with aerosolized administration was 50%, as compared to 23% for intramuscular administration. Combined aerosol and intramuscular administration did not result in a significant increase in survival (58%) over aerosol administration alone.

A later study by Berendt *et al*. examined the treatment of *Klebsiella pneumoniae* respiratory tract infection of squirrel monkeys with aerosol or intramuscular administration of kanamycin.[97] Kanamycin therapy was initiated 24 hours after experimental infection. Monkeys were treated with kanamycin by intramuscular injection (6.9 mg/kg to 22.5 mg/kg divided into two doses daily) or aerosol administration (3.8 to 15.0 mg/kg divided into two doses daily) for five days. The 3.8 mg/kg aerosol dose was ineffective, with 100% mortality observed. No differences in mortality or morbidity were

seen with equivalent dosages of antimicrobial ranging from 15.0 to 6.9 mg/kg of body weight/day, and at 15.0 mg/kg mortality was 0%. In this study the aerosol route of kanamycin administration was as effective as the intramuscular route.

Foal pneumonia

The use of aerosolized antimicrobials, in combination with saline, bronchodilators and mucolytics, has been suggested as an adjunct to systemic antimicrobial treatment of bacterial pneumonia in foals, but no recommendations were made regarding dosage, frequency or method of administration.[98,99] Rhodes and Genetzky[100] reported on the use of aerosolized gentamicin as an adjunct to systemic chloramphenicol therapy (55 mg/kg orally twice daily) in the treatment of a suspected case of bronchopneumonia in a six month old colt. The colt had been previously treated with gentamicin, chloramphenicol and penicillin with no clinical response. The aerosolization solution consisted of 10 ml of acetylcysteine, 20 ml of 1% isoetharine, 10 ml of gentamicin and 50 ml of saline. The aerosol was generated from a jet nebulizer into a plastic bag placed over the foal's head, and treatment was administered for 15 minutes twice daily for 10 days, followed by once daily for five days. They reported the resolution of mucopurulent nasal discharge within two days of initiation of treatment, and normalization of respiratory sounds following seven days of treatment. Unfortunately, the dose of gentamicin was not given in this report, the characteristics of the aerosol generated are unknown, and the method of aerosol administration was potentially very inefficient, making it unclear whether the aerosol administration of gentamicin was involved in the resolution of lower respiratory disease in this case.

Pasteurella haemolytica pneumonia in calves

There have been two studies examining the viability and efficacy of aerosol ceftiofur administration in calves. Vermeersch *et al*. examined the pharmacokinetics of ceftiofur when administered to calves by aerosolization, as compared to intravenous administration.[13] The aerosolization solution consisted of one gram of sodium ceftiofur which was dissolved in 22 ml of a water/ethanol solution (10:1 V/V). This solution, when aerosolized using a jet nebulizer, yielded an aerosol with almost 100% of the particles less than 5 mm in diameter. The aerosol was administered to the calves using a jet nebulizer, a nebulizing chamber and a snug-fitting facemask. Following intravenous administration of ceftiofur (1.0 mg/kg) no antimicrobial activity was detected in the bronchoalveolar lavage fluid at any time point. Immediately following aerosol administration of ceftiofur the antimicrobial activity of ceftiofur in the bronchoalveolar lavage fluid was approximately 4.5 mg/ml. The bronchial lavage fluid values were converted to bronchial secretion activities using the results of an inulin dilution assay (dilution factor = 70.7 +/- 18.8), yielding values greater than the mean inhibitory concentration for *P. haemolytica* for at least 18 – 24 hours following aerosol administration. The plasma concentration of ceftiofur following aerosol administration was low (0 – 0.25 mg/ml), but detectable, for up to eight hours.

The efficacy of intramuscular and aerosol ceftiofur therapy was compared in a *P. haemolytica* bronchopneumonia model in calves.[84] Calves were first subjected to the physical stress of two hours of transport, then inoculated intratracheally *with P. haemolytica type A1*. Treatment with intramuscular ceftiofur (1.0 mg/kg once daily) or

aerosol ceftiofur (1 mg/kg once daily) was given for three days following the development of clinical signs (fever, tachypnea). Calves treated with aerosolized ceftiofur had lower body temperatures than calves treated intramuscularly on days 4 and 5 post-infection. Calves treated with aerosolized ceftiofur had a more rapid return to normal respiratory rates, normal milk intake and normal total white blood count, as compared to calves treated intramuscularly. Mortality was 42% in the intramuscular treatment group, as compared to 8% in the aerosol treatment group. While the high mortality rate seen with intramuscular ceftiofur therapy may be the result of the low dosage rate utilized in this study, it is clear that aerosol ceftiofur therapy was effective for the treatment *of P. haemolytica* pneumonia in this experimental model.

Adult horses

The delivery of gentamicin to the lower respiratory tract of healthy adult horses has been compared following intravenous (6.6 mg/kg gentamicin once) or aerosol administration (1 gram of gentamicin intravenous solution q/s to 20 ml with sterile water).[101] The aerosol was generated using an ultrasonic nebulizer (Ultraneb 99, Devilbiss) and the aerosol was delivered using a commercially available valved facemask (Equine Aeromask, Canadian Monaghan). Horses were exposed to the aerosol for ten minutes. The peak absolute concentration of gentamicin in bronchial lavage fluid was 5.66 mg/ml (+/- 1.03 μg/ml) following aerosol administration, and 0.46 mg/ml (+/- 0.21 μg/ml) following intravenous administration. The gentamicin concentration in the bronchial lavage fluid following aerosol administration was significantly greater than after intravenous administration for at least eight hours.

The aerosol administration of antimicrobials has been advocated as a primary or adjunctive treatment for lower respiratory infectious disease in adult horses.[102] Administration of gentamicin (2.2 mg/kg once daily) and/or ceftiofur (2.2 mg/kg twice daily) was reported to have been highly effective in the treatment of horses with infection of the airway epithelium. Gentamicin concentrations in the bronchial secretions following aerosol administration were reported to be higher than those obtained following intravenous administration (6.6 mg/kg once daily). The gentamicin concentrations in the serum following aerosol gentamicin administration were reported to be less than 1 mg/ml, decreasing the concern regarding potential nephrotoxicity. Parenteral administration of antimicrobials was recommended for treatment of pulmonary parenchymal infections, although aerosol administration of antimicrobials was suggested as an adjunct to parenteral administration.

Viral respiratory disease

The aerosol administration of antiviral agents has been studied in animal models and in humans with viral respiratory disease. Ribavirin is currently the only antiviral agent approved for aerosol administration in humans. Aerosol administration of ribavirin has been demonstrated to be effective in the treatment of influenza A and B infections in young adult humans, and in the treatment of influenza A infections in mice. [103] Efficacy of this treatment has also been demonstrated in respiratory syncytial virus infections in cotton rats and in human infants.[21,103] Aerosol ribavirin administration has been shown to produce very high drug levels within the respiratory secretions with no local or systemic toxicity noted.[21,103]

Fungal respiratory disease

Aerosol administration of antifungal agents is of interest because fungal pneumonia is associated with high mortality rates in humans and horses despite systemic treatment with antifungals.[104,105] Amphotericin B has been one of the most widely used and successful treatments for fungal pneumonia,[106] primarily due to it's broad spectrum of activity.[104,105] Unfortunately this drug also has considerable toxic potential when administered systemically, including nephrotoxicity, hepatotoxicity and phlebitis.[104,105] Aerosol administration of amphotericin B has been demonstrated in sheep to achieve very high drug concentrations in bronchial lavage fluid, while the drug was undetectable in the blood following aerosol administration.[107] Aerosol amphotericin B therapy has been studied both for prophylaxis and treatment of fungal pneumonia, and has been shown to be particularly effective when used prophylactically in both animal models and human patients.[106] Recent investigations have focused on the aerosol administration of amphotericin B in liposomal complexes, which are thought to be taken up by the alveolar macrophages, resulting in prolonged drug release and maintenance of therapeutic intrapulmonary drug concentrations.[107] Aerosolized liposomal amphotericin B has been shown to be more effective than non-liposomal amphotericin B in aspergillus pneumonia models in rats and mice.[107,108]

Limitations

There are several reports documenting the alteration of pulmonary mechanics in human patients following aerosol administration of antimicrobials, primarily as the result of bronchoconstriction, and it has been hypothesized that this effect is the result of irritation induced by the drug itself, the drug carrier, or the tonicity of the solution.[70-72] The administration of bronchodilators prior to the administration of aerosolized medication has been shown to counteract the bronchoconstriction and irritation induced by some aerosolized medications.[20,82] It has been demonstrated that prior treatment with a bronchodilator attenuates the coughing induced by aerosolized medication and, perhaps more significantly, improves the uniformity of pulmonary aerosol deposition.[109,110]

Due to the inability of aerosol administration to deliver medication to areas of the lung which are not ventilated, the administration of antimicrobials by inhalation alone is not appropriate when significant consolidation or parenchymal involvement is present. While this represents a significant limitation of aerosol antimicrobial therapy, this route of administration has a potential benefit as an adjunct to systemic administration.[8,20,102] Inactivation of β-lactam and aminoglycoside antimicrobials has been demonstrated in bronchial secretions, but it is likely that these inhibitions can be overcome by achieving high concentrations of the antimicrobials.[5] It has been demonstrated that very high concentrations of antimicrobials can be attained within the respiratory tract, as described above, and the positive clinical response seen to aerosol antimicrobial therapy in some of these studies suggests that drug inactivation is not a major concern. In fact, it may be a greater concern with systemic administration of antimicrobials, where the concentrations

of antimicrobials within the respiratory tract are lower, and thereby more susceptible to the effects of inactivation.

The development of antimicrobial resistance has been documented in several studies of human patients treated with aerosolized antimicrobials.[83,87,88,111,112] However, the development of resistance was not reported to have any impact on the clinical course in any of these studies. The presence of resistant strains was often transient and did not require alteration of therapy in these studies. It has been suggested that in vitro resistance may have a poor correlation with in vivo resistance, because the antimicrobial concentrations that can be achieved within the lung following aerosol administration are much higher than those concentrations on which standard in vitro susceptibility is based.[104]

Atmospheric contamination has been mentioned frequently in the literature as a possible complicating factor of aerosol antimicrobial administration.[13,17,84] However, there have been no problems attributable to atmospheric contamination reported to date.[104] The potential for contamination of the antimicrobial solution or delivery apparatus with micro-organisms is a valid concern. Most manufacturers of delivery systems give specific recommendations for the cleaning of the equipment to minimize or prevent contamination. In the case of ultrasonic nebulizers, the entire portion of the system exposed to the drug is often disposable. Jet nebulizers and facemasks can typically be gas sterilized between patients to prevent the spread of infectious organisms. The ready availability of snug-fitting valved facemasks for both foals and adult horses (Equine Aeromask®, Canadian Monaghan) allows aerosolized medications to be

delivered routinely in a field or clinical setting.[102,113] Unfortunately, the nebulizers that are currently available for aerosol administration of antimicrobials to horses have the limitations of low efficiency or high cost. The jet nebulizers, while being inexpensive and producing aerosols with desirable particle size distributions, are slow and produce large amounts of noise. The ultrasonic nebulizers tend to produce aerosols with significantly greater density, resulting in much shorter treatment times, but are expensive, limiting their clinical application.

Conclusions

To date the clinical application of aerosol antimicrobial therapy has been limited by technical concerns, concerns regarding adverse effects, an insufficient understanding of the pharmacokinetics and pharmacodynamics of aerosolized medications, and a lack of effective and appropriate means of determining the dosage of these drugs.[21] Many of the concerns have been overcome by improvements in delivery systems and drug formulations, and the increasing body of literature in this area has allowed for a much better understanding of the pharmacokinetics of aerosolized antimicrobials. Aerosolized antimicrobial administration has been shown to be effective in the treatment of lower respiratory infectious diseases where parenchymal involvement is not present, and it may have a role as an adjunct to systemic antimicrobial administration even in the presence of significant parenchymal involvement. The improvements in the equipment available to administer aerosol antimicrobials to horses have made this route of administration more practical.

Administration of gentamicin to the horse by the intravenous and inhaled routes.

Introduction

Respiratory disease is a widespread problem in the equine industry, resulting in considerable expense and loss of use. Respiratory disease is the second most common cause of decreased performance,[114] and lost training days in horses,[115] and bacterial pneumonia has been described as the most common cause of lower respiratory disease in foals and young horses.[116] Treatment of infectious lower respiratory disease has traditionally relied upon administration of enteral and/or parenteral antimicrobials. However, effective antimicrobial therapy requires appropriate concentrations of the antimicrobial agent at the site of infection,[117] and one of the major limitations of systemic antimicrobial therapy is the low activity or bioavailability of many of these medications in the lower respiratory tract.[1-6]

Bacterial respiratory infections in horses are usually precipitated by impairment of local host defenses by viral infections or physiologic stress.[118,119] As a result, the primary site of infection is typically on the bronchial mucosal surface, and the organisms involved are often normal inhabitants of the respiratory tract.[120] As a result, the therapeutic outcome of respiratory infections is typically more closely associated with airway antimicrobial concentrations than with serum antimicrobial concentrations.[1,3] There has been substantial interest in aerosol administration of antimicrobial drugs to the lower respiratory tract because systemically administered antimicrobials may not achieve adequate levels within the respiratory tract whereas the aerosol route of administration is

capable of achieving high concentrations of antimicrobial drugs at the site of infection.[12,13] In addition, there are other potential advantages to delivering medications to the lower respiratory tract via the inhaled route, which include a decrease in the total dose administered, a rapid onset of action, and avoidance or reduction of systemic side effects.[15-17]

Several studies in humans and animals have demonstrated the effectiveness of aerosol antimicrobial administration in achieving high antimicrobial concentrations in the pulmonary epithelial lining fluid and/or respiratory mucosa of the lower respiratory tract.[9-14] Human and animals studies have also demonstrated the effectiveness of aerosol antimicrobial administration in decreasing the severity, or shortening the course of, lower respiratory bacterial infections.[14,80-84] There are two reports in which the aerosol administration of aminoglycosides is suggested to be of potential benefit in foal pneumonia,[99,100] but neither attempted to characterize the dose delivered or determine the effective dosage or optimal frequency of administration. No study to date has investigated the feasibility or efficacy of aerosolized antimicrobial therapy in horses.

The objective of this study was to assess the administration of gentamicin to the lower respiratory tract of horses via the inhaled route by comparing the antimicrobial concentrations achieved in the epithelial lining fluid of the lower respiratory tract following aerosol and intravenous administration. We tested the following hypotheses: 1) the concentrations of gentamicin achieved in the airway epithelial lining fluid of the horse are the same following aerosolized or intravenous administration; 2) the

administration of gentamicin to the lower respiratory tract by aerosolization does not induce lower airway inflammation.

Materials and Methods

Preliminary studies

Aerosol characterization

The particle characteristics and size distribution for the aerosols generated by the ultrasonic nebulizer[a] were determined for the following 7 solutions: gentamicin injectable solution (100 mg/ml), gentamicin injectable solution in sterile water (75 mg/ml, 50 mg/ml, and 25 mg/ml), and gentamicin injectable solution in 0.9% saline (75 mg/ml, 50 mg/ml, and 25 mg/ml). A total volume of twenty ml of solution was placed in the nebulizer cup. Particle size distribution analysis was performed using a laser diffraction aerosol particle analyzer.[b] The refractive index of water (1.330) was utilized in this analysis. The refractive index of each of the eleven solutions was later analyzed using a hand held refractometer.[c] The nebulizer was set up with no inline valving and with the output setting on maximum. The output from the nebulizer cup was directed to the sampling area of the particle analyzer using a 7/8" internal diameter (i.d.) x 36" long section of corrugated plastic nebulizer tubing.[d] The nebulizer was turned on and allowed to run until a steady output of aerosol was visible in the sampling region of the particle analyzer, at which time sampling was begun. Each sampling consisted of 7,500 sweeps of the detector array, and each solution was sampled twice. The results of this analysis were expressed as a volume distribution, with the particle concentration expressed as a percent volume, and with the mass median aerodynamic diameter (MMAD) expressed as that diameter below or above which 50 percent of the volume of particles resided. The changes in particle concentration and mass median aerodynamic diameter between

diluents and concentrations were compared using the Wilcoxon signed rank test. All statistical analysis was performed using a computerized statistical analysis program,[e] and statistical significance was defined as a p-value less than 0.05.

The aerosol generated by the ultrasonic nebulizer[a] and the solution used in the aerosol administration study (10 ml of 100 mg/ml gentamicin injectable solution in 10 ml sterile water, 50 mg/ml final gentamicin concentration) was characterized. Particle size distribution analysis was performed as previously described. Sampling was performed every sixty seconds for the duration of two separate ten minute nebulizer runs. The changes in particle concentration and mass median aerodynamic diameter over time were compared using regression analysis.

Sampling technique validation

The study required that samples of pulmonary epithelial lining fluid be acquired at multiple times following a single administration of gentamicin, and it was necessary to confirm that gentamicin was evenly distributed throughout the lungs after aerosol administration. Six adult horses, 3 to 15 years of age, were used. These horses belonged to the Marion duPont Scott Equine Medical Center research herd. Horses were maintained on pasture except for the twelve hour period prior to and including the sampling period, at which time the horses were housed in 12' x 12' box stalls and bedded on pine wood shavings. Water and timothy hay were available at all times. Each horse was administered gentamicin aerosol once. A close fitting facemask[f] was placed over the muzzle of the horse and attached to an ultrasonic nebulizer[a] by smoothbore flexible plastic tubing.[g] The test solution (10 ml of 100 mg/ml gentamicin in 10 ml sterile water-50 mg/ml final gentamicin concentration) was placed into the disposable nebulizer cup, and the nebulizer was run for ten minutes. Following completion of aerosol administration 0.02 mg/kg acepromazine was administered intravenously. Five minutes prior to bronchial lavage each horse was sedated further with 0.5 mg /kg xylazine intravenously. Bronchial lavage was performed 15 to 20 minutes after the completion of aerosol administration, with four sites being lavaged sequentially. These sites were designated R1 (right caudoventral lung), R2 (right caudodorsal lung), L1 (left caudoventral lung), and L2 (left caudodorsal lung).

A 2 meter-long video endoscope[h] was utilized for all lavage procedures. Prior to endoscopy a #205 polyethylene tube,[i] with an i.d. of 1.57 mm, was inserted into the

biopsy channel of the endoscope, and a 16 gauge blunt needle was inserted into the outer end of this tube to enable attachment of a Luer tip syringe. As the endoscope was advanced down the trachea 20 to 40 ml of a 0.2% mepivacaine solution was infused into the trachea via the polyethylene tubing, to ensure adequate local anesthesia of the tracheal surface. Upon reaching the carina 5 ml of a 0.4% mepivacaine solution was infused into the airway as the endoscope was advanced to the area where it was to be wedged. The scope was withdrawn to the carina for 30 to 60 seconds to allow for the mepivacaine to anesthetize the area, and the polyethylene tubing was withdrawn from the biopsy channel of the endoscope. The endoscope was then advanced to the desired area, and the tip was wedged in the bronchus. A 60 milliliter syringe containing 30 ml of normal sterile saline and 30 ml of air was attached to the biopsy channel of the endoscope and the contents were infused into the lung such that the air immediately followed the saline to ensure that the full volume was delivered to the bronchi. A small volume (30 milliliter) lavage technique was chosen for the lavage procedure in order to preferentially sample the proximal bronchial tree and to minimize the dilution of the pulmonary epithelial lining fluid.[121,122] Aspiration was immediately performed with the attached syringe to remove as much of the infused volume as possible, and aspiration was repeated five times. The endoscope was then withdrawn to the carina and topical anesthesia and bronchial lavage were repeated at each lavage site.

Following completion of the bronchial lavage procedures, the bronchial lavage fluid samples were centrifuged at 3,000 rpm for 10 minutes (1,900 G) at 4°C. The supernatant was removed, filtered through a 0.2 micron polyethersulfone filter[j] and 2

aliquots of fluid were frozen at -70°C until the assays were performed. Gentamicin concentrations were determined by fluorescence polarization immunoassay.[k] The working range for this assay is 1 μg/ml to 10 μg/ml, with a lower limit of sensitivity of 0.27 μg/ml gentamicin in serum.[l] Results of the gentamicin assays were compared using the Wilcoxon signed rank test.

Gentamicin standards (10 μg/ml, 1 μg/ml and 0.1 μg/ml) were formulated initially in gentamicin-free equine serum and bronchial lavage fluid utilizing the commercial gentamicin 100 mg/ml solution[m] utilized for gentamicin administration in this study. Substantial errors in gentamicin concentration were observed with the 10 μg/ml and 0.1 μg/ml standards with both diluents. These errors were most likely due to the presence of additives in the commercial solution that interfered with the fluorescence polarization immunoassay for gentamicin determination. A 100 mg/ml gentamicin stock solution was then formulated using reagent grade gentamicin sulfate[n] and sterile water as a diluent. Gentamicin standards of 10 μg/ml, 1 μg/ml and 0.1 μg/ml concentration were formulated by serial dilution in gentamicin-free equine serum and bronchial lavage fluid utilizing the 100 mg/ml gentamicin stock solution. The serum controls yielded mean gentamicin concentrations of 9.76 ± 0.13 μg/ml, 1.16 ± 0.05 μg/ml and 0.21 ± 0.21 μg/ml for the 10, 1 and 0.1 μg/ml solutions, respectively. The bronchial lavage fluid controls yielded mean gentamicin concentrations of 9.54 ± 0.47 μg/ml, 1.19 ± 0.01 μg/ml and 0.32 ± 0.01 μg/ml for the 10, 1 and 0.1 μg/ml solutions, respectively.

Primary study

Nine adult horses, 3 to 27 years of age, were used in a crossover design. Six horses belonged to the Marion duPont Scott Equine Medical Center research herd and three horses had been donated for medical reasons unrelated to the respiratory tract. Gross evidence of respiratory disease, such as nasal discharge, labored respirations or coughing were the only criteria for an individual not being included into the study. Horses were turned out onto pasture for a minimum of 2 weeks between treatments. Aerosol administration of gentamicin was performed as previously described. Intravenous administration consisted of one 6.6 mg/kg bwt dose of gentamicin administered via a 14 gauge, indwelling intravenous Teflon® catheter[o] placed into the right jugular vein. Samples of pulmonary epithelial lining fluid were collected by bronchial lavage via videoendoscope, as previously described, performed at 0.5, 4, 8 and 24 hours following gentamicin administration. The lavage procedure was performed at site L1 at 0.5 hours, site L2 at 4 hours, site R1 at 8 hours and site R2 at 24 hours. Bronchial lavage samples were processed (as previously described). Serum samples were collected at 0.5, 4, 8 and 24 hours following gentamicin administration. Ten ml of blood was collected from the left jugular vein into a plain vacuum collection tube.[p] After being allowed to clot, each blood sample was centrifuged at 3,000 rpm (1,900 G) for 10 minutes at 4°C. The serum was removed and two aliquots of serum were frozen at -70°C until the gentamicin assays were performed.

All bronchial lavage fluid and serum samples were analyzed for gentamicin concentration as previously described. Nucleated cell counts and cytologic examinations

were performed on aliquots of bronchial lavage fluid obtained at 0.5, 8 and 24 hours. The nucleated cell counts were performed manually using a commercial pipetting and dilution system[q] and a Neubauer ruled hemacytometer. Two separate counts were made from each sample and the results were averaged. Slides were prepared for cytologic examination using a cytology centrifuge[r] and were stained with eosin and thiazine stain.[s] Cytologic samples were analyzed microscopically for nucleated cell differential on a total of 200 nucleated cells for each sample.

Gentamicin concentration data were compared by route of administration (aerosol and intravenous), time following gentamicin administration (times 0.5, 4, 8 and 24 hours) and sampling site (bronchial lavage fluid and serum). Bronchial lavage fluid cytology data were compared by route of administration and time following gentamicin administration. The Wilcoxon signed rank test was used for all comparisons.

Results

Preliminary studies

Aerosol characterization

The refractive indices of the test solutions ranged from 1.363 for the 100 mg/ml gentamicin solution, to 1.341 for the 25 mg/ml gentamicin solutions. These values were close to the value for water (1.330), and as a result should not have significantly affected the results of the particle size analysis. The results of the initial aerosol characterization demonstrated a significant ($p<0.05$) decrease in the mass median aerodynamic diameter (MMAD) with increasing gentamicin concentration, regardless of diluent. (Figure 1) There was a significant decrease ($p<0.05$) in the particle concentration (aerosol density) with increasing gentamicin concentration, with the mean percent volume ranging from $4x10^{-3}$% at 25 mg/ml to $4x10^{-4}$% at 100 mg/ml, with no significant difference between diluents. (Figure 2)

Further characterization of the test solution (50 mg/ml gentamicin in sterile water) yielded results which differed from the previous analysis. The mean MMAD was 5.51 µm, as compared to 4.53 µm in the previous analysis. Over the course of the ten-minute nebulizer run there was a statistically significant ($p<0.05$) decrease in MMAD, from 5.74 µm to 5.28 µm (Figure 3). The mean particle concentration observed at five minutes (2.8×10^{-3} %) was significantly greater ($p<0.05$) than the values observed at the beginning (1.95×10^{-3} %) and end (1.8×10^{-3} %) of the ten-minute nebulizer run (Figure 4).

Sampling technique validation

No significant differences were observed in the bronchial lavage fluid gentamicin concentrations obtained from the four sites R1, R2, L1 and L2 immediately following aerosol administration of gentamicin. The yield of bronchial lavage fluid was 11.1 ± 0.4 ml (37% of the volume infused ± 1.3%). The mean bronchial lavage fluid gentamicin concentration from all of the sites was 5.56 ± 0.61 µg/ml (mean ± SEM). The bronchial lavage fluid gentamicin concentrations (mean ± SEM) for each site were: R1, 6.74 ± 1.40 µg/ml; R2, 5.28 µg/ml ± 0.89 µg/ml; L1, 4.76 µg/ml ± 1.14 µg/ml; and L2, 5.46 ± 1.59 µg/ml.

Primary study

The yield of bronchial lavage fluid was 10.8 ml ± 0.2 ml (mean ± SEM), representing 36% ± 0.6% of the volume infused. Mean bronchial lavage fluid gentamicin concentrations (mean ± SEM) at 0.5, 4, 8 and 24 hours after aerosol administration were 5.66 μg/ml ± 1.03 μg/ml, 2.80 μg/ml ± 0.54 μg/ml, 0.80 μg/ml ± 0.04 μg/ml, and 0.18 μg/ml ± 0.02 μg/ml, respectively. Mean bronchial lavage fluid gentamicin concentrations at 0.5, 4, 8 and 24 hours after intravenous administration were 0.46 μg/ml ± 0.21 μg/ml, 0.44 μg/ml ± 0.17 μg/ml, 0.3 μg/ml ± 0.03 μg/ml, and 0.17 μg/ml ± 0.05 μg/ml, respectively. Mean serum gentamicin concentrations at 0.5, 4, 8 and 24 hours after aerosol administration were 0.31 μg/ml ± 0.04 μg/ml, 0.15 μg/ml ± 0.02 μg/ml, 0.17 μg/ml ± 0.13 μg/ml, and 0.01 μg/ml ± 0.01 μg/ml, respectively. Mean serum gentamicin concentrations at 0.5, 4, 8 and 24 hours after intravenous administration were 39.8 μg/ml ± 1.66 μg/ml, 7.93 μg/ml ± 0.49 μg/ml, 2.3 μg/ml ± 0.23 μg/ml, and 0.21 μg/ml ± 0.05 μg/ml, respectively. The mean bronchial lavage fluid gentamicin concentrations following aerosol administration were significantly greater ($p<0.05$) than the bronchial lavage fluid gentamicin concentrations following intravenous administration at 0.5, 4 and 8 hours after administration (Figure 5). The gentamicin concentrations in the serum following aerosol administration of gentamicin were < 0.5 μg/ml at all times (Figure 6).

The total nucleated cell count in the bronchial lavage fluid demonstrated a significant ($p<0.05$) increase over the course of the 24-hour sampling period, from 251 ± 42 cells/μl (mean ± SEM) at 0.5 hours to 464 ± 64 cells/μl at 8 hours and 433 ± 53

cells/µl at 24 hours (Figure 7). There was a significant difference ($p<0.05$) in the increase in total nucleated cell count between routes of gentamicin administration, with higher counts following aerosol administration. The neutrophil count in the bronchial lavage fluid increased significantly ($p<0.05$) over the course of the 24-hour sampling period regardless of the route of gentamicin administration. The increase in neutrophil count was, however, significantly greater ($p<0.05$) following aerosol gentamicin administration as compared to intravenous gentamicin administration. The neutrophil count observed following aerosol gentamicin administration increased from 31 ± 10 cells/µl to 90 ± 15 cells/µl from 0.5 to 24 hours, (Figure 8) while the number of neutrophils observed following intravenous administration of gentamicin increased from 30 ± 8 cells/µl to 61 ± 12 cells/µl from 0.5 to 24 hours (Figure 9).

Discussion

The clinical efficacy of a therapeutic aerosol depends on the amount of the therapeutic substance which is deposited within the respiratory tract.[23] The pattern of deposition of aerosol particles and the efficiency of aerosol delivery are profoundly influenced by the characteristics of the aerosol itself, including the size distribution of the aerosol particles and the aerosol density.[27] The aerosols produced by nebulization show very significant intra- and inter-device variability with identical solutions,[18] and the use of different solutions only increases this variability. In order to fully characterize the delivery of an aerosolized drug it is essential to determine the characteristics of the aerosol produced by the test solution in the delivery system utilized.[73]

The refractive indices of the test solutions ranged from 1.363 for the 100 mg/ml gentamicin solution, to 1.341 for the 25 mg/ml gentamicin solutions. These values were close to the value for water, and it was considered unlikely that this was an important source of error in the particle size analysis.[t] The significant increase in aerosol density with decreasing gentamicin concentration may have clinical relevance, because increasing the density of the aerosol results in an increase in aerosol delivery per unit time. Because the increased density coincided with decreasing drug concentration, we elected to compromise between maximum drug concentration and maximum aerosol density and utilize the 50 mg/ml solution for the remainder of the test protocol. The MMAD decreased significantly with increasing gentamicin concentration, but this was not important from a clinical standpoint, because the nebulizer produced an aerosol with

greater than 50% of the particles in the respirable range of 1-5 μm diameter with all of the gentamicin solutions.

The characterization of the test solution yielded a 1 μm increase in mean MMAD, to 5.51 μm, as compared to the initial analysis. The only change in technique between these analyses was a change in the type of nebulizer tubing, from corrugated tubing to smoothbore tubing. It is likely that the corrugated tubing created turbulent airflow, resulting in the increased deposition of large particles, while the more laminar airflow expected in the smoothbore tubing allowed these particles to reach the test chamber, resulting in a skewing of the particle distribution towards a greater MMAD. Over the course of the ten-minute nebulizer run there was a statistically significant decrease in MMAD, from 5.735 μm to 5.275 μm, likely due to warming of the nebulizer solution and/or the increasing gentamicin concentration in the nebulizer solution during the course of the ten minute nebulizer run.[23,36] This resulted in an increase in the percentage of particles produced within the respirable range.

Gentamicin concentrations in the bronchial lavage fluid are reported here with no correction attempted for the dilution of the pulmonary epithelial lining fluid by the lavage fluid. Several techniques have been proposed for determining the volume of pulmonary epithelial fluid in bronchoalveolar lavage fluid. These techniques have utilized endogenous albumin or urea,[78,123-125] or exogenous substances such as 99mTc-DTPA, 51Cr-EDTA, inulin, urea, and methylene blue, as markers of dilution.[126] It has been demonstrated that such correction techniques introduce significant error into the reported concentration of pulmonary epithelial lining fluid constituents,[126,127] and for that reason

these correction techniques were not utilized in this study. The yield of bronchial lavage fluid was very consistent, indicating that the lavage technique was consistent and reproducible. This consistency in yield suggests that there should be minimal variation in the dilution of the pulmonary epithelial lining fluid by the lavage fluid. The sampling technique validation study demonstrated that there was no significant difference in the bronchial lavage fluid gentamicin concentration obtained from the four sites R1, R2, L1 and L2 immediately following aerosol administration of gentamicin. The mean gentamicin concentrations from all of the sites were not significantly different, validating the sampling technique for determining bronchial lavage fluid gentamicin concentration at different sites within the lungs.

The antimicrobial gentamicin was selected for this investigation because the aminoglycosides have characteristics that are potentially beneficial when locally administered. Aminoglycosides exhibit concentration-dependent bactericidal activity and produce prolonged post-antibiotic effects against susceptible organisms.[128] High concentrations of aminoglycosides produce more rapid and extensive bacterial killing than lower levels, as well as prolonging the duration of the post-antibiotic effects.[128] High peak concentrations (greater than 8-10 times the minimum inhibitory concentration) have also been shown to decrease the emergence of resistant strains.[128]

In this study we demonstrated that the aerosol administration of gentamicin to the equine lower respiratory tract achieves bronchial lavage fluid gentamicin levels that are significantly higher than those obtained following intravenous administration for at least the first eight hours following gentamicin administration. The bronchial lavage fluid

gentamicin concentrations reported in this study were lower than those reported by Godber *et al*. in a study using a paper disc absorption technique for sampling equine bronchial secretions.[6] In that study the maximum gentamicin concentration in bronchial secretions following intravenous administration of a single 6.6 mg/kg gentamicin dose ranged from 3.67 µg/ml to 5.08 µg/ml,[6] approximately 8 to 11 times greater than the mean bronchial lavage fluid gentamicin concentration following intravenous administration reported here. Because the dose of intravenous gentamicin was the same in both studies this suggests that the dilutional effect of the lavage fluid in this study was approximately ten fold.

Godber *et al*. concluded that the time period during which bronchial gentamicin concentrations were greater than minimum inhibitory concentrations, plus the duration of the post-antibiotic effect, was approximately 8 hours, and that the intravenous administration of gentamicin could not be recommended for treatment of airway infections.[6] Because the maximum bronchial lavage fluid gentamicin concentrations reported here after aerosol administration were 12 times greater than those measured following intravenous administration, we believe that aerosol administration achieves sufficiently high airway concentrations of gentamicin to be of clinical benefit.

The administration of medications by aerosolization and inhalation has several limitations. These include: 1) the inability to deliver medication to areas of the lung which are not ventilated, 2) potential inactivation of the medication 3) time consuming administration, 4) the potential for pulmonary tissue irritation or injury, 5) atmospheric contamination and 6) potential contamination of the antimicrobial solution with micro-

organisms.[17-21] As a result of the inability of aerosol administration to deliver medication to areas of the lung which are not ventilated, the administration of antimicrobials by inhalation alone is not appropriate when significant consolidation or parenchymal involvement is present, but may be of benefit as an adjunct to systemic administration.[8,20] Inactivation of β-lactam and aminoglycoside antimicrobials has been demonstrated in bronchial secretions, but it is likely that these inhibitions can be overcome by achieving high concentrations of the antimicrobials.[5] There are several reports documenting the alteration of pulmonary mechanics in human patients following aerosol administration of antimicrobials, primarily as the result of bronchoconstriction, and it has been hypothesized that this effect is the result of irritation induced by the drug itself, the drug carrier, or the tonicity of the solution.[70-72]

The serum concentrations of gentamicin observed following aerosol administration are primarily the result of pulmonary absorption.[16] While gastrointestinal absorption of ingested gentamicin is a potential source of serum gentamicin following nebulized administration, gentamicin is poorly absorbed from the gastrointestinal tract due to its polarity.[16] The pulmonary absorption of the drug appears to be minimal, and the low serum concentrations of gentamicin detected following aerosol administration in this study are consistent with the results of other studies in human beings and laboratory animals.[9,11,16,129] Studies of short and long term aerosol administration of aminoglycoside antimicrobials to human beings have found no evidence of pulmonary, renal or neural toxicity.[88,90,111,112] The concurrent administration of gentamicin by the aerosol and intravenous routes could potentially alter the pharmacokinetics of gentamicin in the

serum. Studies in human beings have found no statistical difference, however, in the serum peak and trough aminoglycoside concentrations in patients receiving intravenous or concurrent intravenous and aerosol administration of aminoglycosides.[90,91]

The significant increase in the total nucleated cell count and neutrophil count in the bronchial lavage fluid that occurred over the course of the sampling period suggests that there was an inflammatory response to the testing procedure. Because increases in total nucleated cell count and neutrophil count occurred with both routes of gentamicin administration a component of this response was due to the repeated bronchial lavage. This was expected, as bronchoalveolar lavage has been documented to cause mild neutrophilic pulmonary inflammation in the horse.[130] There was a significantly greater increase in the total nucleated cell count and neutrophil count in the bronchial lavage fluid following aerosol administration of gentamicin, although the magnitude of this difference was not great. This finding indicates that there is an inflammatory response within the respiratory tract to the aerosol administration of gentamicin. Previous investigators have indicated that the preservatives contained in the gentamicin solution, such as sodium sulfite, sodium metabisulfite and EDTA, are known bronchoconstrictors and could cause pulmonary inflammation if delivered to the respiratory tract by aerosolization.[36,56,59,73,104] The clinical relevance of the inflammatory response to gentamicin nebulization is unclear, however, as the total nucleated cell counts observed at all time points were within normal limits for small volume bronchoalveolar lavage (50 ml) in healthy horses.[131]

Summary

This study demonstrated that the aerosol administration of gentamicin to the equine respiratory tract achieves pulmonary epithelial lining fluid gentamicin levels that are significantly greater than those obtained following intravenous administration for at least the first eight hours following administration. The low concentration of gentamicin in the bronchial lavage fluid at all times following intravenous administration is consistent with the reports of poor penetration of aminoglycosides into bronchial secretions following systemic administration.[3,9,16,75,77,78,132] There was a mild increase in bronchial lavage fluid nucleated cell and neutrophil counts, which reflected a mild inflammatory response to the lavage procedure and to aerosol administration of gentamicin. We conclude that aerosol administration of gentamicin to the equine respiratory tract achieves bronchial lavage fluid gentamicin concentrations that are significantly greater than the concentrations obtained following intravenous administration for at least the first 8 hours after administration.

Figures

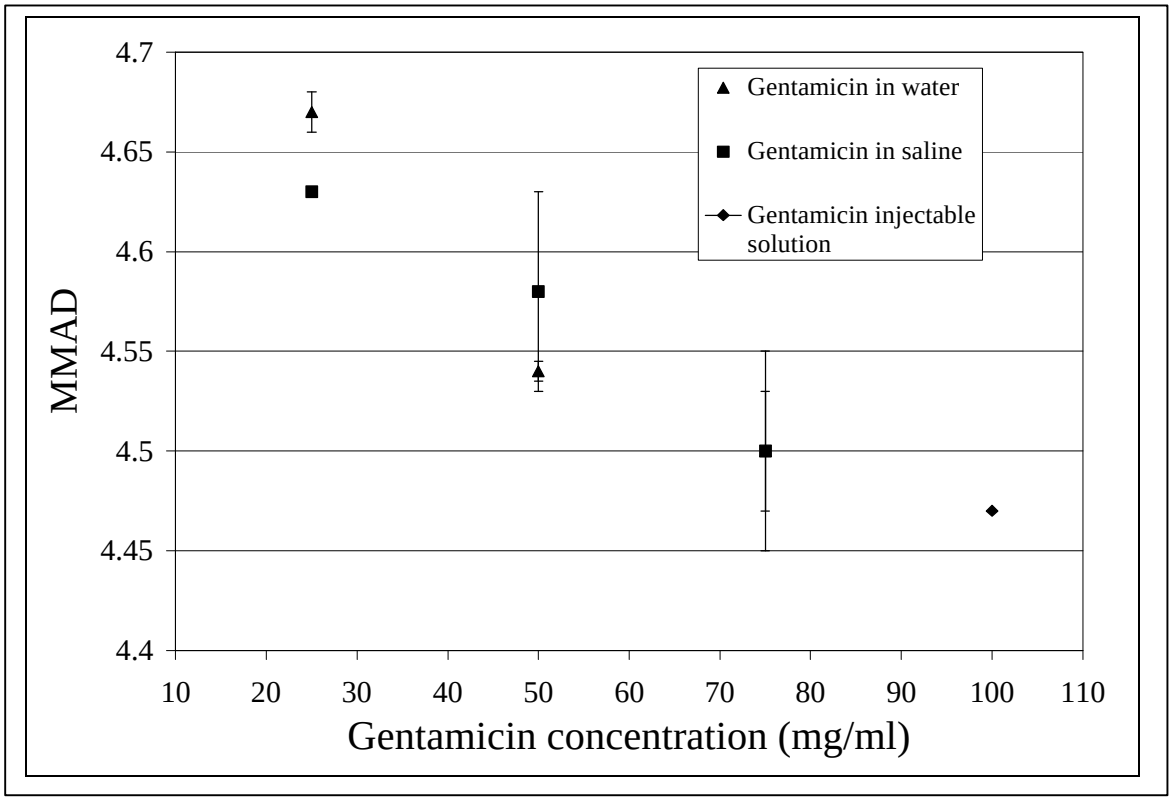

Figure 1. Aerosol particle size (mean ± SEM) of gentamicin solutions aerosolized using an ultrasonic nebulizer.[a]

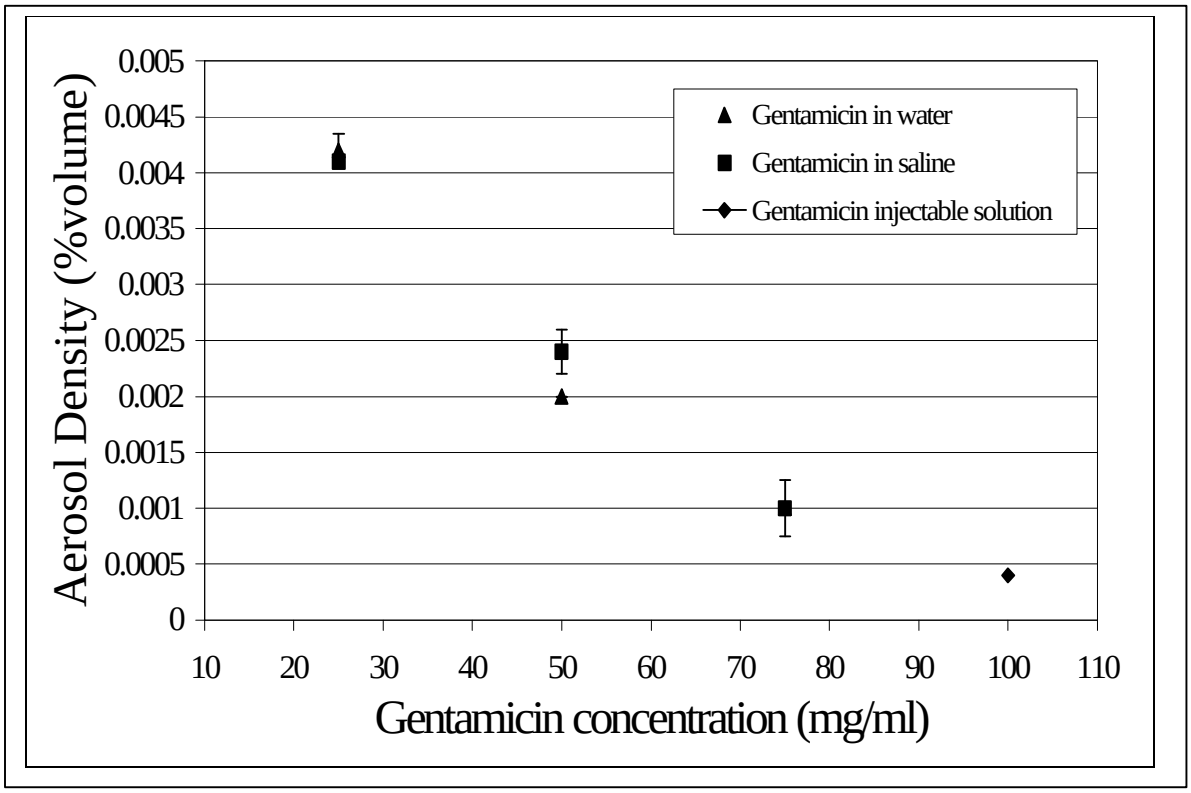

Figure 2. Aerosol density (mean ± SEM) of gentamicin solutions aerosolized using an ultrasonic nebulizer.[a]

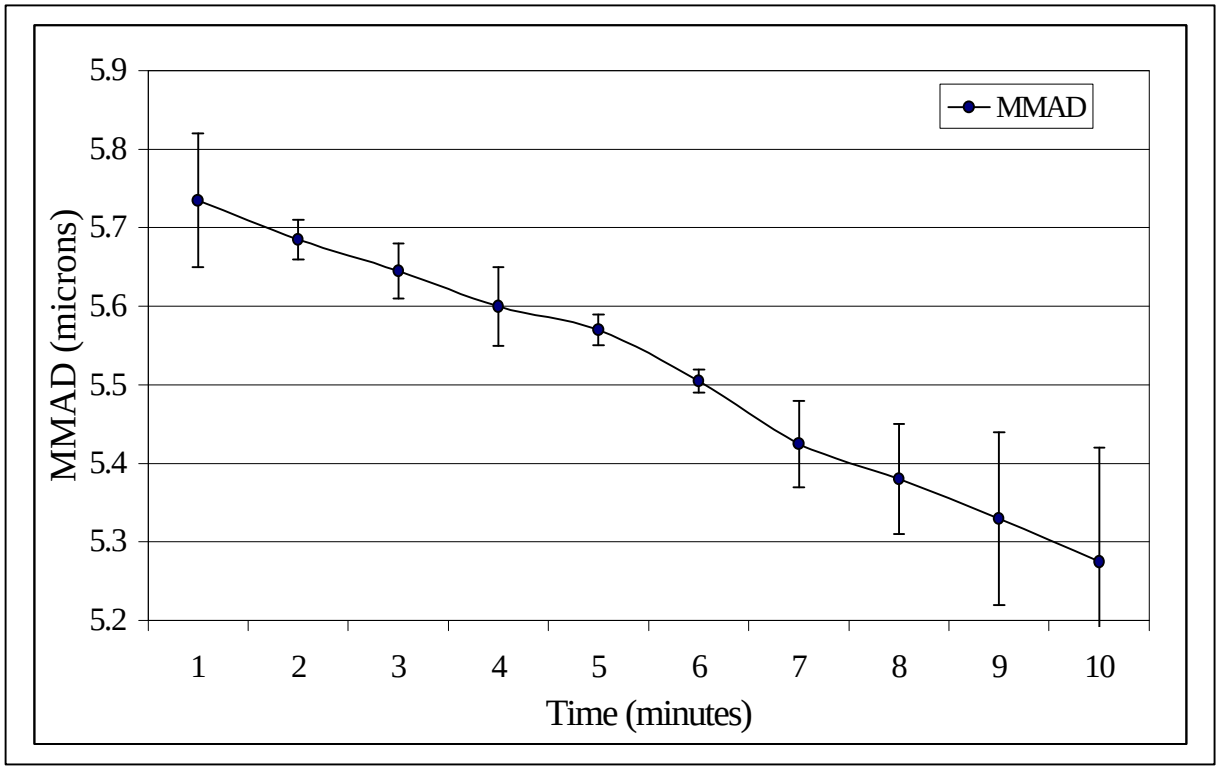

Figure 3. Aerosol particle size (MMAD, mean ± SEM) of the aerosol generated using the 50 mg/ml gentamicin in sterile water solution in an ultrasonic nebulizer.[a]

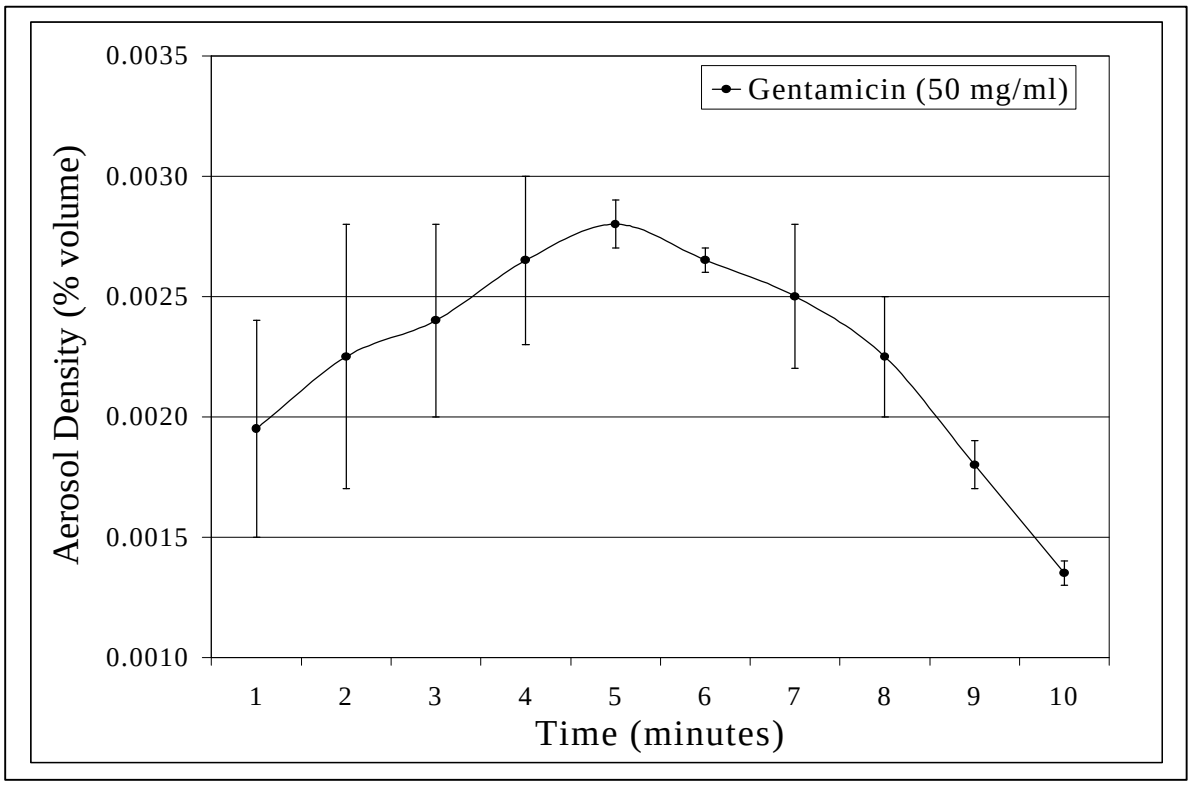

Figure 4. Aerosol density (mean ± SEM) of the aerosol generated using the 50 mg/ml gentamicin in sterile water solution in an ultrasonic nebulizer.[a]

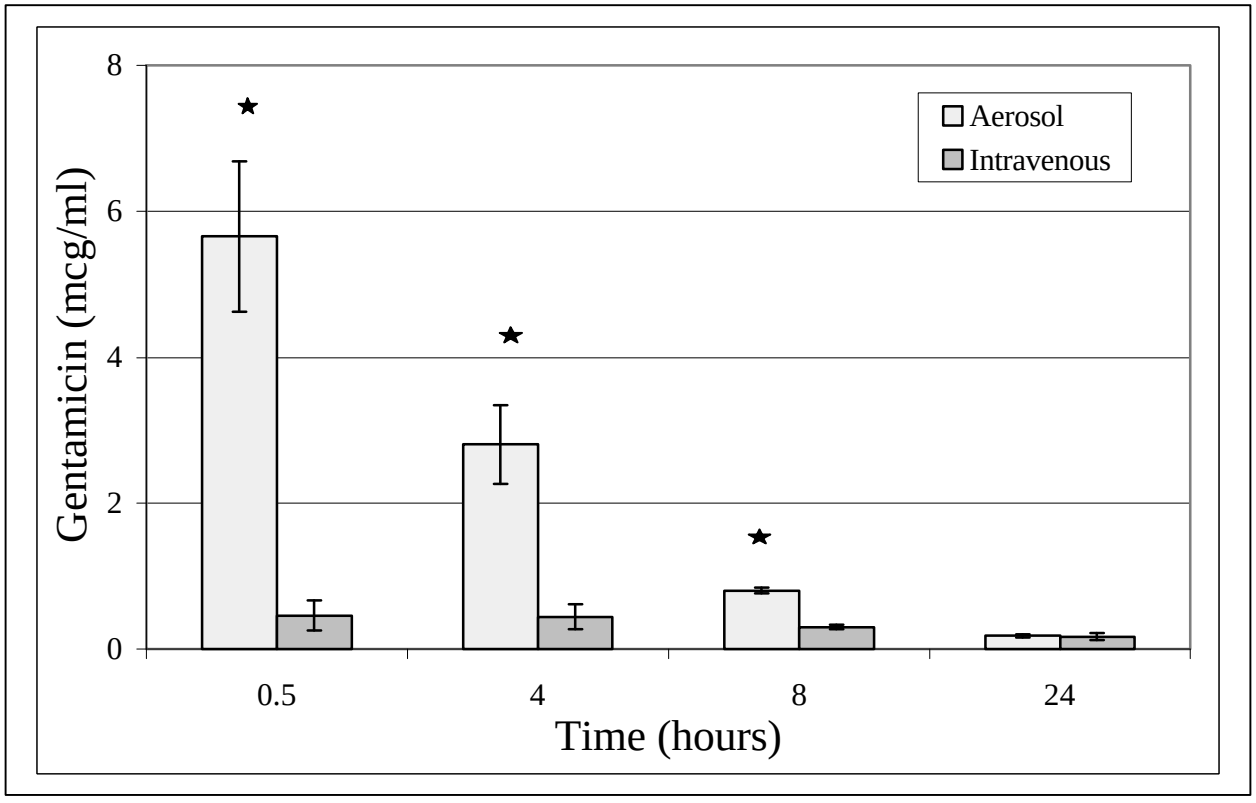

Figure 5. Bronchial lavage fluid concentrations of gentamicin (mean ± SEM) after aerosol or intravenous administration of gentamicin to horses (n=9). ★ indicates significant difference ($p \leq 0.05$) between routes of administration.

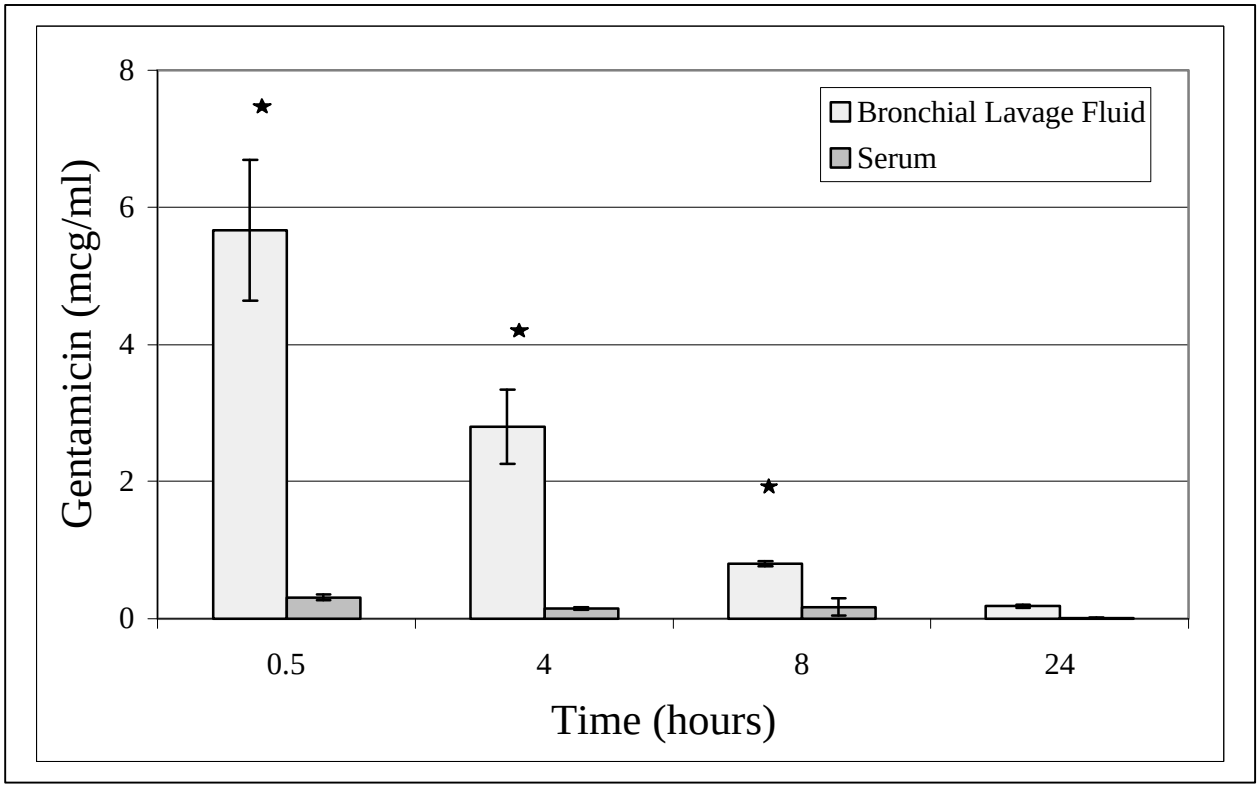

Figure 6. Gentamicin concentrations (mean ± SEM) in bronchial lavage fluid and serum after aerosol administration of gentamicin to horses (n = 9). ★ indicates significant difference ($p \leq 0.05$) between sampling sites.

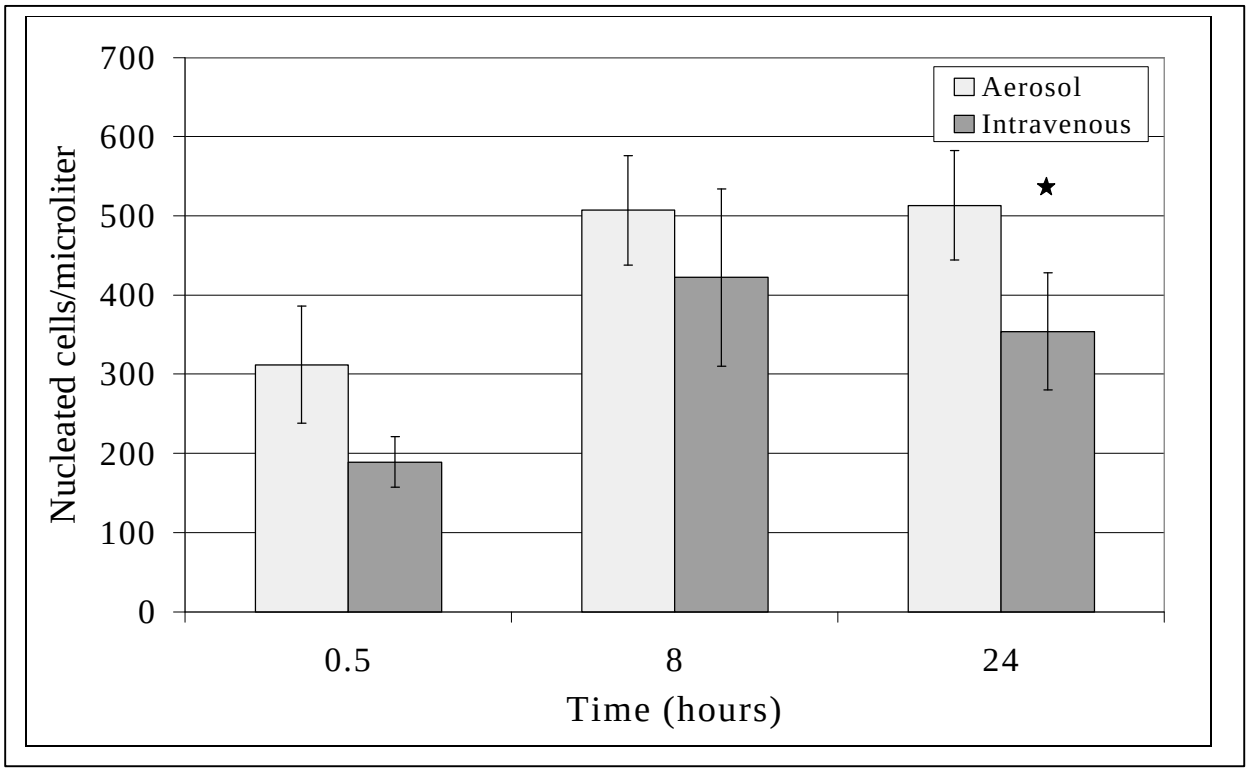

Figure 7. Total nucleated cell counts in bronchial lavage fluid after aerosol and intravenous administration of gentamicin to horses (n = 9). ★ indicates significant difference ($p \leq 0.05$) between routes of administration.

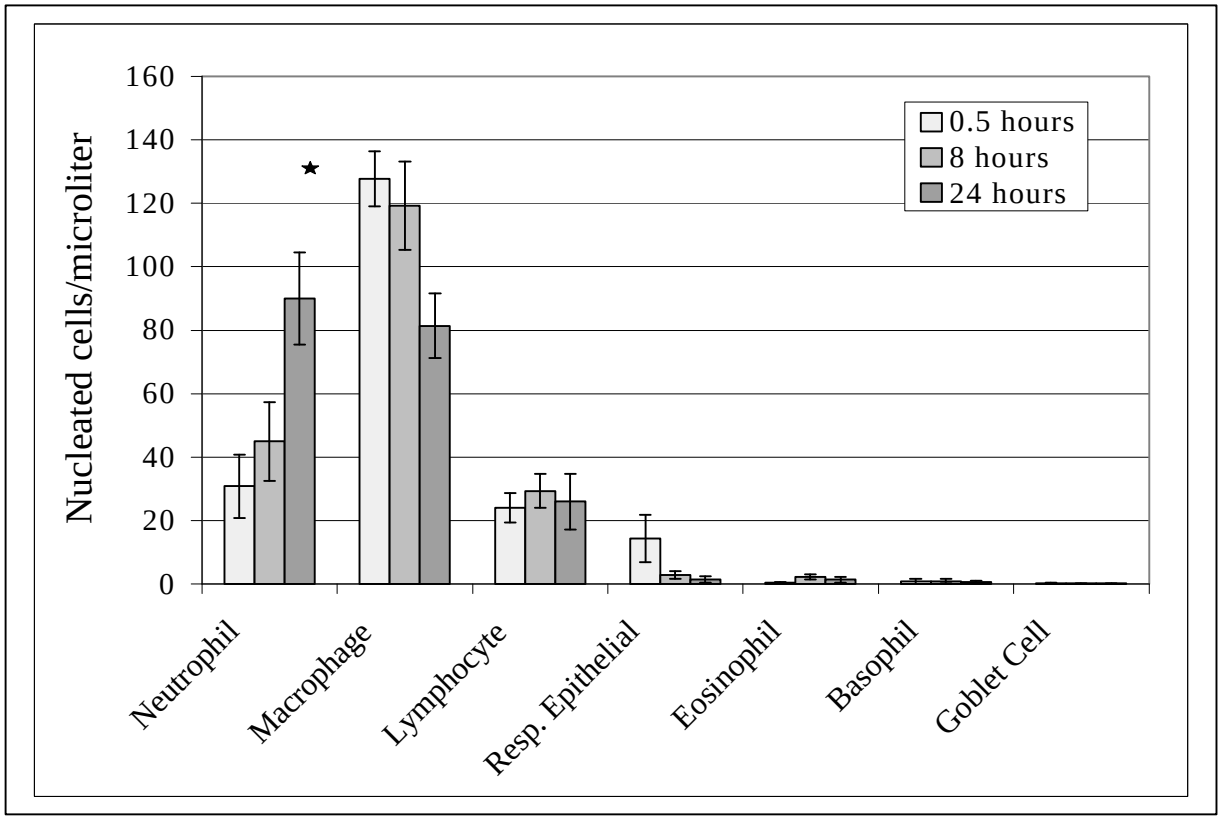

Figure 8. Bronchial lavage fluid cytology after aerosol administration of gentamicin to horses (n = 9). ★ indicates significant difference ($p \leq 0.05$) between times following administration.

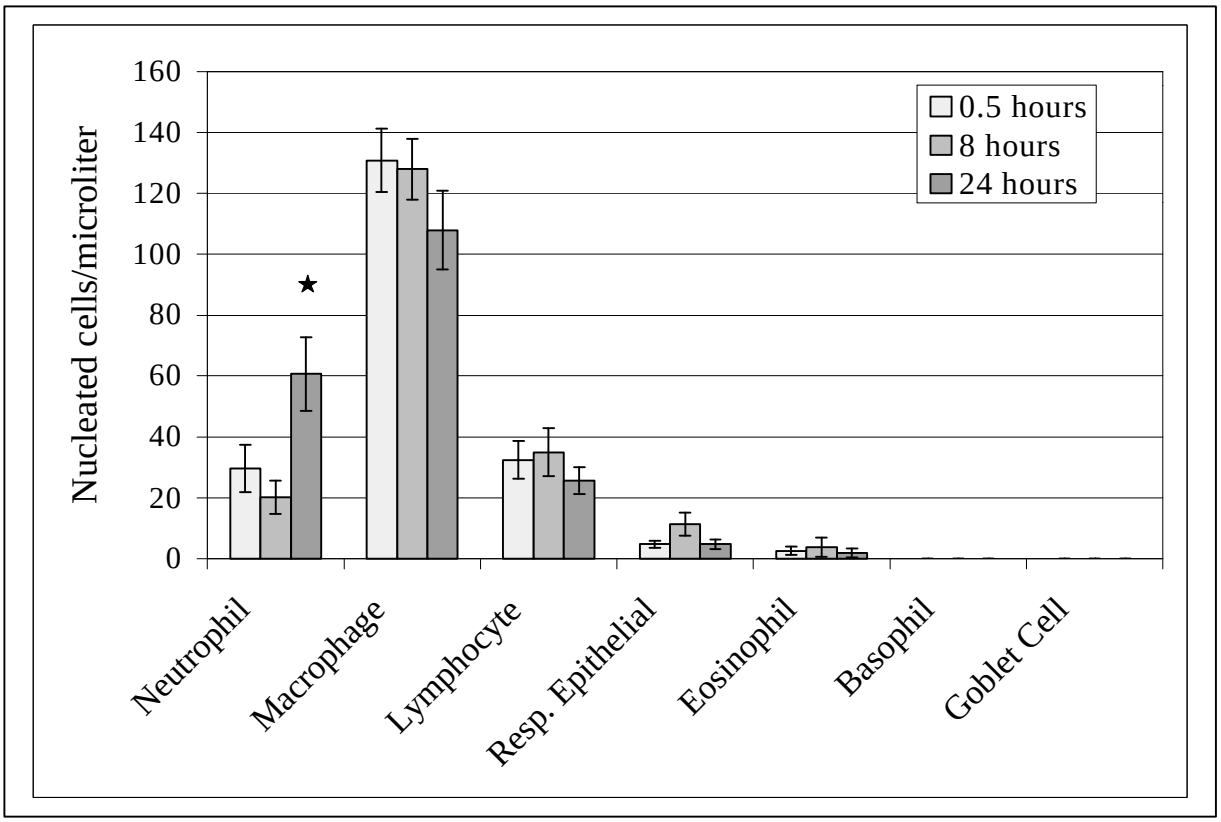

Figure 9. Bronchial lavage fluid cytology after intravenous administration of gentamicin to horses (n = 9). See figure 8 for key.

Literature Cited

1. Wong GA, Pierce TH, Goldstein E, et al. Penetration of antimicrobial agents into bronchial secretions. *Am J Med* 1975;59:219-23.
2. Dixon PM, Thomson,J.R., Brown, R., Galbraith, A. and Bogan, J.A. Some observations on the penetration of antimicrobial drugs into the respiratory secretions of horses. Lung Function and Respiratory Diseases in the Horse. 1985;35-37.
3. Valcke Y, Pauwels R, Van der Straeten M. Pharmacokinetics of antibiotics in the lungs. *Eur Respir J* 1990;3:715-22.
4. Baldwin DR, Honeybourne D, Wise R. Pulmonary disposition of antimicrobial agents: methodological considerations. *Antimicrob Agents Chemother* 1992;36:1171-5.
5. Thys J-P, Aoun M, Klastersky J. Local antibiotic therapy for bronchopulmonary infections In: J. E. Pennington, ed. *Respiratory Infections: Diagnosis and Management*. 3rd ed. New York: Raven Press, Ltd., 1994;741- 766.
6. Godber LM, Walker RD, Stein GE, et al. Pharmacokinetics, nephrotoxicosis, and in vitro antibacterial activity associated with single versus multiple (three times) daily gentamicin treatments in horses. *Am J Vet Res* 1995;56:613 - 618.
7. Bryson V, Sansome E, Laskin S. Aerosolization of penicillin solutions. *Science* 1944;100:33-35.

8. Hiller FC. Therapeutic aerosols: An overview from a clinical perspective. In: A. J. Hickey, ed. *Pharmaceutical inhalation aerosol technology.* New York: Marcel Dekker, Inc., 1992;289 - 306.

9. Le Conte P, G. P, Peltier P, et al. Lung distribution and pharmacokinetics of aerosolized tobramycin. *Am Rev Respir Dis* 1993;147:1279 - 1282.

10. Chaleva EI, Vasileva IV, Savova MD. Absorption of lincomycin through the respiratory pathways and its influence on alveolar macrophages after aerosol administration to chickens. *Res Vet Sci* 1994;57:245-7.

11. Baran D, de Vuyst P, Ooms HA. Concentration of tobramycin given by aerosol in the fluid obtained by bronchoalveolar lavage. *Respir Med* 1990;84:203-4.

12. Montgomery AB, Debs RJ, Luce JM, et al. Selective delivery of pentamidine to the lung by aerosol. *Am Rev Respir Dis* 1988;137:477-8.

13. Vermeersch H, Vandenbossche G, Remon JP, et al. Pharmacokinetics of nebulized sodium ceftiofur in calves. *J Vet Pharmacol Therap* 1996;19:152- 154.

14. Palmer LB, Smaldone GC, Simon SR, et al. Aerosolized antibiotics in mechanically ventilated patients: Delivery and response. *Crit Care Med* 1998;26:31 - 39.

15. Whelan AM, Hahn NW. Optimizing drug delivery from metered-dose inhalers. *Dicp* 1991;25:638-45.

16. Lipworth BJ. Pharmacokinetics of inhaled drugs. *Br J Clin Pharmacol* 1996;42:697-705.

17. Duvivier DH, Votion D, Vandenput S, et al. Aerosol therapy in the equine species. *Vet Journal* 1997;154:189- 202.

18. Smith AL, Ramsey B. Aerosol administration of antibiotics. *Pediatr Pulmonol Suppl* 1995;11:68-9.

19. Votion D, Ghafir Y, Munsters K, et al. Aerosol deposition in equine lungs following ultrasonic nebulisation versus jet aerosol delivery system. *Eq Vet J* 1997;29:388 - 393.

20. Gough PA, Jordan NS. A review of the therapeutic efficacy of aerosolized and endotracheally instilled antibiotics. *Pharmacotherapy* 1982;2:367-77.

21. Hall CB, McBride JT. Pulmonary infections. In: F. Moren, M. B. Dolovich, M. T. Newhouse and S. P. Newman, eds. *Aerosols in medicine. Principles, diagnosis and therapy.*: Elsevier Science Publishers B. V., 1993;291 - 301.

22. Moren F. Aerosol dosage forms and formulations. In: F. Moren, M. B. Dolovich, M. T. Newhouse and S. P. Newman, eds. *Aerosols in medicine: Principles, diagnosis and therapy.*: Elsevier Science Publishers B. V., 1993;321 - 349.

23. Phipps PR, Gonda I. Droplets produced by medical nebulizers. Some factors affecting their size and solute concentration. *Chest* 1990;97:1327-32.

24. Martonen T, Yang Y. Deposition mechanics of pharmaceutical particles in human airways. In: A. J. Hickey, ed. *Inhalation aerosols: physical and biological basis for therapy.* New York, NY: Marcel Dekker, Inc., 1996.

25. Huchon G. Aerosol deposition in the alveolar space. *Lung* 1990;168 Suppl:672-6.

26. Brain JD, Valberg PA. Deposition of aerosol in the respiratory tract. *Am Rev Respir Dis* 1979;120:1325 - 1373.

27. Gupta P, Hickey A. Contemporary approaches in aerosolized drug delivery to the lung. *J Controlled Release* 1991;17:129 - 148.

28. Hardy JG, Newman SP, Knoch M. Lung deposition from four nebulizers. *Respir Med* 1993;87:461-5.

29. Dolovich M. Lung dose, distribution, and clinical response to therapeutic aerosols. *Aerosol Sci Tech* 1993;18:230 - 240.

30. Brain JD, Blanchard JD. Mechanisms of particle deposition and clearance. In: F. Moren, M. B. Dolovich, M. T. Newhouse and S. P. Newman, eds. *Aerosols in Medicine. Principles, diagnosis and therapy.*: Elsevier Science Publishers B. V., 1993;117-156.

31. Clarke AF. A review of environmental and host factors in relation to equine respiratory disease. *Equine Vet J* 1987;19:435-41.

32. Skerrett SJ. Host defenses against respiratory infection. *Medical Clinics of North America* 1994;78:941-966.

33. Nelson S, Mason CM, Kolls J, et al. Pathophysiology of pneumonia. *Clin Chest Med* 1995;16:1-12.

34. Newman SP. Aerosol deposition considerations in inhalation therapy. *Chest* 1985;88:152S-160S.

35. Adjei AL, Qiu Y, Gupta PK. Bioavailability and pharmacokinetics of inhaled drugs. In: A. J. Hickey, ed. *Inhalation aerosols: physical and biological basis for therapy.* New York: Marcel Dekker, Inc., 1996;197 - 231.

36. Weber A, Morlin G, Cohen M, et al. Effect of nebulizer type and antibiotic concentration on device performance. *Ped Pulm* 1997;23:249 - 260.

37. Ferron GA, Busch B. Deposition of hygroscopic aerosol particles in the lungs. In: J. C. M. Marijnissen and L. Gradon, eds. *Aerosol inhalation: Recent research frontiers.* Netherlands: Kluwer Academic Publishers, 1996;143 - 152.

38. Gonda I. The use of aerosol medication in respiratory disease. *Aust Fam Physician* 1992;21:575-6.

39. Dolovich M, Ryan G, Newhouse MT. Aerosol penetration into the lung; influence on airway responses. *Chest* 1981;80:834-6.

40. Derksen FJ, Robinson NE. Effective use of bronchodilators in the horse. *Proc* World equine airway symposium. 1998;18.

41. Thompson PJ. Drug delivery to the small airways. *Am J Respir Crit Care Med* 1998;157:S199 - S202.

42. Chang HK, Menon AS. Airflow dynamics in the human airways. In: F. Moren, M. B. Dolovich, M. T. Newhouse and S. P. Newman, eds. *Aerosols in Medicine. Principles, diagnosis and therapy.*: Elsevier Science Publishers B. V., 1993;85 116.

43. Rush BR. Immune therapy in respiratory disease. *Vet Clin North Amer: Equine Pract* 1997;13:531-548.

44. Derksen F. Applied respiratory physiology In: J. Beech, ed. *Equine respiratory disorders*. 1st ed. Philadelphia: Lea and Febiger, 1991;1 - 25.

45. Morrow PE, Yu CP. Models of aerosol behavior in airways and alveoli. In: F. Moren, M. B. Dolovich, M. T. Newhouse and S. P. Newman, eds. *Aerosols in Medicine. Principles, diagnosis and therapy.*: Elsevier Science Publishers B. V., 1993;157-193.

46. Yates WDG. Respiratory System In: R. G. Thomson, ed. *Special Veterinary Pathology*. Toronto: B. C. Decker, 1988.

47. Thompson DC. Pharmacology of therapeutic aerosols. In: A. J. Hickey, ed. *Pharmaceutical inhalation aerosol technology.* New York: Marcel Dekker, Inc., 1992;29 - 59.

48. Niven RW. Modulated drug therapy with inhalation aerosols. In: A. J. Hickey, ed. *Pharmaceutical inhalation aerosol technology.* New York: Marcel Dekker, Inc., 1992;321 - 359.

49. Lippmann M, Yeates DB, Albert RE. Deposition, retention, and clearance of inhaled particles. *Br J Industr Med* 1980;37:337 - 362.

50. Wolff RK. Experimental investigation of deposition and fate of particles: animal models and interspecies differences. In: J. C. M. Marijnissen and L. Gradon, eds. *Aerosol inhalation: Recent research frontiers.* Netherlands: Kluwer Academic Publishers, 1996;247 - 263.

51. Levy J. Antibiotic activity in sputum. *J Pediatr* 1986;108:841 - 846.

52. Crooks PA. Drug targeting to the lung: chemical and biochemical considerations. In: A. J. Hickey, ed. *Pharmaceutical inhalation aerosol technology.* New York: Marcel Dekker, Inc., 1992;83 - 127.

53. Effros RM. Solute transport following aerosol deposition in the lungs. In: A. J. Hickey, ed. *Inhalation aerosols: physical and biological basis for therapy.* New York: Marcel Dekker, Inc., 1996;139 - 155.

54. Dixon PM. Respiratory mucociliary clearance in the horse in health and disease, and its pharmaceutical modification. *Vet Rec* 1992;131:229-35.

55. Forrest JB. Lower airway: structure and function. In: F. Moren, M. B. Dolovich, M. T. Newhouse and S. P. Newman, eds. *Aerosols in Medicine: Principles, diagnosis and therapy.*: Elsevier Science Publishers B. V., 1993;27-59.

56. Dalby RN, Tiano SL, Hickey AJ. Medical devices for the delivery of therapeutic aerosols to the lungs. In: A. J. Hickey, ed. *Inhalation aerosols: physical and biological basis for therapy.* New York: Marcel Dekker, Inc., 1996;441 - 473.

57. Newman SP, Clarke SW. Therapeutic aerosols 1--physical and practical considerations. *Thorax* 1983;38:881-6.

58. Smith SJ, Bernstein JA. Therapeutic uses of lung aerosols. In: A. J. Hickey, ed. *Inhalation aerosols: physical and biological basis for therapy.* New York: Marcel Dekker, Inc., 1996;233 - 269.

59. Atkins PJ, Barker NP, Mathisen D. The design and development of inhalation drug delivery systems. In: A. J. Hickey, ed. *Pharmaceutical inhalation aerosol technology.* New York: Marcel Dekker, Inc., 1992;155 - 185.

60. Newman SP. Scintigraphic assessment of therapeutic aerosols. *Crit Rev Ther Drug Carrier Syst* 1993;10:65-109.

61. Hickey AJ. Summary of common approaches to pharmaceutical aerosol administration. In: A. J. Hickey, ed. *Pharmaceutical inhalation aerosol technology.* New York: Marcel Dekker, Inc., 1992;255 - 288.

62. Viel L, Tesarowski D. Radioaerosol deposition in equids. *Proc* 40th Convention Am Assoc Eq Pract 1994.

63. Tesarowski DB, Viel L, McDonell WN, et al. The rapid and effective administration of a beta 2-agonist to horses with heaves using a compact inhalation device and metered-dose inhalers. *Can Vet J.* 1994;35:170-173.

64. Derksen FJ, Olszewski M, Robinson NE, et al. Use of a hand-held, metered-dose aerosol delivery device to administer pirbuterol acetate to horses with 'heaves'. *Equine Vet J* 1996;28:306-10.

65. Duvivier D, Votion D, Vandenput S, et al. Airway response of horses with COPD to dry powder inhalationo of ipratroprium bromide. *Vet Jour* 1997;154:149-153.

66. Goldman JM, Bayston SM, O'Connor S, et al. Inhaled micronised gentamicin powder: a new delivery system. *Thorax* 1990;45:939-40.

67. Greenspan BJ. Ultrasonic and electrohydrodynamic methods for aerosol generation. In: A. J. Hickey, ed. *Inhalation aerosols: physical and biological basis for therapy.* New York: Marcel Dekker, Inc., 1996;313 - 335.

68. Niven RW. Delivery of biotherapeutics by inhalation aerosol. *Crit Rev Ther Drug Carr Syst* 1995;12:151 - 231.

69. Faurisson F, Dessanges JF, Grimfeld A, et al. Nebulizer performance: AFLM study. *Respiration* 1995;62 Suppl 1:13-8.

70. Chua HL, Collis GG, Le Souef PN. Bronchial response to nebulized antibiotics in children with cystic fibrosis. *Eur Respir J* 1990;3:1114- 1116.

71. Dally MB, Kurrle S, Breslin ABX. Ventilatory effects of aerosol gentamicin. *Thorax* 1978;33:54 - 56.

72. Dickie KJ, de Groot WJ. Ventilatory effects of aerosolized kanamycin and polymyxin. *Chest* 1973;63:694 - 697.

73. O'Callaghan C, Barry PW. The science of nebulized drug delivery. *Thorax* 1997;52:S31 - S44.

74. MacLusky I, Levison H, Gold R, et al. Inhaled antibiotics in cystic fibrosis: is there a therapeutic effect? *J Pediatr* 1986;108:861-5.

75. Pennington JE. Penetration of antibiotics into respiratory secretions. *Rev Infect Dis* 1981;3:67-73.

76. Pennington JE, Reynolds HY. Concentrations of gentamicin and carbenicillin in bronchial secretions. *J Infect Dis* 1973;128:63 - 68.

77. Smith BR, LeFrock JL. Bronchial tree penetration of antibiotics. *Chest* 1983;83:904-8.

78. Valcke Y, Pauwels R, Van der Straeten M. The penetration of aminoglycosides into the alveolar lining fluid of rats. The effect of airway inflammation. *Am Rev Respir Dis* 1990;142:1099-103.

79. Baldwin DR, Honeybourne D, Wise R. Pulmonary disposition of antimicrobial agents: In vivo observations and clinical relevance. *Antimicrob Agents Chemother* 1992;36:1176-1180.

80. Berendt RF, Long GG, Walker JS. Treatment of respiratory *Klebsiella pneumoniae* infection in mice with aerosols of kanamycin. *Antimicrob Agents Chemother* 1975;8:585 - 590.

81. Jensen T, Pedersen SS, Garne S, et al. Colistin inhalation therapy in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection. *J Antimicrob Chemother* 1987;19:831 - 838.

82. Orcutt TA, Godwin CR, Pizzo PA, et al. Aerosolized pentamidine: a well-tolerated mode of prophylaxis against *Pneumocystis carinii* pneumonia in older children with human immunodeficiency virus infection. *Pediatr Infect Dis J* 1992;11:290 - 294.

83. Ramsey BW, Dorkin HL, Eisenberg JD, et al. Efficacy of aerosolized tobramycin in patients with cystic fibrosis. *N Engl J Med* 1993;328:1740-6.

84. Sustronck B, Deprez P, Muylle E, et al. Evaluation of the nebulisation of sodium ceftiofur in the treatment of experimental *Pasteurella haemolytica* bronchopneumonia in calves. *Res Vet Sci* 1995;59:267-71.

85. Chartrand SA, Marks MI. Pulmonary infections in cystic fibrosis: pathogenesis and therapy. In: J. E. Pennington, ed. *Respiratory infections: diagnosis and management.* 3rd ed. New York: Raven Press, 1994;323 - 347.

86. Hoff GE, Schiotz PO, Paulsen J. Tobramycin treatment of *Pseudomonas aeruginosa* infections in cystic fibrosis. *Scand J Infect Dis* 1974;6:333 - 337.

87. Hodson ME, Penketh AR, Batten JC. Aerosol carbenicillin and gentamicin treatment of *Pseudomonas aeruginosa* infection in patients with cystic fibrosis. *Lancet* 1981;2:1137-9.

88. MacLusky IB, Gold R, Corey M, et al. Long-term effects of inhaled tobramycin in patients with cystic fibrosis colonized with *Pseudomonas aeruginosa*. *Pediatr Pulmonol* 1989;7:42-8.

89. Wiesemann HG, Steinkamp G, Ratjen F, et al. Placebo-controlled, double-blind, randomized study of aerosolized tobramycin for early treatment of *Pseudomonas aeruginosa* colonization in cystic fibrosis. *Pediatr Pulmonol* 1998;25:88 - 92.

90. Stephens D, Garey N, Isles A, et al. Efficacy of inhaled tobramycin in the treatment of pulmonary exacerbations in children with cystic fibrosis. *Pediatr Infect Dis* 1983;2:209-11.

91. Schaad UB, Wedgwood-Krucko J, Suter S, et al. Efficacy of inhaled amikacin as adjunct to intravenous combination therapy (ceftazidime and amikacin) in cystic fibrosis. *J Pediatr* 1987;111:599-605.

92. Debs RJ, Straubinger RM, Brunette EN, et al. Selective enhancement of pentamidine uptake in the lung by aerosolization and delivery in liposomes. *Am Rev Respir Dis* 1987;135:731 - 737.

93. Leoung GS, Feigal DW, Montgomery AB, et al. Aerosolized pentamidine for prophylaxis against *Pneumocystis carinii* pneumonia. *N Engl J Med* 1990;323:767 - 775.

94. Hardy WD, Feinberg J, Finkelstein CM, et al. A controlled trial of trimethoprim-sulfamethoxazole or aerosolized pentamidine for secondary prophylaxis of *Pneumocystis carinii* pneumonia in patients with the acquired immunodeficiency syndrome. AIDS Clinical Trials Group Protocol 021. *N Engl J Med* 1992;327:1842 - 1848.

95. Rouby JJ, Poete P, Martin de Lassale E, et al. Prevention of gram negative nosocomial bronchopneumonia by intratracheal colistin in critically ill patients. Histologic and bacteriologic study. *Intensive Care Med* 1994;20:187-92.

96. Beech J. Therapeutic strategies involving antimicrobial treatment of the lower respiratory tract in large animals. *J Am Vet Med Assoc* 1984;185:1199 - 1202.

97. Berendt RF, Magruder RD, Frola FR. Treatment of *Klebsiella pneumoniae* respiratory tract infection of squirrel monkeys with aerosol administration of kanamycin. *Am J Vet Res* 1980;41:1492-4.

98. Wilson WD. Bacterial lung diseases in foals. *Proc* World equine airway symposium. 1998;24.

99. Wilson WD. Foal pneumonia. In: N. E. Robinson, ed. *Current therapy in equine medicine.* 3rd ed. Philadelphia: W. B. Saunders Company, 1992;466 - 473.

100. Rhodes CH, Genetzky RM. Nebulization therapy in the foal. *Iowa State Univ Vet* 1982;44:104-108.

101. McKenzie HC, Murray MJ. Time/concentration relationships of gentamicin in serum and bronchial lavage fluid of horses administered gentamicin intravenously and by aerosol. *Proc* World Equine Airway Symposium. 1998;29.

102. Murray MJ. New diagnostic techniques and treatment for lower respiratory disease. *Proc* 17th Amer Coll Vet Intern Med Forum 1997.

103. Gilbert B, Wyde P, Ambrose M, et al. Further studies wth short duration ribavirin aerosol for the treatment of influenza virus infection in mice and respiratory syncytial virus infection in cotton rats. *Antiviral Res* 1992;17:33-42.

104. Saiman L. Selected topics in novel methods of antibiotic delivery: aerosolized antibiotics and lipid preparations. *Adv Pediatr Infect Dis* 1998;13:349-76.

105. Ruoff WW. Fungal pneumonia in horses. *Proc* 34th Annual Convention Am Assoc Eq Pract 1988;423 - 426.

106. Koizumi T, Kubo K, Kaneki T, et al. Pharmacokinetic evaluation of amphotericin B in lung tissue: lung lymph distribution after intravenous injection and airspace distribution after aerosolization and inhalation of amphotericin B. *Antimicrob Agents Chemother* 1998;42:1597-600.

107. Cicogna CE, White MH, Bernard EM, et al. Efficacy of prophylactic aerosol amphotericin B lipid complex in a rat model of pulmonary aspergillosis. *Antimicrob Agents Chemother* 1997;41:259-61.

108. Allen SD, Sorensen KN, Nejdl MJ, et al. Prophylactic efficacy of aerosolized liposomal (AmBisome) and non- liposomal (Fungizone) amphotericin B in murine pulmonary aspergillosis. *J Antimicrob Chemother* 1994;34:1001-13.

109. Harrison KS, Laube BL. Bronchodilator pretreatment improves aerosol deposition uniformity in HIV-positive patients who cough while inhaling aerosolized pentamidine. *Chest* 1994;106:421-6.

110. Rush BR, Hoskinson JJ, Matson CJ, et al. Pulmonary distribution of aerosolized 99m Technetium-DTPA in horses with 'heaves' after single-dose aerosolized albuterol sulfate. *Proc* 15th Veterinary Respiratory Symposium 1997.

111. Smith AL, Ramsey BW, Hedges DL, et al. Safety of aerosol tobramycin administration for 3 months to patients with cystic fibrosis. *Pediatr Pulmonol* 1989;7:265-71.

112. Steinkamp G, Tummler B, Gappa M, et al. Long-term tobramycin aerosol therapy in cystic fibrosis. *Pediatr Pulmonol* 1989;6:91-8.

113. Hoffman A. Inhaled medication for small airway disease in horses. *Proc* 13th Amer Coll Vet Intern Med Forum 1995;758-760.

114. Geor RJ. Performance-limiting conditions of the lungs. In: C. N. Kobluk, T. R. Ames and R. J. Geor, eds. *The horse: Diseases and clinical management.* 1st ed. Philadelphia: W. B. Saunders Company, 1995;263-273.

115. Burrell MH, Wood JL, Whitwell KE, et al. Respiratory disease in thoroughbred horses in training: the relationships between disease and viruses, bacteria and environment. *Vet Rec* 1996;139:308-13.

116. Reed SR. Pneumonia in horses. *Proc* Dubai International Equine Symposium 1997;265 - 274.

117. Bergogne-Berezin E, Vallee E. Pharmacokinetics of antibiotics in respiratory tissues and fluids. In: J. E. Pennington, ed. *Respiratory Infections: Diagnosis and Management*. 3rd ed. New York: Raven Press, Ltd., 1994;715- 740.

118. Bayly W, Liggitt H, Huston L, et al. Stress and its effect on equine pulmonary mucosal defenses. *Proc* 32nd Convention Am As Eq Pract 1986;253-262.

119. Rose RJ, Hodgson DR. Lower respiratory tract diseases. In: R. J. Rose and D. R. Hodgson, eds. *Manual of Equine Practice*. Philadelphia: W. B. Saunders Company, 1993;160 - 169.

120. Folz SD, Hanson BJ, Griffin AK, et al. Treatment of respiratory infections in horses with ceftiofur sodium. *Equine Vet J* 1992;24:300-304.

121. Schmekel B. A critical view on lavage sampling techniques. *Agents Actions Suppl* 1990;30:133-43.

122. Chinard FP. Quantitative assessment of epithelial lining fluid in the lung. *Am J Physiol* 1992;263:L617-8.

123. Baldwin DR, Wise R, Andrews JM, et al. Microlavage: a technique for determining the volume of epithelial lining fluid. *Thorax* 1991;46:658-62.

124. McGorum BC, Dixon PM, Halliwell REW, et al. Evaluation of urea and albumen as endogenous markers of dilution of equine bronchoalveolar lavage fluid. *Res Vet Sci* 1993;55:52-56.

125. Valcke YJ, Vogelaers DP, Colardyn FA, et al. Penetration of netilmicin in the lower respiratory tract after once-daily dosing. *Chest* 1992;101:1028-32.

126. Von Wichert P, Joseph K, Muller B, et al. Bronchoalveolar lavage. Quantitation of intraalveolar fluid? *Am Rev Respir Dis* 1993;147:148-52.

127. Marcy TW, Merrill WW, Rankin JA, et al. Limitations of using urea to quantify epithelial lining fluid recovered by bronchoalveolar lavage. *Am Rev Respir Dis* 1987;135:1276-80.

128. Craig WA. Once-daily versus multiple-daily dosing of aminoglycosides. *J Chemother* 1995;7 Suppl 2:47-52.

129. Ilowite JS, Gorvoy JD, Smaldone GC. Quantitative deposition of aerosolized gentamicin in cystic fibrosis. *Am Rev Respir Dis* 1987;136:1445-9.

130. Sweeney CR, Rossier Y, Ziemer EL, et al. Effect of prior lavage on bronchoalveolar lavage fluid cell population of lavaged and unlavaged lung segments in horses. *Am J Vet Res* 1994;55:1501-4.

131. Sweeney C, Rossier Y, Ziemer EL, et al. Effects of lung site and fluid volume on results of bronchoalveolar lavage fluid analysis in horses. *Am J Vet Res* 1992;53:1376-1379.

132. Braude AC, Hornstein A, Klein M, et al. Pulmonary disposition of tobramycin. *Am Rev Respir Dis* 1983;127:563-5.

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[a] UltraNeb 99, DeVilbiss, Sunrise Medical, Somerset, PA, USA
[b] Malvern Mastersizer, Malvern Instruments Ltd., Malvern, UK c/o DeVilbiss
[c] Spartan, Japan
[d] Aerosol Hose, Professional Medical Co., Greenwood, SC, USA
[e] Minitab for Windows, Release 10.1, Minitab Inc., State College, PA
[f] Equine Aeromask, Canadian Monaghan Ltd., London, Ontario, Canada
[g] 7/8" i.d. x 48" length Stackhouse® tubing, Stackhouse Inc., Riverside, CA, USA
[h] Welch-Allyn Corp., Skaneateles, NY, USA

[i] Becton Dickinson and Co., Franklin Lakes, NJ, USA
[j] Gelman Supor 200, Gelman Sciences Inc., Ann Arbor, MI, USA
[k] Abbot TdX, Abbott Laboratories, Abbott Park, IL, USA
[l] Abbot TdX Manual, Abbot Laboratories, Abbot Park, IL, USA
[m] Gentocin, Schering-Plough Animal Health Corp., Kenilworth, NJ, USA
[n] Sigma, St. Louis, MO, USA
[o] Abbocath-T, Abbot Ireland, Sligo, IRE
[p] Vacutainer, Becton Dickinson and Co.
[q] Unopette 5855, Becton Dickinson and Co.
[r] Cytospin, Shandon Lipshaw USA., Pittsburgh, PA, USA
[s] Hemacolor 65044/93, EM Diagnostic Systems, EM Industries, Inc., Gibbstown, NJ, USA
[t] Personal communication: Frank Clementi, DeVilbiss Corp.