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Three-minute nebulization of gentamicin in healthy dogs results in therapeutic concentrations in bronchoalveolar lavage fluid while remaining below the toxic range values in blood

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OBJECTIVE
To determine and compare the concentration of gentamicin in the lower airways and serum of healthy spontaneously breathing dogs after nebulization with 5% undiluted gentamicin during 3 versus 10 minutes.

ANIMALS
10 healthy experimental Beagles.

METHODS
This was a prospective crossover study. A standardized bronchoalveolar lavage (BAL) procedure was performed in each dog after 1 week of administration of each of 2 different gentamicin nebulization protocols separated by a 1-week washout period. The 2 protocols consisted of nebulization of 5% undiluted gentamicin (50 mg/mL) twice daily either during 10 minutes per session (± 95 mg; 10-minute protocol) or 3 minutes per session (± 28 mg; 3-minute protocol). BAL fluid (BALF) was obtained under general anesthesia using a bronchoscope within 15 minutes after administration of the last nebulization. Blood was collected within 5 minutes after BALF collection. BALF and serum gentamicin concentrations were determined by particle-enhanced turbidimetric inhibition immunoassay. Concentrations between protocols were compared using a paired *t* test.

RESULTS
Both BALF and serum gentamicin concentrations were higher after the 10-minute protocol compared with the 3-minute protocol (mean ± SD: 2.41 ± 0.87 mg/L vs 1.25 ± 0.31 mg/L, $P = .001$; and 1.02 ± 0.59 mg/L vs 0.31 ± 0.24 mg/L, $P < .0001$ in BALF and serum, respectively), while the BALF-to-serum ratio did not differ between the protocols (3.75 [1.37 to 5.75] (median [IQR]) in the 3-minute protocol vs 2.48 [2.02 to 2.67] in the 10-minute protocol; $P = .754$).

CLINICAL RELEVANCE
A 3-minute nebulization of gentamicin seems to achieve sufficient concentrations of gentamicin in the BALF to have good efficacy against aminoglycoside-sensitive bacteria while remaining below the toxic range values in blood.

Keywords: small animal respiratory tract, nebulization, bronchoalveolar lavage

Infectious pulmonary diseases are traditionally treated with oral or parenteral administration of antimicrobials in human and veterinary medicine. However, for antimicrobial therapy to be effective, appropriate concentrations of the product need to be delivered at the site of infection.[1] In some cases, oral and parenteral administrations of antimicrobials may result in a low bioavailability of the drug in the lungs and lower airways, which might not achieve adequate concentrations at the site of infection. In contrast, inhalation of antimicrobials might be more

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efficacious in some cases.[2,3] Inhalation therapy can provide high intrapulmonary drug concentrations with limited systemic exposure and thus limited systemic adverse effects.[4] Nebulization is reported for the delivery of hydrophilic antimicrobials, such as gentamicin.[5] Gentamicin is a broad-spectrum aminoglycoside that is effective against the majority of pathogenic aerobic bacteria (particularly against gram-positive cocci), isolated from dogs.[6,7] IV administration of gentamicin is known to be potentially nephrotoxic and ototoxic in humans and dogs.[8,9] The toxic serum concentration of gentamicin reported in humans ranges from 2 to 4 mg/L.[5,8–10] In dogs, the MIC of gentamicin in the epithelial lining fluid (ELF) of small animals for *Bordetella bronchiseptica* has been reported to range from 1 to 4 mg/L.[11]

Nebulization of gentamicin is often used in human medicine in cases of *Pseudomonas* sp infection in patients with cystic bronchiectasis, cystic fibrosis, or ventilator-associated pneumonia.[5] Also, nebulization of gentamicin is now considered one of the cornerstones of treatment in bacterial infections in patients with various bronchopneumopathies, while using nebulizers produces an adequate size of droplets to reach the lower airways.[12] The pharmacokinetics of gentamicin after nebulization remain unclear to date. One study[5] in human medicine described and compared the pulmonary and systemic pharmacokinetics of gentamicin after nebulization versus IV administration in mechanically ventilated patients. After nebulization, gentamicin concentrations were much higher (3,800-fold) in ELF than in plasma, with a reported proportion of ELF in bronchoalveolar lavage fluid (BALF) of about 1% to 2%.[13] The average systemic bioavailability of nebulized gentamicin was estimated to be 5%.[5] The efficacy of gentamicin has been linked to the ratio of the maximal concentration (C_{max}) divided by the MIC (C_{max}/MIC) of the pathogen, with the optimum bactericidal activity being achieved when C_{max}/MIC is greater than 7 to 10.[14] The optimal use of the administration route of nebulized gentamicin, or other drugs, also remains unknown to date in human medicine. Delivery of nebulized drugs is influenced by variable factors such as the type of patient, the type of device used, and the type of drug.[15,16]

In veterinary medicine, a limited number of experimental and clinical studies[3,17–20] using nebulization therapy for treating lower inflammatory airway diseases have been published over the last 2 decades. The use of nebulization of corticosteroids is well described in cats and dogs.[17–20] On the other hand, little is known about the use of nebulized antimicrobials, such as gentamicin in dogs with infectious lower airway disease, such as *B bronchiseptica*, including their ideal dosing protocol, administration protocol, and duration of treatment. In small animals, there are limited studies[21,22] available related to the performance of various delivery devices and the impact of varying duration of nebulization on airway concentration.

One study[3] performed in 46 dogs with a *B bronchiseptica* infection that were resistant to conventional oral antimicrobial treatment compared the clinical response to 2 empirical protocols of nebulized gentamicin in dogs infected with *B bronchiseptica.* This study showed that administration of 5% undiluted gentamicin twice daily for 10 minutes for 3 to 4 weeks showed a better clinical response compared to dogs that received the classical protocol of 4 mg/kg of gentamicin, diluted with saline (0.9% NaCl) for 10 minutes twice daily for 3 to 4 weeks. In this study,[3] no investigations regarding the duration of each nebulization session were performed.

Since a 10-minute nebulization protocol could cause practical problems for the dog's owners, it would be interesting to know if the nebulization protocol could be shortened.

The first aim of this study was to measure gentamicin concentration in the lower airways after 2 nebulization protocols for 3 and 10 minutes with 5% undiluted gentamicin during spontaneous breathing to determine if the MIC of *B bronchiseptica* can be reached. The second aim of this study was to determine the impact of the 2 nebulization protocols of 5% undiluted gentamicin on the gentamicin concentration in the serum and to determine if nebulization could possibly cause toxic effects.

Methods

Study population

Ten healthy experimental intact male Beagle dogs were included in this study. At inclusion, the dogs were considered healthy based on the absence of clinical signs, normal physical examination, normal CBC count, normal serum biochemistry, and normal thoracic radiographs. No history of respiratory tract problems in the past 4 weeks was reported. The study protocol was approved by the ethical committee of the university hospital (approval No. 2299).

Study design

This prospective crossover study was conducted over a 28-day period and consisted of BALF and serum collection sessions on day 0, day 14, and day 28 **(Figure 1)**. Before the start of the gentamicin nebulization, each dog was acclimated to a close-fitting face mask placed over the dog's muzzle. This acclimation consisted of a short training during the first week of the study design. Two nebulization protocols with a fixed concentration of gentamicin solution (Genta-Kel 5%; KELA) were used over the study period, using an ultrasonic nebulizer (Aeroflaem; Martino). A volume of 5 mL of 5% undiluted gentamicin (50 mg/mL) was nebulized, independently of the dog size. If the cupule of the nebulizer was empty before the end of the nebulization, an additional volume of 5% undiluted gentamicin was added. From day 7 to day 14, gentamicin was nebulized twice daily for 10 minutes (10-minute protocol). After a 1-week washout, gentamicin was nebulized again twice daily for 3 minutes (3-minute protocol) from day 21 to day 28. To standardize each nebulization protocol, BALF and serum were collected after 1 week of nebulization. Each 3- and 10-minute protocol resulted in the

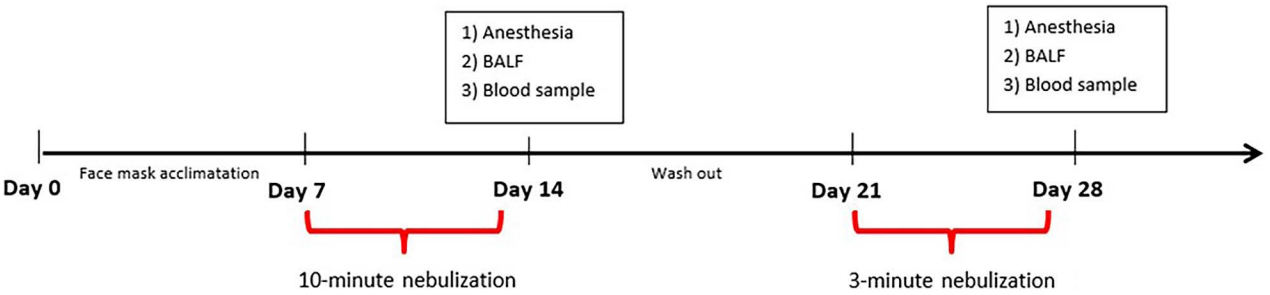

Figure 1—Broncho-alveolar lavage fluid (BALF) and serum collection sessions.

nebulization of a total amount of approximately 28 and 95 mg of gentamicin, respectively.

Sample collection

In each dog, at the end of each week of nebulization, a standardized BAL procedure was performed under general anesthesia.[23] Dogs were anesthetized using butorphanol (0.2 mg/kg; Butomidor; Richter Pharma) in combination with medetomidine (5 mg/kg; Medetor; CP-Pharma) both IV. Propofol (2 to 4 mg/kg to effect; Propovet; Zoetis) was used for induction and was administered IV. Anaesthesia was maintained with isoflurane (Iso-Vet; Eurovet) in oxygen. Dogs were placed in sternal recumbency. BALF was obtained within 15 minutes after administration of the last nebulization using a flexible pediatric endoscope (FUJINON Paediatric Video-Bronchoscope EB-530S) cleaned and disinfected before each use. Two and one 20-mL aliquots of sterile saline solution (0.9% NaCl) were instilled into, and retrieved from, the right and the left caudal lung lobes, respectively. The recovered BALF was pooled.

Venous blood samples were obtained in each dog by jugular venepuncture using 5-mL plastic syringes and 21-G needles and placed into 5-mL serum collection tubes within 5 minutes after BALF collection.

Sample processing

Each BALF sample was centrifuged at 35,000 X *g* for 5 minutes. Then, 1- mL aliquots of BALF supernatant were transferred into cryotubes and stored at 6 °C until batched analysis the same day. After being allowed to clot, each venous blood sample was centrifuged at 35,000 X *g* for 5 minutes. Serum was removed, placed in cryotubes, and stored at 6 °C until batched analysis the same day. All BALF and serum samples were transported on ice to the Centre for Interdisciplinary Research on Medicines, University Hospital of Liège, Liège, Belgium. Gentamicin quantification was performed by a Particle-Enhanced Turbidimetric Inhibition Immunoassay on an Alinity analyzer (Abbott), as described earlier.[24] With the use of this technique, the lower limit of quantification (LOQ) of gentamicin was validated on human serum samples at 0.5 µg/mL.[24]

Statistical analysis

All statistical analyses were performed with XLSTAT software (version 2022.4.1; Lumivero) for Windows. Gentamicin concentrations below LOQ were assimilated as LOQ/2 (0.25 µg/mL) for statistical analysis.[25] In the absence of detection, the values were set at zero. Normality was checked with Shapiro-Wilk tests. A paired *t* test was used to compare the concentrations of gentamicin in BALF and in serum between the 2 protocols. The BALF-to-serum gentamicin concentration ratio was compared between the 2 protocols using the Wilcoxon signed rank test. Data normally distributed were expressed in mean ± SD, while data not normally distributed were expressed in median and IQR. For all tests, a *P* value less than .05 was considered significant.

Results

Study population

The age of the included dogs ranged between 2 and 15 years (mean ± SD: 6.2 ± 6.6). Mean body weight was 16.8 kg (17.1 ± 1.41).

BAL and sample collection

The whole BAL procedure time was ± 2 minutes. BAL was performed within 15 minutes (mean: 13.6 ± 1.17) after the last gentamicin administration. The volume of BALF recovered varied between 23 to 32 mL (mean: 27.9 ± 3.14 mL) representing a mean of 46.4% of the instilled liquid. BAL sampling technique was well tolerated, and no adverse side-effect was observed during or after the procedure. Serum was sampled within 36 minutes (mean: 30 ± 2.78 min) after the last gentamicin administration.

Gentamicin concentration in BALF and serum

Gentamicin was detected in the BALF of all dogs. The mean concentration of gentamicin in BALF was significantly higher in the 10-minute protocol (2.41 ± 0.87 mg/L) compared with the 3-minute protocol (1.25 ± 0.31 mg/L; $P < .001$; **Figure 2**). Serum gentamicin was detected in all dogs with the 10-minute protocol but was below the LOQ in serum in 2 dogs. Serum gentamicin concentration was below the LOQ in 5 dogs and undetectable in 2 dogs after the 3-minute protocol. The mean serum gentamicin concentration was significantly higher in the 10-minute protocol (1.02 ± 0.59 mg/L) compared to the 3-minute protocol (0.31 ± 0.24 mg/L; $P < .0001$; **Figure 3**).

The BALF-to-serum ratio did not differ between the protocols (3.75 [1.37 to 5.75] in the 3-minute

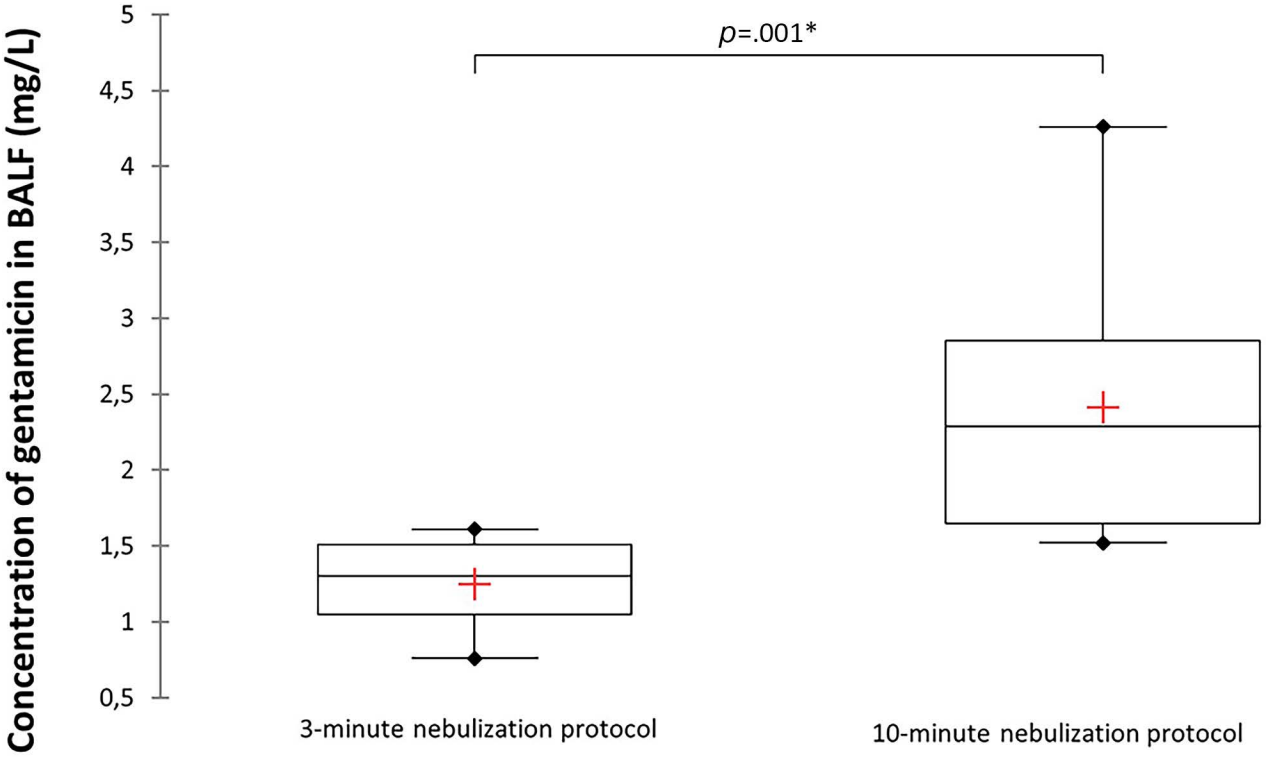

Figure 2—Gentamicin concentrations in bronchoalveolar lavage fluid after a 10-minute nebulization protocol and after a 3-minute nebulization protocol. The mean concentration of gentamicin in BALF was significantly higher in the 10-minute protocol (2.41 ± 0.87 mg/L) compared with the 3-minute protocol (1.25 ± 0.31 mg/L; $P < .001$). Red cross indicates the mean value.

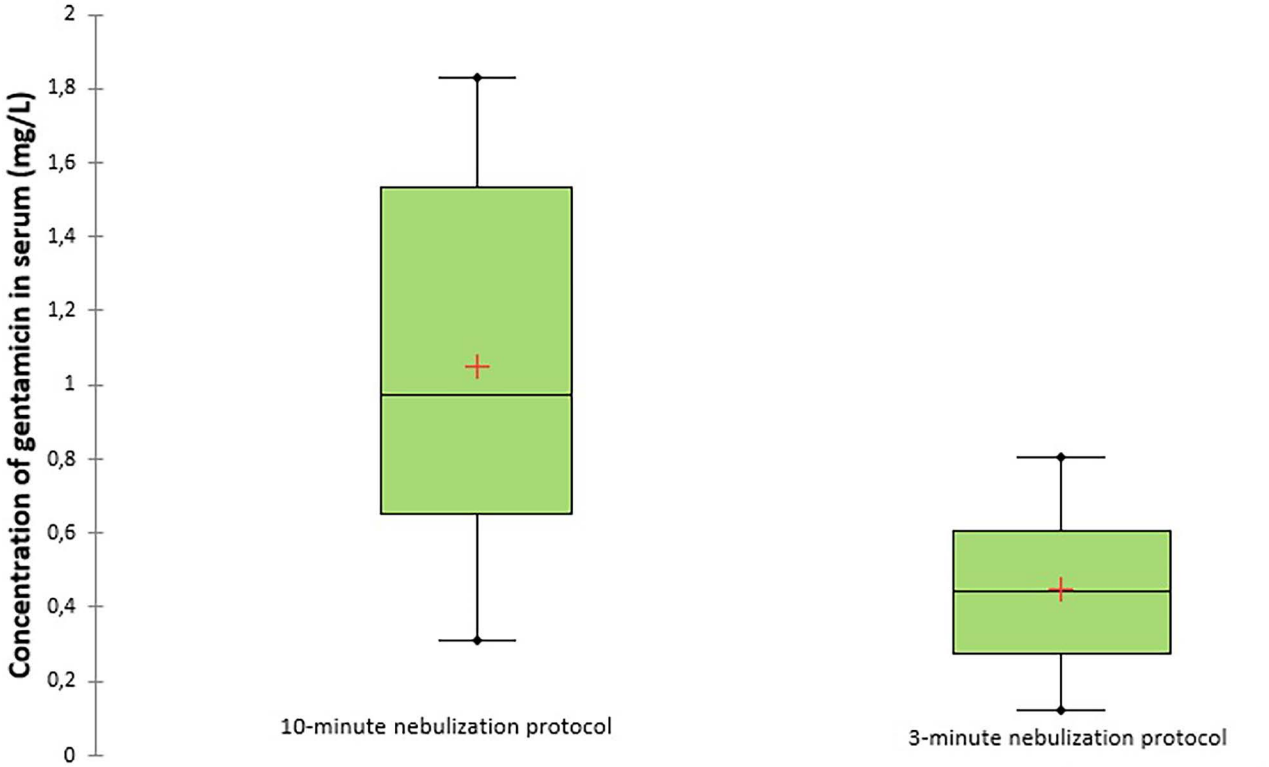

Figure 3—Gentamicin concentrations in serum after a 10-minute nebulization protocol and after a 3-minute nebulization protocol. The mean serum gentamicin concentration was significantly higher in the 10-minute protocol (1.02 ± 0.59 mg/L) compared to the 3-minute protocol (0.31 ± 0.24 mg/L; $P < .0001$). Red cross indicates the mean value.

protocol vs 2.48 [2.02 to 2.67] in the 10-minute protocol; $P = .754$). In both protocols, the serum concentrations of gentamicin remained below the toxic ranges.

Discussion

This study assessed the concentration of gentamicin in the BALF and serum after a 10-minute nebulization protocol and after a 3-minute nebulization protocol in healthy dogs. BALF gentamicin concentrations were superior to the reported minimal effective dose for aminoglycoside-sensitive bacteria such as *B bronchiseptica* after both the 10-minute and the 3-minute protocols, while remaining below the toxic range values in blood.

Bordetella bronchiseptica is described as one of the primary causes of canine infectious respiratory disease complex. In most cases, oral antimicrobial treatment is sufficient to treat this bacterium. However, dogs can be chronically and refractorily infected with *B bronchiseptica* due to mechanisms such as adherence of the bacteria to the respiratory cilia, ciliostasis, production of biofilms, and local immunosuppression.[26–28] For those reasons, conventional oral antimicrobial treatment might be insufficient to reach the apical surface of bronchial epithelium and nebulized delivery of antimicrobials might be required.[3] The MIC of gentamicin for *B bronchiseptica* isolates in dogs is described to be 1 to 4 mg/L in ELF.[11] In this study, the mean concentration of gentamicin in BALF after 10 minutes of nebulization was 2.41 ± 0.87 mg/L versus 1.25 ± 0.31 mg/L after 3 minutes of nebulization. A study from Rennard et al[13] in humans and Hawkins et al[29] in dogs reported that, based on BALF-to-plasma urea and albumin ratios, the apparent volume of ELF in a healthy man or dog is 1% to 2% of the recovered BALF. One study from Melamies et al[30] showed that the mean (± SD) proportion of ELF in BALF was 2.28 ± 0.39% (range, 1.5% to 5%) when using a fixed-amount technique and 2.89 ± 0.89% (range, 1% to 3%) when using a weight-adjusted technique. If we extrapolate this method to our dogs and consider the dilutional effect of a standard BAL procedure in dogs, based on the values of Melamies et al,[30] using the fixed-amount technique and even based on the 5% proportion if ELF in BALF, which is the most pessimistic scenario, we may estimate that the 10-minute protocol and the 3-minute protocol easily provide at least 5 times the minimum level of gentamicin concentrations required in the ELF (ie, 48.2 and 25 mg/L gentamicin in ELF, respectively) to kill *B bronchiseptica* in the lower airways of dogs. However, the urea dilution method, used for calculating the volume of ELF in BALF, is known to overestimate the ELF volume, which in turn might cause an underestimation of the drug concentrations.[29–31]

To obtain high local pulmonary drug concentrations and to minimize high serum concentrations, which might have possible systemic repercussions, a high BALF-to-serum ratio is preferred. In this study, the BALF-to-serum ratio did not differ significantly between both protocols. This means that both nebulization protocols were equivalent. Other bacterial agents that are described to contribute to the canine infectious respiratory disease complex include *Mycoplasma cynos.*[32] However, the exact role of *M cynos* in this disease complex remains unknown.[33] Some *Mycoplasma* spp might be susceptible to aminoglycosides; for example, *M pneumoniae* is susceptible to streptomycin and *My pneumoniae* to gentamicin. However, no proof has been found in the literature that gentamicin might be indicative for treating *M cynos* infections.[34] In case of a suspicion or confirmed *M cynos* infection, an appropriate treatment should be initiated.

In humans and dogs, IV administration of gentamicin is known to be potentially ototoxic, but the major toxic effect is nephrotoxicosis due to the accumulation of gentamicin in renal tubular epithelial cells, which might limit its prolonged use.[35] Risk factors associated with gentamicin-induced nephrotoxicity include prolonged therapy (> 7 to 10 days), multiple doses per day, electrolyte disturbances, acidosis, volume depletion, administration of other concurrent nephrotoxic drugs, and preexisting renal disease.[35] Serum concentrations of aminoglycosides can be monitored to confirm therapeutic antimicrobial levels and to reduce possible toxicity.[36] The results of our study show that serum gentamicin concentrations remained below the toxic value (> 2 mg/L) in all 10 dogs for both nebulization protocols (1.02 mg/L for the 10-minute protocol and 0.31 mg/L for the 3-minute protocol). However, the effects of the above-mentioned risk factors associated with gentamicin-induced nephrotoxicity by using nebulized gentamicin remain unknown but seem to be of minor importance based on human medicine literature. They were therefore not evaluated in this study.

The timing for sample collection remains controversial, and no established method has found universal acceptance.[37] In our study, serum was obtained within 36 minutes (mean: 30 ± 2.78 minutes) after the last gentamicin administration. In most human studies, on the other hand, therapeutic drug monitoring is obtained 30 to 60 minutes after IV administration of aminoglycosides with a second sample just before administering the next dose ,while other studies[37,38] obtained a second serum sample 6 to 14 hours after administration. A study from Bucki et al[36] from 2004 in horses receiving amikacin found that the first serum sample should be taken 90 to 120 minutes after administration and the second serum sample 8 to 16 hours after drug administration. It might thus be possible that our toxicity results are not reflective of reality. However, as the timing to determine gentamicin concentrations after nebulization remains unknown, the serum concentration observed in this study may not reflect the peak concentrations. In addition, in this study, we have no idea about the kinetics of gentamicin. However, since it is known that the bactericidal capacity of gentamicin correlates with the peak concentration, gentamicin is preferable in high-dose regimens associated with extended-interval doses.[14] Indeed, it is accepted that

peak concentrations above 2 mg/L in serum are a risk factor for nephrotoxicity and ototoxicity while the peak toxic concentration is above 12 mg/L.[9,10] Since gentamicin exhibits a narrow range between toxic and therapeutic dosages, the guarantee of low concentration in the serum is a must before advising any therapeutic protocol using this drug. The risk of toxicity of the 2 protocols investigated here in normal dogs appears therefore negligible. In serum, gentamicin was always detectable after a 10-minute nebulization, while after a 3-minute nebulization gentamicin serum concentrations were not detected twice. One study[39] in 18 dogs receiving 15.2 to 17.5 mg/mL gentamicin nebulization for 15 minutes failed to detect gentamicin in serum by radioimmunoassay in all dogs. In our opinion, the absence of knowledge of the pharmacokinetics of this drug in dogs did not influence our study conclusions.

Administering drugs by nebulization is not without any risk and should be performed under adapted conditions and by informing the owners. An adapted close-fitting face mask should be available to avoid losses of the drug by aerosol around the face mask. Nebulization therapy has the potential of fugitive emissions due to the generation of aerosols and to the environment and to the owner that is around the dogs while keeping the face mask around the dog's muzzle. Different studies[40,41] in human medicine have shown that up to 50% of the generated aerosol during therapy remained in the indoor environment for several hours. It has been shown in human medicine that conventional nebulizers might spew two-thirds of the emitted aerosol in the ambient environment.[42] This may also lead to indirect exposure and uptake of potentially minimal concentrations of antimicrobials, leading to an increased risk of antimicrobial resistance in humans. Nebulization therapy may create practical problems for the owners since the dog's cooperation is necessary and a 10-minute nebulization protocol may be too long for some dogs. For these reasons, a 3-minute nebulization protocol can be considered a good alternative since the BALF, and estimated ELF, gentamicin concentrations remain superior to the MIC of gentamicin to kill *B bronchiseptica* in the lower airways of dogs.

This study has some limitations. First, BALF was collected within 10 to 15 minutes after the last nebulization, and serum was collected within 5 minutes after the BALF collection. Small differences in the timing of collection between the 10 dogs could have led to small differences in data, despite using a standardized protocol. Second, it might have been interesting to measure gentamicin concentrations in ELF as well. This has not been performed in this study since urea concentrations in BALF were not available. Gentamicin concentrations were measured only in BALF and were only estimated in ELF. However, the estimated values of gentamicin in ELF are sufficiently high to provide the minimum level of gentamicin concentrations required to kill *B bronchiseptica* in the lower airways of dogs.

There are also some limitations of this study when extrapolating the study design in practice. First, this study was performed in healthy experimental dogs. Distribution of gentamicin might be altered in dogs with lower airway disease. For example, dogs with lower airway disease might have impaired mucociliary clearance and mucous retention. Since aminoglycosides such as gentamicin are known to have a poor transmucous permeability, this potentially leads to reduced drug delivery and impaired efficacy of the drug.[43] Also, chronic inflammatory airways may result in airway remodeling, leading to changes in airflow dynamics and reduced drug deposition.[44] Second, lung diseases often cause a heterogeneous airflow, leading to a lower aerosol deposition of the antimicrobial.[45] Further research is required in dogs with pulmonary disease before providing recommendations regarding the nebulization procedure. Third, different aerosol systems will be used by the dog owners compared to our nebulization systems. This can lead to potential variations in droplet size, power of aerosolization, and the deepness of the drug deposition. Finally, the concentration of the inhaled gentamicin will depend on the cooperation of the dogs. In our study, all dogs were cooperative and received the complete aerosol therapy. In practice, however, it is possible that less cooperative dogs will sometimes move their muzzle out of the face mask leading to incomplete aerosol therapy. For this reason, a 3-minute nebulization protocol should be easier to administer.

We can conclude that although the most adequate and efficacious nebulization protocols still need to be determined, the treatment of dogs, diagnosed with a *B bronchiseptica* infection and reluctant or resistant to oral antimicrobial therapy, with a 3-minute nebulization of 5% undiluted gentamicin is safe and should be as efficacious as the 10-minute protocol so far described.

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