Inhalation Therapy in Horses

Mandy L. Cha, BS, BVSc[a], Lais R.R. Costa, MV, MS, PhD[b,*]

KEYWORDS

• Inhalation • Pharmacology • Respiratory • Equine • Nebulizer

KEY POINTS

- Inhalation therapy in horses can be accomplished with nebulizers or pressured metered dose inhalers.
- Nasal or muzzle masks can be used for delivery of inhalation therapy in horses.
- Inhalation therapy is important in the treatment of inflammatory airway disease in horses.
- Inhaled antimicrobial therapy may be adjunctive to systemic therapy for treatment of pneumonia.

INTRODUCTION

Inhalation therapy has been practiced in humans since ancient times in many cultures, including Chinese, Indian, Greek, Egyptian, Roman, and Hebrew.[1] In all of these cultures, inhalational therapy was performed using condensation of vapor by steaming liquids and combustion of plants or their derivatives, creating a smoke containing aerosolized droplets and solid particles.[1]

Inhalation therapy is the administration of aerosols into the airway and is a means of delivering topical pulmonary therapy. An aerosol is a suspension of liquid or solid particles dispersed in gas. The physical characteristics of the particles, including size, hydrophobicity, and shape, affect their ability to travel within the airways.[1,2] The size of the particles is regarded as one of the most critical physical characteristics of aerosol therapy. **Fig. 1** depicts a schematic representation of the aerosol deposition throughout the airways of the horse. Large aerosols (>10 μm) are filtered out in the upper respiratory tract (URT) or deposited in larger airways in association with turbulent airflow and do not effectively reach the lower airways. Midsized particles (10 to 6 μm) deposit in the larynx, trachea, bronchi, and large-caliber bronchioles. Particles 5 μm or

[a] Kulshan Veterinary Hospital, 8880 Benson Rd, Lynden, WA 98264, USA; [b] William R. Pritchard Veterinary Medical Teaching Hospital, University of California-Davis, One Shields Avenue, Davis, CA 95616, USA
* Corresponding author.
E-mail address: lais.costa65@gmail.com

Vet Clin Equine 33 (2017) 29–46
http://dx.doi.org/10.1016/j.cveq.2016.11.007
vetequine.theclinics.com

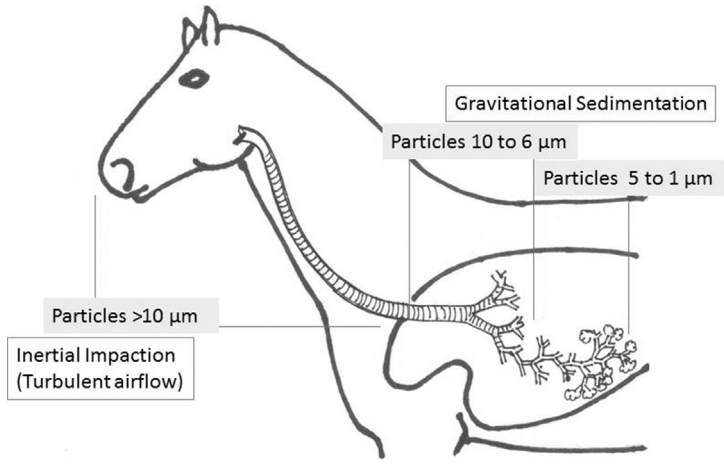

Fig. 1. Particle deposition throughout the airways of the horse. Large aerosols (>10 μm) deposit in the upper respiratory tract and larger airways; midsized aerosols (10 to 6 μm) deposit in the larynx, trachea, bronchi, and large-caliber bronchioles; aerosols less than 5 μm are deposited in smaller diameter bronchioles and in alveolar acini by gravitational sedimentation; and very small aerosols (<1 μm) remain in suspension, with part being deposited in alveoli and part exhaled.

less are deposited in smaller diameter bronchioles and in alveolar acini by gravitational sedimentation. Very small particles (<1 μm) tend to remain in suspension, and approximately 50% are deposited in alveoli and 50% are exhaled. Small particles may coalesce to make larger particles, which affects deposition. Hydrophilic particles attract water, promoting their deposition deeper in the tracheobronchial tree. More aerodynamically shaped particles are also deposited deeper within the respiratory tract. Patient factors that influence aerosol particle deposition include the depth of breathing, airway patency and reactivity, bronchospasm, and coughing.

Inhalation medications may be in the form of solutions, powders, vapors, or pressurized cartridges, when solutions or powders are administered with a propellant. The onset of action of aerosolized drugs is relatively rapid; however, the effects are usually short-lived; this is because the aerosolized drugs are partially degraded in the lung, cleared from the respiratory tract by the mucociliary escalator, and absorbed into the blood stream where they are disposed of by breakdown and excretion as are systemic drugs.

ADVANTAGES AND DISADVANTAGES OF INHALATION VERSUS SYSTEMIC ROUTE OF DRUG ADMINISTRATION

The inhalation route has long been perceived as the best route to affect the components of the airways and alveoli. The blood-bronchial barrier limits the access of systemically administered drugs to the airway lumen and to the cells lining the lower respiratory tract (LRT). In order to achieve drug penetration, high systemic doses are often required.

Some drugs, such as sympathomimetic and parasympatholytic agents used in the management of equine asthma, including recurrent airway obstruction (RAO) and inflammatory airway disease (IAD), may result in undesirable and sometimes life-threatening

side effects, such as tachycardia, tremors, sweating, anxiety, decreased gastrointestinal motility, and cardiac arrhythmias, when used in large systemic doses. Administration of these drugs through the inhalational route minimizes the risks of these side effects.

In equine asthma, the potent inflammatory responses in the small airways lead to a cascade of pathophysiologic events, including the accumulation of inflammatory cells, primarily neutrophils and mucus, as well as a decrease in mucociliary clearance. Bronchoconstriction and coughing are common features of airway disease. Many of the drugs recommended for treating equine asthma are available in inhaled formulations, and current consensus indicates that inhalation is the route of choice to manage these conditions.[3,4] Directly delivering potent antiinflammatory therapy in the form of inhaled corticosteroids provides pharmacotherapy and also reduces the likelihood of adverse systemic effects. The inflammatory cascade can be modulated by the preventative use of cromones, such as cromoglycate and nedocromil sodium, medications which are only available for inhalational administration.

Drugs administered by aerosol obviate absorption, bypass degradation in the gastrointestinal tract and liver, avoid the detrimental effects on the gut flora, and allow the use of drugs that are not bioavailable when administered orally. One example is the aminoglycoside class of antimicrobials, which can be administered through the inhalational route.

Although drugs administered through the inhalational route can still cause adverse reactions, such as anaphylaxis, hypersensitivity, idiosyncratic reactions, overdose, cumulative effects, and toxicity, they are less likely to do so than the same drugs administered systemically. Tolerance, defined as resistance to standard dosages of drugs, can also occur with inhalational therapy. Tolerance of inhaled drugs may be manifested as tachyphylaxis, resistance, paradoxic effect, and rebound phenomenon.[1]

Horses with chronic pulmonary diseases, particularly severe asthma (RAO), require long-term management. Aerosol treatment is often a more acceptable means by which owners can manage their horses at home with bronchodilators and corticosteroids as opposed to injections or even oral medications.

Treatment of infections of the LRT with aerosolized antimicrobial drugs may avoid antibiotic-induced colitis associated with systemic administration. Infectious agents of bacterial pneumonia, even when secondary, are often inhaled or aspirated rather than established by a hematogenous route in adult horses. Administration of antimicrobials directly to the site where the pathogen resides potentially enhances efficacy and decreases undesirable effects.

Delivering a precise dose of a medication by inhalation therapy is challenging. Because of variable drug deposition, the determination of half-life of inhaled drugs is difficult. The efficacy of drugs administered through inhalation is best gauged by the observation of a desirable drug effect. Improvement of airflow in the case of bronchodilators is a better indicator than, for example, the measured decline in activity of the drug in bronchoalveolar lavage fluid (BALF). Knowledge of the half-life is helpful, because for an adequate response, many drugs are administered at intervals that are approximately twice that of the half-life. Drugs administered through inhalation tend to have a shorter duration of effect than when administered through the systemic route. Thus, more frequent administration may be necessary to attain a similar desired effect. Drug deposition of the inhaled drug may be even more unpredictable in diseased lung. Proper drug distribution is hampered by abnormal breathing patterns, bronchoconstriction, airway secretions, and coughing. Thus, control of bronchospasm and coughing is important to obtain an effective distribution of any drug through the inhalation route.

DELIVERY OF AEROSOLS IN INHALATION THERAPY

Particles as large as 50 μm can be suspended in gas and administered as aerosol; however, only small particles effectively reach more distal airways. Particles ranging from 1 to 5 μm suspended in gas, referred to as therapeutic aerosol, maximize deposition in lower airways deeper in the lung.[1,2,5] The relationship between particle size and deposition pattern holds true across species.

The patients' pattern of breathing impacts drug distribution with the inhalation route. Maximal distal deposition occurs when patients take slow, deep breaths, with large tidal volumes. Unlike humans, it is difficult to control the breathing pattern of animals because it is impossible to request a voluntary deep breath from equine patients.

Forms of Administration

The available forms of drug delivery by the inhalation route include nebulizers and pressurized cartridge dispensers for administration of aqueous or alcoholic solutions as well as powders in the form of aerosol. Nebulization is used for the delivery of medications formulated as a liquid, either as a solution or suspension. There are different kinds of nebulizers, including vaporizers, jet nebulizers, and ultrasonic nebulizers.

Vaporizers deliver the drug vaporized in steam and are the oldest form of inhalation therapy. With vaporizers, the particle size and drug deposition are highly variable. Vaporizers are currently used primarily for the purpose of humidification of airway secretions because they have been replaced by other devices for medication delivery. Steam vaporizers are thought to increase the fluidity of airway secretions, thereby aiding with tenacious mucus and favoring their elimination by the ciliary escalator and coughing.

Jet nebulizers draw up liquid and produce a spray, breaking up the liquid into small particles in the process. Larger droplets are returned to the reservoir and enter the next cycle, whereas smaller particles are carried in inhaled air. The primary advantage of jet nebulizers is that they are economical. The disadvantages are that they are loud, provide a slow delivery rate, particle size is highly variable, and relatively large volumes of liquid are required for a small amount of actual delivery.[6] In humans, the reported deposition of drug by jet nebulizers is approximately 7.0% to 7.5% of nebulized volume.[7] The airflow rate should be set at 6 to 8 L/min to optimize output.[6,8] If the airflow rate is too slow, there is an increase in droplet size, whereas if the flow rate is too fast, turbulent flow favors pharyngeal deposition and the droplets do not reach the lower airways.

Ultrasonic nebulizers use piezoelectric crystal vibrations to nebulize a pool of liquid into a cloud of mist. The vibration frequency is the primary determinant of particle size. Higher frequencies create smaller droplets, but droplet size and deposition are determined by several factors, including individual drug characteristics, nebulizer specifications, and tubing length and diameter. The disadvantages of ultrasonic nebulizers are that they are more expensive than jet nebulizers and they generate heat, which may degrade the medication. The primary advantage is faster delivery and creation of more specific droplet size than jet nebulizers.[9] In humans, the reported deposition of drug is around 5% of nebulized volume.[7] Both jet and ultrasonic nebulizers require good sanitation, as unhygienic use can result in deposition of bacteria into lower airways.

Pressurized cartridge dispensers or pressurized metered dose inhalers (pMDIs) are the other primary means of providing inhalation therapy. They provide a method of ensuring administration of an accurate amount of medication by delivering a set amount of drug per actuation or puff. Although this method is thought to be precise,

some variability of delivery may still occur.[10] Failure to adequately shake the pMDI before use is a very common source of this variability.[10] The type of propellant and the form of delivery influence the relative deposition of the drug in the lung. Coordination of actuation of the pMDI with inhalation is important for successful delivery; failure to coordinate is the most common cause of therapeutic failure, making the use of a delivery device a requirement.[11] In humans, incorrect coordination results in oropharyngeal deposition of the drug rather than pulmonary deposition, but correct coordination results in 60% of the drug reaching the lungs.[12] Therefore, for adequate delivery by pMDI, a spacer is required in the delivery device for use in horses. Most pMDI drugs labeled for human use, including bronchodilators and corticosteroids for treatment of asthma, are designed to be inhaled through the mouth. Because horses are obligate nasal breathers, the delivery devices have been modified for nasal delivery. The type of propellant influences the relative deposition of drug in the lung. Currently, chlorofluorocarbon (CFC) propellant is no longer available, and only the hydrofluoroalkane (HFA) propellant is available in the United States.

Commercial Spacers for Use in Horses

The inability to coax a horse to breathe on command necessitates the use of spacers to deliver the pMDI-packaged therapies. These aerosol delivery devices create a reservoir of drug in a chamber that is released on subsequent inspirations. The use of a delivery device adapts the commercially available human formulations of pMDI-delivered drugs to horses. This adaptation eliminates the need to manually synchronize the actuation of the pMDI with the horse's inspirations by automatically releasing the drug from the device when there is airflow (ie, when the horse breathes in). The pMDI delivery with a spacer is thought to be a great advance as compared with the initial nebulizer systems, which deposited only small amounts into equine lungs.[13] These aerosol delivery devices are generally well tolerated and effective in horses.[14–18] If a particular horse reacts to the sound of the actuation of the pMDI, the drug may be puffed into the chamber away from horse's face. Then the spacer can be held up to the nostril until the next inhalation. Currently there are several products used as spacer devices in the horse. It is important to ensure the device is properly cleaned after each use. The devices should be cleaned with warm water and mild soap and rinsed with sterile or distilled water. The valves and crevices should be thoroughly cleaned.

There are both single nostril and entire muzzle masks available for use in horses, along with spacer devices. Single nostril masks for horses include the Equine Haler and the AeroHippus. The Equine Haler (Equine Health Care Aps Jorgensen Labs, Inc., Loveland, Colorado, USA) is a single-nostril mask connected to a handheld large ellipsoid chamber, the spacer, into which the pMDI is inserted (**Fig. 2**). On actuation of the pMDI, the aerosol particles are suspended in the chamber, which contains a one-way valve that allows flow of medication on inspiration. This device was shown to effectively provide deposition of radiolabeled fluticasone propionate with propellant CFC in lung tissue at a rate of 8.2% $\pm$ 5.2% of the administered dose (250 μg per actuation) indicating successful deposition of the drug in small airways and alveoli.[17]

The AeroHippus Equine Aerosol Chamber (Trudell Medical, London, Ontario, Canada) (https://www.trudellmed.com/animal-health/aerohippus) is also a single-nostril device (**Fig. 3**).[19] A proprietary visible valve called a Flow-Vu (Trudell Medical, London, Ontario, Canada) serves as a visual indicator of inspiration so that the operator knows when to puff the pMDI. The drug deposition of radiolabeled beclomethasone dipropionate with propellant HFA was reported to be 18.2% using 10 actuations at 80 μg per actuation with this device.[18]

Fig. 2. Equine Haler: The handheld, single-nostril mask is connected to a large ellipsoid chamber, and the pMDI is inserted into the opposite end. On actuation of the pMDI, the suspended aerosols remain in the chamber until the horse inspires. (Jorgensen Laboratories, Loveland, CO. *Courtesy of* Dr Laurent Couetil, West Lafayette, IN.)

The studies mentioned earlier were completed using different propellants, drugs, and amounts of drug per actuation; therefore, the 2 studies cannot be directly compared. In a direct comparison between the AeroHippus and the Equine Haler as devices for the delivery of albuterol with the same propellant, at the same dose, similar improvements in clinical scores and pulmonary function were reported.[15] However, this study did not evaluate drug deposition. Nonetheless, it was concluded that both devices effectively delivered the bronchodilator albuterol and reversed the abnormalities associated with airway obstruction.[15] With either of the single-nostril devices, it is recommended to occlude the contralateral nostril temporarily in order to optimize deposition.

The AeroMask by Trudell Medical has been discontinued, but it is still in use by many veterinarians in clinical and research settings. It consists of a fitted mask placed on the horse's muzzle, thus including both nostrils (**Fig. 4**).

The Flexineb Equine Nebulizer (Flexineb Inc, Union City, TN, USA) is a fitted mask placed on the horse's muzzle (http://flexineb.us/; **Fig. 5**). The mask should be snug over the horse's face, with both nostrils exposed to the system. The mask is strapped behind the horse's ears, to ensure a tight fit around the horse's nose and minimize drug leakage. The Flexineb mask is best suited for use with nebulizers. The mask is connected to a vertical cylindrical chamber, the spacer, to which a container is attached. The solution to be nebulized is added to the container and aerosolized; the aerosols remain in the spacer until the horse inspires. The control

Fig. 3. Aerohippus Equine Aerosol Chamber: The handheld, single-nostril mask is connected to a small cylindrical chamber, and the pMDI is inserted into the opposite end. On actuation of the pMDI, the suspended aerosols remain in the chamber until the horse inspires. (Trudell Medical, Inc, London, ON. *Courtesy of* Dr Laurent Couetil, West Lafayette, IN.)

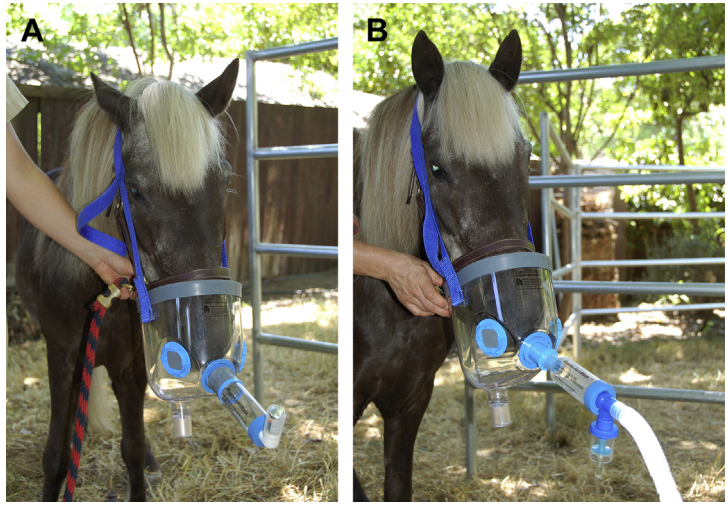

Fig. 4. AeroMask: The fitted mask is placed on the horse's muzzle, such that both nostrils are exposed to the system, and the mask is strapped behind the horse's ears. (*A*) The mask is connected to a small cylindrical chamber, and the pMDI is inserted into the opposite end. On actuation of the pMDI, the suspended aerosols remain in the chamber until the horse inspires. (*B*) The mask can be connected to a nebulization delivery system. (Trudell Medical, Inc, London, ON.)

Fig. 5. Flexineb Equine Nebulizer: The fitted mask is placed on the horse's muzzle, such that both nostrils are exposed to the system, and the mask is strapped behind the horse's ears. The mask is connected to a vertical cylindrical chamber, to which the solution to be nebulized is added. (Flexineb, Inc, Union City, TN. *Courtesy of* Dr Laurent Couetil, West Lafayette, IN.)

is attached to the side of the mask. This mask can also be adapted for use with pMDIs.

PHARMACOKINETICS OF DRUGS ADMINISTERED THROUGH THE INHALATION ROUTE TO HORSES

Dead Space, Drug Absorption, and Drug Clearance

Inhaled drug administration systems contain dead space. This dead space occurs in the reservoir chamber or in the tubing of the nebulizer. Dead space in nebulizers and spacers serves to create a reservoir of drug available to patients on a subsequent inspiration. Reducing dead space volume and using a reservoir with a round or conical shape is best for recuperating any lost droplets.[9]

The airway epithelial lining readily absorbs drugs in aqueous form.[20,21] Most drugs administered through the inhalation route into the lung are relatively bioavailable.[21] In contrast to aqueous solutions, a suspension of drug still in particulate form may not be readily absorbed. Any delay in absorption may result in mucociliary clearance of the drug before it has a chance to achieve full effect.[20] The more soluble the drug, the faster it is absorbed. The lungs, although possessing some ability of drug clearance, do not do so as efficiently or as rapidly as the liver.

Drugs administered into the airway can be systemically absorbed through alveolar capillaries into the bloodstream. The absorption of the drug into the systemic

circulation is of concern with drugs that have undesirable side effects, such as immunosuppression from corticosteroids or the anticholinergic effects of atropinelike drugs (ie, ipratropium). However, the zona occludens that limits entry of systemically administered drugs into the airways generally allows little of the inhaled drug to reach the circulation. Indeed, several studies have shown only small increases in plasma concentrations of inhaled drugs.[22–24]

Physiologic changes in the lungs, such as age-related changes or pathologic conditions as a result of the disease process, may decrease the effectiveness of inhalation therapy and, thus, the amount of drug that can be deposited in the lung. Although this is outside the control of the operator, it should be considered when designing therapy. For example, a 25-year-old horse with severe equine asthma (RAO) may require more drug than a 7-year-old horse with mild asthma (IAD).

Therapy Design and Drugs Used for Inhalation

In order to maximize efficacy, the therapeutic plan must be designed with appropriate duration of treatment and frequency of administration in mind. This point is especially important for inhalation treatments. Patient compliance and tolerance are maximized when nebulization time is short.[10] There is an inverse correlation between droplet size and time to aerosolize the drug; thus, larger droplets get aerosolized faster. Viscous substances take longer to nebulize and have larger droplet sizes.[25] Frequency of administration must be feasible for the client to comply with.

Reported values of effective doses of inhaled drugs serve as general guidelines, because each drug will interact with the delivery system and create a particle size distribution unique to that combination. **Tables 1** and **2** depict recommended doses for commonly used drugs administered by inhalation to horses. Some drugs used in humans and small animals, such as xanthine derivatives (eg, theophylline), are not currently used in horses.

Mucolytic drugs

Mucus is held together by covalent, ionic, and hydrogen bonds, van der Waals forces and parallel DNA network formation. Mucus binds bacteria and other debris and is then cleared through the ciliary escalator. Excessive mucus, resulting from increased production due to inflammation or decreased clearance, can be detrimental by

Table 1
Bronchodilators used as inhalation therapy in horses

Type of Bronchodilator	Drug	Formulations Brand Names (in the United States)	Dosage
Short-acting beta2 adrenergic agonists	Albuterol (Salbutamol)	Proventil-HFA Ventolin-HFA ProAir-HFA	1–2 μg/kg, q 1–4 h
	Levalbuterol	Xopenex-HFA	0.5 μg/kg, q 4 h
Long-acting beta2 adrenergic agonists	Salmeterol	Serevent-CFC free	0.25–1.0 μg/kg, q 6–8 h
Muscarinic cholinergic antagonist	Ipratropium	Atrovent-HFA	1–3 μg/kg, q 6–8 h

These inhalers are formulated as pMDIs and should be used with a delivery device, such as Equine Haler, AeroHippus, AeroMask, or Flexineb.

Table 2
Antiinflammatories used as inhalation therapy in horses

Type of Antiinflammatory	Drug Formulations	Brand Names (in the United States)	Dosage
Corticosteroid	Beclomethasone: HFA	QVAR Beclazone	2–8 μg/kg q 12 h
	Fluticasone: HFA	Flovent Flixotide	2–4 μg/kg q 12 h
	Flunisolide: HFA	Aerospan	1–4 μg/kg q 8 h (anecdotal)
Mast cell stabilizer	Nedocromil: CFC free	Tilade (2 mg per actuation)	8–14 mg per 500 kg q 4–8 h
	Cromoglycate: CFC free	Intal (5 mg per actuation)	10–15 mg per 500 kg q 4–8 h

These inhalers are formulated as pMDIs and should be used with a delivery device, such as Equine Haler, AeroHippus, AeroMask, or Flexineb.

altering gas exchange properties and retaining harmful substances or bacteria within the respiratory tract.

The amount and viscosity of mucus, along with ciliary activity, determine mucus clearance. Mucus production and viscosity are increased during pulmonary diseases, such as severe equine asthma. Mucokinetic agents that reduce mucus in airways are candidates for inhalation therapy in the management of diseases like severe asthma.

Physiologic saline exerts mucolytic activity when applied topically to respiratory mucus. Inhalation of droplets of isotonic fluid can help to breakdown the oligosaccharide cross-linking and disrupt van der Waals forces, thus, reducing the viscosity of mucus and making it easier to clear.[26] Diseases such as pneumonia and moderate to severe equine asthma result in increased mucus secretion. The inhalation of vaporized or nebulized isotonic fluid in such cases can have a therapeutic effect by facilitating clearance of these secretions.[27] Although isotonic solutions are beneficial, the use of vaporized or nebulized sterile water or hypertonic saline is controversial because of possible bronchoconstriction in response to the altered osmolariy.[28]

Other mucokinetic agents that have been administered by inhalation include hygroscopic agents, such as propylene glycol, as well as true mucolytics, such as acetylcysteine.[1,8]

Bronchodilators

The 2 major classes of inhaled bronchodilators are the beta-2-adrenergic agonists and the muscarinic antagonists. Beta-2 agonists include albuterol (salbutamol), levalbuterol, salmeterol, and fenoterol. The muscarinic antagonists include ipratropium, oxitropium, and tiotropium. Inhaled nitric oxide (NO) also promotes bronchodilation.

Beta-2 adrenergic agonists The beta-2 adrenergic agonists are sympathomimetic drugs that inhibit smooth muscle contraction by decreasing intracellular calcium stores and the activity of smooth muscle cell protein kinase. This inhibition of smooth muscle contraction is done through activation of adenyl cyclase, which in turn stimulates cyclic adenosine monophosphate and protein kinase A. Beta-2 agonists enhance mucociliary clearance by stimulating ciliary activity and increasing the fluidity of the mucus secretion. In addition, these drugs may also decrease proinflammatory cytokine release from mononuclear cells and neutrophils.[29]

Beta-2 agonists mediate smooth muscle relaxation leading to bronchodilation.[30] Short-acting beta-2 agonists have a quick onset of action and are the most effective

drug class for relieving acute bronchoconstriction in patients with asthma.[31] Diseases associated with smooth muscle hypertrophy, hyperplasia, or metaplasia are particularly prone to the occurrence of life-threatening bronchoconstriction, making short-acting beta-2 agonists critical for the management of these diseases in several animal species.[18]

Short-acting beta-2 adrenergic agonists have a rapid onset of action within 5 to 15 minutes, and duration as short as 2 to 4 hours. Long-acting beta-2 agonists have a slower onset time of 30 minutes, with peak activity 3 hours after administration, and duration of 8 hours.[32] Available formulations are listed in **Table 1**.

Reversing bronchoconstriction is symptomatic therapy only, and it does not treat the underlying disease. Nonetheless, it helps to reestablish normal gas exchange and comfort. In addition, provision of bronchodilation first will aid in improved delivery of inhalational corticosteroids or other therapies.

The systemic side effects of beta-2 adrenergic agonists include trembling, sweating, anxiousness, tachycardia, and cardiac arrhythmias. These side effects are very uncommon when the drugs are administered through the inhalation route as compared with systemic routes.

Muscarinic cholinergic antagonists Muscarinic cholinergic antagonists are parasympatholytic drugs. They block muscarinic receptors in a fashion similar to atropine, resulting in inhibition of calcium release from myocytes, and thus preventing contraction of airway smooth muscles. They are therefore very potent inhibitors of bronchoconstriction. Side effects include decreased salivation, tachycardia, mydriasis, and decreased gastrointestinal motility; however, they are significantly less common after inhalation as compared with systemic administration. The onset of action is slow, 30 to 60 minutes, making these drugs less suitable as rescue remedies for acute respiratory distress associated with bronchospasm as compared with short-acting beta-2 agonists.

Formulations of muscarinic cholinergic antagonists for inhalation therapy include ipratropium, oxitropium and tiotropium. Currently only ipratropium is recommended for use in the horse. **Table 1** depicts details in the formulation and dosage of inhalational ipratropium in horses. Muscarinic cholinergic antagonists can be given in combination with beta-2 adrenergic agonists; a combination of ipratropium and albuterol inhalation aerosol (Combivent Respimat) is commercially available in the United States.

Nitric oxide NO is a gaseous, highly reactive molecule with a free radical electron, produced in neuronal and non-neuronal tissues from the conversion of L-arginine to L-citrulline. In lungs its exact source is uncertain, but it may be produced by pneumocytes, pulmonary macrophages, or from the respiratory epithelium. At the cellular level, NO binds to guanylyl cyclase, changing its shape and increasing its activity to increase production of cyclic guanosine 3′5′-monophosphate (cGMP). cGMP then acts on smooth muscle, causing vasodilation and bronchodilation. The pulmonary effects of inhaled NO are pulmonary vasodilation, with an accompanying decrease in pulmonary blood pressures. Therefore, NO can reverse pulmonary vasoconstriction and may be used as a therapy in acute respiratory distress syndrome and pulmonary hypertension.[33] It also serves as a bronchodilator by acting directly on airway smooth muscles and may even play a role in mast cell stabilization, which would assist in controlling exacerbation of asthma and other obstructive airway diseases.[34] It must be remembered that NO in large amounts can be toxic because of the formation of nitrite and peroxynitrite radicals, particularly in the presence of oxygen. These toxic byproducts may cause increased airway responsiveness.[35]

Mast cell stabilizers

The cromone drugs inhibit mast cell degranulation, thus preventing the release of several mediators of inflammation, including prostaglandins, leukotrienes and histamine by blocking calcium channels. The overall effect is to inhibit bronchoconstriction by means of decreasing the inflammatory cascade. These drugs are considered prophylactic or preventative. For optimal results, they require a period of 1 to 2 weeks of steady use before the onset of signs in order to provide any notable benefit. Therefore, they are particularly indicated in horses with seasonally recurrent asthma, particularly when an increased percentage of mast cells is present on BALF cytology. Available inhalational formulations of cromones include nedocromil (Tilade) and cromoglycate (Intal) (see **Table 2**).

Corticosteroids

Corticosteroids bind to intracellular glucocorticoid receptors leading to inhibition of nuclear factor–kβ and ultimate downregulation of the gene expression of inflammatory cytokines. The side effects of glucocorticoids include hyperglycemia, sodium and water retention, hypokalemia, suppression of the hypothalamic pituitary axis, impaired healing, immunosuppression, gastrointestinal ulceration and laminitis. Because of the concerns about the use of systemic corticosteroids, inhaled corticosteroids have become quintessential to the treatment of inflammatory bronchoconstrictive diseases, such as RAO and IAD in horses, especially for long-term management. One limiting factor for the use of inhaled corticosteroids is that they are more expensive than the formulations available for systemic use. The adverse effects are significantly less likely with the inhaled versus systemic formulations.[36] Inhaled corticosteroids can be delivered through various spacers. Formulations of pMDI currently available include beclomethasone, fluticasone and flunisolide (see **Table 2**).

Beclomethasone dipropionate Beclomethasone dipropionate is currently available in various formulations with an HFA propellant. The receptor binding half-life of beclomethasone administered through this route is 7.5 hours. Very fine particle size can be achieved with the propellant, which increases lower airway and lung deposition.[37,38] Beclomethasone dipropionate is transformed to the more active 17-beclamethasone dipropionate on absorption.[39] It reverses airway obstruction and improves pulmonary function in horses affected with severe equine asthma.[40,41] Inhaled beclomethasone at doses greater than 1500 μg per 500 kg of body weight in horses has been shown to cause adrenal suppression.[42] Because of this, the dose must be tapered at cessation of treatment.[41,42] In humans, the most common side effect of inhalational beclomethasone is oral candidiasis, possibly associated with local immunosuppression.[43] In horses, mild respiratory infections have been reported after inhaled beclomethasone therapy.[40]

Fluticasone Fluticasone is currently formulated with HPA propellants, such as Flovent or Flixotide. The receptor binding half-life in humans is 10.5 hours.[38] In humans, fluticasone is detectable in the lungs for up to 20 hours after inhalation.[23] In comparing the two inhalational corticosteroids, fluticasone has better potency, less systemic absorption, and a longer half-life than beclomethasone. The primary disadvantage of fluticasone is the increased cost. The recommended dosage is similar to beclomethasone for resolution of airway obstruction during severe equine asthma attacks.[4,36,44,45] No evidence of immunosuppression was noted in horses treated for as long as 11 months based on markers of immune function.[36] Administration of 2000 μg per 500 kg of body weight through inhalation does not result in adrenal suppression, whereas higher doses, including 3000 μg per 500 kg of body weight, may do so.[25]

Flunisolide Flunisolide is also formulated with an HPA propellant. It has a receptor-binding half-life of 3.5 hours and is less potent than fluticasone.[38] There are no published recommended doses for flunisolide in horses.

Antimicrobials

There is great interest in inhaled antimicrobials for use in lower airway infections in human medicine, especially in patients affected with cystic fibrosis and non–cystic fibrosis bronchiectasis. Several classes of antibiotics are reported to be administered through the inhalation route in humans with these infections. These classes include tobramycin, amikacin, and gentamicin (aminoglycoside antibiotics); aztreonam (monobactam antibiotic); colistin (polymyxin antibiotic); ciprofloxacin (fluoroquinolone antibiotic); and ceftazidime (third-generation cephalosporin).[46,47] Only tobramycin, colistin, and aztreonam are specifically formulated for aerosol delivery.

Selection of an antibiotic for use as inhalation therapy should be made with care, as some drug formulations contain preservatives or stabilizers that could be harmful if inhaled.[48] Among these are phenols, thioglycerol, cresol, and disodium edetate, which can cause bronchial irritation, coughing, and bronchospasm.[47,48] Use of sterile water as a diluent should be avoided because hypotonicity of the solution can cause bronchoconstriction. Drugs may be reconstituted in saline (either 0.23% or 0.45%) instead of water to avoid the ill effects of hypotonicity.[24] Hyperosmolarity can also induce bronchial irritation.

Inhalation allows delivery of antibiotics that are not well absorbed orally without having to perform an injection. In addition, it reduces systemic side effects of antimicrobials. None of the antibiotics discussed are labeled for aerosol use (**Table 3**).

Gentamicin Gentamicin is an aminoglycoside antibiotic with primarily aerobic gram-negative coverage.[49] It may also affect some aerobic gram-positive bacteria, such as *Staphylococcus* species and *Rhodococcus equi*.[49,50] Resistance to gentamicin is developing in many genera of bacteria.[51]

The bactericidal activity of gentamicin is concentration dependent. When administered systemically, gentamicin is primarily distributed in extracellular fluid. As such, it easily reaches therapeutic levels in fluids associated with the respiratory tract, like pleural fluid, sputum, and bronchial secretions.[49] It also reaches therapeutic concentrations in lung tissue.[49] The most significant side effect of gentamicin administered systemically is nephrotoxicity. Administration of gentamicin in an aerosol can lower

Table 3
Antimicrobials studied for inhalation administration in horses

Antimicrobial	Delivery Method	Volume of Injectable and Diluent	Dosage
Gentamicin	Ultrasonic nebulizer[22,24]	Gentamicin diluted to 50 mg/mL (from 100 mg/mL) using sterile water[22] or sterile saline[24]	20 mL total volume of diluted solution per adult horse q 24 h (approximately 2 mg/kg daily)
Ceftiofur (Naxcel)	Nebulized with Flexineb[53]	Diluted with sterile water to a concentration of 50 mg/mL[53]	2.2 mg/kg q 24 h[53] (or 1.1 mg/kg q 12 h)
Cefquinome	Jet nebulizer[57]	5 mL injectable cefquinome + 2.5 mL saline[57]	0.5 mg/kg q 24 h

the total-body dose while providing a high concentration of drug within the respiratory system, an important consideration for a concentration-dependent drug.[22]

The recommended concentration for nebulization of gentamicin is 50 mg/mL.[22] When gentamicin was administered to adult horses through inhalation of 20 ml of 50 mg/ml or intravenous injection at 6.6 mg/kg, the concentration of gentamicin in BALF was higher after inhalation than after systemic administration.[22] Seven days of once-a-day administration of gentamicin through inhalation at 1000 mg (20 ml of 50 mg/ml) per day per horse did not result in side effects. There was also no evidence of inflammation in BALF.[24] The primary disadvantage of gentamicin and other aminoglycosides is that they poorly penetrate abscessed pulmonary tissue.[32]

Ceftiofur Ceftiofur has the spectrum of a third-generation cephalosporin, which provides broad-spectrum antimicrobial coverage against gram-positive and gram-negative bacteria, including *Streptococcus equi ss zooepidemicus*. Ceftiofur is effective in the treatment of respiratory infections in adult horses when given systemically and is labeled for treatment of *Streptococcus equi ss zooepidemicus*.[52] The recommended concentration for nebulization is 25 mg/mL, but the concentration of 50 mg/mL did not result in any adverse effects in one study.[53] In a comparison of ceftiofur administered to foals through inhalation using a Flexineb (Flexineb North America, Jiffy Steamer Equine Division, Union City, Tennessee) mask and systemic administration, the inhalation route was found to be adequate. Ceftiofur was administered with the nebulization system or through intramuscular injection at a dosage of 2.2 mg/kg once daily for 5 days; the concentration in BALF was higher, and the BALF concentrations lasted longer after inhalation than after the intramuscular injection.[52] No adverse effects were reported following the use of ceftiofur given through inhalation.[53]

Cefquinome Cefquinome is a fourth-generation cephalosporin that provides broad-spectrum coverage, particularly against gram-negative aerobes and cephalosporinase-producing bacteria.[54] When given systemically, it has efficacy against common equine respiratory pathogens.[55] It is also used to treat septicemia in neonatal foals.[56] In a comparison study, cefquinome was given to adult horses through inhalation at 225 mg (0.5 mg/kg) or systemically at 1 mg/kg (intramuscularly or intravenously).[57] The inhalation route resulted in BAL concentrations that were much higher than after systemic administration. For aerosol administration, the total dose of 225 mg resulted in BALF concentrations that were greater than the reported minimal inhibitory concentrations (MICs) for respiratory pathogens within 30 minutes after nebulization. After 5 days of treatment, the horses showed no adverse effects.[57] In pulmonary epithelial lining, high concentrations were detected immediately after nebulization, but they were rapidly cleared (less than 4 hours after administration).[58] As cephalosporins are time-dependent antimicrobials, this may affect their ability to control infections in the lower airways unless given at frequent intervals.[55]

Marbofloxacin Marbofloxacin is a fluoroquinolone antimicrobial that exhibits broad-spectrum bactericidal activity, although it does not have anaerobic or adequate antistreptococcal activity.[49] It frequently has activity against multidrug-resistant bacteria, such as *Pseudomonas aeruginosa*, *Klebsiella spp*, and methicillin-resistant *Staphylococcus aureus*.[49] When administered at a dose of 300 mg with nebulization, using a concentration of 25 mg/mL, the concentrations of marbofloxacin in BALF were higher compared with those after systemic administration.[59] However, concentrations did not reach published MIC values for most bacterial pathogens.[39,60] Therefore, studies regarding dose and safety are required before it can be recommended for

administration. No adverse pulmonary effects have been noted after a single administration, as determined by pulmonary function testing.[59]

ADJUNCTIVE MEASURES

Environmental and Feed Management

In airway disease, such as mild, moderate, and severe equine asthma syndromes, identifying and controlling environmental breathable particulates, including organic and inorganic dust, mold, and pollen, are critical in reducing the exacerbation of disease. Factors such as seasonality (summer, winter), indoor and outdoor management, and dusty or moldy hay are important triggers of clinical signs of RAO and IAD. Soaking hay, using low-dust bedding (such as shredded cardboard), and providing misters and adequate ventilation to decrease particulate matter in the affected horse's breathing zone are some of the important environmental management measures. In fact, environmental and feeding management are even more important than medical therapy in the treatment of horses with airway disease.[45]

Key Points

Inhaled therapy provides 2 particular benefits: (a) the ability to deliver a drug directly to the airways for immediate or relatively rapid effects and (b) decreased systemic absorption of medication, which helps to avoid unwanted drug side effects. In addition, inhaled drugs can be delivered at a maximal concentration to the target site, as compared with systemic administration of the drug.

Inhalational drug therapy depends on a complex combination of drug characteristics (such as size, solubility, and hydrophilicity), the method of delivery, and patient breathing pattern. There are numerous potential combinations of drugs and delivery forms.

Some general rules to use during inhalational therapy include the following:

- Use equipment known to have optimal droplet size and flow rate.
- Ensure drug formulation is of optimal viscosity, dilution, and tonicity for aerosolization.
- Minimize dead space volume in the nebulization delivery system.
- Optimize actuation of pMDIs with inhalation, by using a delivery device appropriate for horses.
- Ensure delivery apparatus is clean before use.

REFERENCES

1. Ziment I. Respiratory pharmacology and therapeutics. Philadelphia (PA): Saunders; 1978.
2. Derksen F. Applied respiratory physiology. Beech J, editor. Equine respiratory disorders. Philadelphia (PA): Lea & Febiger; 1991. p. 1–26.
3. Couëtil LL, Hoffman AM, Hodgson J, et al. ACVIM consensus statement inflammatory airway disease of horses. J Vet Intern Med 2007;21:356–61.
4. Couëtil L, Cardwell J, Gerber V, et al. Inflammatory airway disease of horses—revised consensus statement. J Vet Intern Med 2016;30(2):503–15.
5. Clay MM, Clarke SW. Effect of nebulised aerosol size on lung deposition in patients with mild asthma. Thorax 1987;42(3):190–4.
6. Clay MM, Newman SP, Pavia D, et al. Assessment of jet nebulisers for lung aerosol therapy. Lancet 1983;2(8350):592–4.

7. Votion D, Ghafir Y, Munsters K, et al. Aerosol deposition in equine lungs following ultrasonic nebulisation versus jet aerosol delivery system. Equine Vet J 1997; 29(5):388–93.
8. Duvivier DH, Votion D, Roberts C, et al. Inhalation therapy of equine respiratory disorders. Equine Veterinary Education 1999;11(3):124–31.
9. Flament M-P, Leterme P, Gayot A. Factors influencing nebulizing efficiency. Drug Dev Ind Pharm 1995;21(20):2263–85.
10. Durham A. Inhalation therapy for LRT disease. Paper presented at: Proceedings of the 50th British Equine Veterinary Association Congress. Liverpool, United Kingdom, September 7–10, 2011.
11. Lenney J, Innes J, Crompton G. Inappropriate inhaler use: assessment of use and patient preference of seven inhalation devices. Respir Med 2000;94(5): 496–500.
12. Leach CL, Davidson PJ, Hasselquist BE, et al. Influence of particle size and patient dosing technique on lung deposition of HFA-beclomethasone from a metered dose inhaler. J Aerosol Med 2005;18(4):379–85.
13. Viel L, Tesarowski D. Radioaerosol deposition in equids. Paper presented at: 40th Annual American Association Equine Practitioners Convention. Vancouver, December 4–7, 1994.
14. 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(3):170.
15. Bertin F, Ivester K, Couetil L. Comparative efficacy of inhaled albuterol between two hand-held delivery devices in horses with recurrent airway obstruction. Equine Vet J 2011;43(4):393–8.
16. Derksen F, Olszewski M, Robinson N, 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(4):306–10.
17. Funch-Nielsen H, Roberts C, Weekes J, et al. Evaluation of a new spacer device for delivery of drugs into the equine respiratory tract. Paper presented at: Proceedings of the World Equine Airways Society Symposium. Edinburgh, Scotland, July 19–23, 2001.
18. Hoffman A, Foley M, Spendlove P. Respiratory medicine: new advances in interspecies aerosol delivery. Paper presented at: Proceedings of the Australasian Equine Science Symposium. Queensland, Australia, June 4–6, 2008.
19. Anonymous. AeroHippus equine aerosol chamber. 2016. Available at: https://www.trudellmed.com/animal-health/aerohippus. Accessed May 5, 2016.
20. Olsson B, Bondesson E, Borgström L, et al. Pulmonary drug metabolism, clearance, and absorption. Controlled pulmonary drug delivery. New York: Springer; 2011. p. 21–50.
21. Brown R, Schanker L. Absorption of aerosolized drugs from the rat lung. Drug Metab Dispos 1983;11(4):355–60.
22. McKenzie HC III, Murray MJ. Concentrations of gentamicin in serum and bronchial lavage fluid after intravenous and aerosol administration of gentamicin to horses. Am J Vet Res 2000;61(10):1185–90.
23. Esmailpour N, Hogger P, Rabe K, et al. Distribution of inhaled fluticasone propionate between human lung tissue and serum in vivo. Eur Respir J 1997;10(7): 1496–9.
24. McKenzie HC III, Murray MJ. Concentrations of gentamicin in serum and bronchial lavage fluid after once-daily aerosol administration to horses for seven days. Am J Vet Res 2004;65(2):173–8.

25. Munoz T, Leclere M, Jean D, et al. Serum cortisol concentration in horses with heaves treated with fluticasone propionate over a 1 year period. Res Vet Sci 2015;98:112–4.
26. King M. Relationship between mucus viscoelasticity and ciliary transport in guaran gel/frog palate model system. Biorheology 1979;17(3):249–54.
27. King M, Rubin BK. Pharmacological approaches to discovery and development of new mucolytic agents. Adv Drug Deliv Rev 2002;54(11):1475–90.
28. Mann J, Howarth P, Holgate S. Bronchoconstriction induced by ipratropium bromide in asthma: relation to hypotonicity. Br Med J (Clinical Res Ed) 1984; 289(6443):469.
29. Robinson NE. Clenbuterol and the horse. Paper presented at: Proceedings of the American Association of Equine Practitioners Annual Convention. San Antonio, Texas, November 26–29, 2000.
30. Olson L, Perkowski S, Mason D, et al. Isoproterenol-and salbutamol-induced relaxation of acetylcholine-and histamine-induced contraction of equine trachealis muscle in vitro. Am J Vet Res 1989;50(10):1715–9.
31. US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Heart, Lung, and Blood Institute. National Asthma Education and Prevention Program: Expert panel report: guidelines for the diagnosis and management of asthma: update on selected topics, 2002. Washington D.C.
32. Mazan MR, Ceresia ML, Cole C, et al. Clinical pharmacology of the respiratory system. Equine Pharmacology: John Wiley & Sons, Inc; 2014. p. 137–82.
33. Rossaint R, Falke KJ, Lopez F, et al. Inhaled nitric oxide for the adult respiratory distress syndrome. N Engl J Med 1993;328(6):399–405.
34. Valentovic M, Ball J, Morenas M, et al. Influence of nitrovasodilators on bovine pulmonary histamine release. Pulm Pharmacol 1992;5(2):97–102.
35. Barnes PJ, Belvisi M. Nitric oxide and lung disease. Thorax 1993;48(10):1034.
36. Dauvillier J, Felippe M, Lunn DP, et al. Effect of long-term fluticasone treatment on immune function in horses with heaves. J Vet Intern Med 2011;25(3):549–57.
37. Kelly HW. Comparison of inhaled corticosteroids: an update. Ann Pharmacother 2009;43(3):519–27.
38. Dubois EF. Clinical potencies of glucocorticoids: what do we really measure? Curr Respir Med Rev 2005;1(1):103–8.
39. Würthwein G, Rohdewald P. Activation of beclomethasone dipropionate by hydrolysis to beclomethasone-17-monopropionate. Biopharm Drug Dispos 1990;11(5): 381–94.
40. Ammann V, Vrins A, Lavoie JP. Effects of inhaled beclomethasone dipropionate on respiratory function in horses with chronic obstructive pulmonary disease (COPD). Equine Vet J 1998;30(2):152–7.
41. Rush BR, Raub ES, Thomsen MM, et al. Pulmonary function and adrenal gland suppression with incremental doses of aerosolized beclomethasone dipropionate in horses with recurrent airway obstruction. J Am Vet Med Assoc 2000;217(3): 359–64.
42. Rush BR, Trevino I, Matson C, et al. Serum cortisol concentrations in response to incremental doses of inhaled beclomethasone dipropionate. Equine Vet J 1999; 31(3):258–61.
43. Pedersen S, O'Byrne P. A comparison of the efficacy and safety of inhaled corticosteroids in asthma. Allergy 1997;52(s39):1–34.

44. Giguère S, Viel L, Lee E, et al. Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate. Vet Immunol Immunopathol 2002;85(3):147–58.
45. Couëtil LL, Chilcoat CD, DeNicola DB, et al. Randomized, controlled study of inhaled fluticasone propionate, oral administration of prednisone, and environmental management of horses with recurrent airway obstruction. Am J Vet Res 2005;66(10):1665–74.
46. Brodt AM, Stovold E, Zhang L. Inhaled antibiotics for stable non-cystic fibrosis bronchiectasis: a systematic review. Eur Respir J 2014;44:382–93.
47. Quon BS, Goss CH, Ramsey BW. Inhaled antibiotics for lower airway infections. Ann Am Thorac Soc 2014;11(3):425–34.
48. Kuhn RJ. Formulation of aerosolized therapeutics. Chest 2001;120(supplement 3):94S–8S.
49. Plumb DC. Plumb's veterinary drug handbook. Stockholm (WI); Ames (IA): PharmaVet; Distributed by Wiley; 2011.
50. McNeil M, Brown J. Distribution and antimicrobial susceptibility of Rhodococcus equi from clinical specimens. Eur J Epidemiol 1992;8(3):437–43.
51. Erol E, Locke SJ, Donahoe JK, et al. Beta-hemolytic Streptococcus spp. from horses a retrospective study (2000–2010). J Vet Diagn Invest 2012;24(1):142–7.
52. Folz S, Hanson B, Griffin A, et al. Treatment of respiratory infections in horses with ceftiofur sodium. Equine Vet J 1992;24(4):300–4.
53. Fultz L, Giguère S, Berghaus LJ, et al. Pulmonary pharmacokinetics of desfuroylceftiofur acetamide after nebulisation or intramuscular administration of ceftiofur sodium to weanling foals. Equine Vet J 2015;47(4):473–7.
54. Rose M, Thomas V, Nordmann P. In vitro activity of third and fourth generation cephalosporins against E. coli strains expressing plasmid-encoded AmpC cephalosporinases. Risk management for the limitation of antibiotic resistance. Berlin, Germany: Federal Office of Consumer Protection and Food Safety (BVL); 2004.
55. Thomas E, Thomas V, Wilhelm C. Antibacterial activity of cefquinome against equine bacterial pathogens. Vet Microbiol 2006;115(1):140–7.
56. Rohdich N, Zschiesche E, Heckeroth A, et al. Treatment of septicaemia and severe bacterial infections in foals with a new cefquinome formulation: a field study. Dtsch Tierarztl Wochenschr 2009;116(9):316–20.
57. Art T, Ramery E, Fraipont A, et al. Pulmonary function, airway cytology and bronchoalveolar lavage fluid drug concentration after aerosol administration of cefquinome to horses. Equine Vet Education 2010;22(9):473–9.
58. Winther L, Baptiste KE, Friis C. Antimicrobial disposition in pulmonary epithelial lining fluid of horses, part III. cefquinome. J Vet Pharmacol Ther 2011;34(5):482–6.
59. Art T, De Moffarts B, Bedoret D, et al. Pulmonary function and antimicrobial concentration after marbofloxacin inhalation in horses. Vet Rec 2007;161(10):348.
60. Carretero M, Rodriguez C, Andrés MS, et al. Pharmacokinetics of marbofloxacin in mature horses after single intravenous and intramuscular administration. Equine Vet J 2002;34(4):360–5.