THE COMPARATIVE EVALUATION OF ANTIBIOTIC THERAPY IN THE TREATMENT EFFICIENCY OF CHRONIC BRONCHITIS IN HORSES

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Abstract
The susceptibility testing of microbial pathogens obtained by transtracheal lavage and bronchoalveolar lavage from the respiratory system of horses and cultured were compared. The antibiotics were compared with each other (inhibition zone diameter and resistance/sensibility of etiologic agents) to determine the best antibiotic that can be administered in horses with respiratory infections. Comparison of different families of antibiotics were also done (by average inhibition area diameter). Among the samples obtained by means of a bronchoalveolar lavage there were no microorganisms with resistance to florfenicol, enrofloxacin, and amoxicillin. The highest average inhibition zone diameters were: penicillin (36 mm), ciprofloxacine (25 mm), enrofloxacin (22.5 mm) and tetracycline (20 mm). The best antibiotics for treating the pathogens in samples obtained by means of a transtracheal lavage were: cefquinome (23 mm), tetracycline (21 mm), trimetoprim (21 mm) and florfenicol (19.83 mm). Further (and more elaborate) studies are required to determine the best antibiotics for the treatment of respiratory infections.
Key words*: antibiotic, resistance, horse, respiratory infection.*

Introduction

The respiratory system is indispensable to mammalian life, and each species has developed unique adaptations to the necessities its interaction with its environment requires. The flow rate generated by the equine respiratory system during exercise is about 64-79 l/s, a comparatively large amount for mammals. For comparative reasons, humans only generate about 4 l/s of air flow during exercise. (Oke, 2010).

The definition of bronchitis is the inflammation of the parts of the respiratory system located between the nose and the lungs as well as the bronchi. Chronic bronchitis is a type of bronchitis that occurs as a consequence of long term exposure to particular irritants of the respiratory system, as described by medical dictionary.

The high prevalence of bronchitis in horses is due to the chronic exposure of animals to irritants such as allergens among which the fungi present in low quality hay and bedding found in barns (i.e *Aspergilius* and *Saccharopolyspora spp.*). This leads to symptoms such as recurrent dyspnea, chronic cough, reduced stamina etc. *Aspergilius fumigatus* targets damaged epithelial cells of the upper airways. It is believed that fumigillin as well as gliotoxin and helvolic acid are used by this fungus to impair the respiratory epithelium's defenses (specifically to decrease the function of the cilia involved in moving particulates up and towards the proximal end of the trachea). (Latge, J.P., 1999).

Pneumonia is an inflammation of the alveolar component of the lungs. The symptoms of pneumonia are as follows: weakness and lethargic behavior, lack of appetite, respiratory distress, depression, high fever, diarrhea, rapid pulse, cough, nasal discharge and loss of gum color due to poor oxygen intake. (McLuckie, 2009). Bacterial (caused by a bacterial agent colonizing the lower airways. *Klebsiella spp*., is a common pathogenic agent that may be multi-drug resistant; guarded prognosis when dealing with *Klebsiella pneumonie*) (Estell, 2016).

The aim of this study was to indicate the most efficient antibiotic for the respiratory tract in horses combined with the ultimate valuable barn management in order to keep the respiratory apparatus at his optimal capacity. The main objectives of the research were represented by the comparative evaluation of two sampling methods for microbiological - evaluation: bronchoalveolar lavage and transtracheal lavage and the comparison of the efficiency of antibiotics frequently used in respiratory diseases of horses.

Materials and methods

The research was conducted in the microbiology laboratory of the Faculty of Veterinary Medicine Cluj-Napoca during the 2015-2016 time period. The samples included in the study were both broncheoalveolar lavage and transtracheal lavage samples collected from the horses with respiratory disease in the clinics of the Faculty of Veterinary Medicine and private owners from Cluj County. The inclusion criteria were met by a total of 8 subjects whose samples were obtained by means of a tracheal lavage (40%) and a total of 12 subjects whose sample was obtained by means of a bronchoalveolar lavagae. Of these 12 subjects, 2 had sterile cultural results.

The samples were collected and conditioned in sterile containers such as swabs, syringes, urine containers and blood dry vacutainers, always from the middle aspirating fluid. The samples that have not been processed within 2 hours from collection were initially refrigerated, but after bacterial development became difficult in such conditions, duplicate samples were sent to the laboratory: one stored at 4°C and the other in room temperature. The samples were than inoculated on blood agar, incubated overnight at 37°C and the colonies evaluated microscopically after the Gram-staining process. The antibiotic sensitivity was tested on Mueller Hinton agar, using the Kirby-Bauer Disk Susceptibility Test.

Bronchoalveolar lavage (BAL) consists of a saline wash of the airways and alveoli with the recovery of the lavage liquid (along with any inflammatory cells that might be present at this level). Bronchoalveolar lavage is indicated for recovering cytological recovery in inflammatory processes: unexplained chronic cough, bleeding; fungal pneumonias; interstitial lung diseases. Transtracheal lavage (TL) is used for the collection of tracheal respiratory secretions for cytology and bacteriology by means of a fiber optic endoscope or video endoscope and a collection catheter, to aid in the investigation of pulmonary disease.

Results and discussions

The average inhibition zone diameter for antibiotics which elicited resistance from samples obtained through bronchoalveolar lavage may be seen in Fig. 1. Column height (as well as the number on the column) indicates the average size of the inhibition zone for the antibiotic in question.

The average inhibition zone diameter for antibiotics which elicited resistance from samples obtained through bronchoalveolar lavage may be seen in Fig. 2. Column height (as well as the number on the column) indicates the average size of the inhibition zone for the antibiotic in question.

When comparing the different families of antibiotics used to test samples obtained through transtracheal lavage (by average inhibition zone diameter) we obtained the graph in Fig. 3 (column height as well as the number above the column represents the average inhibition zone diameter for different families of antibiotics tested).

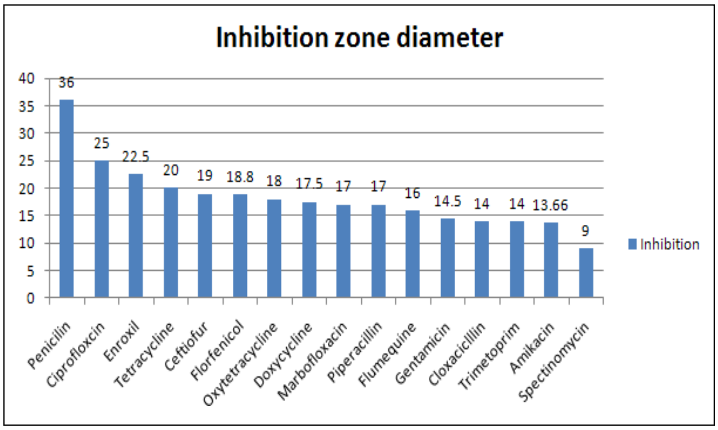

Fig 1. Average inhibition zone diameter for antibiotics in susceptibility of samples obtained through bronchoalveolar lavage

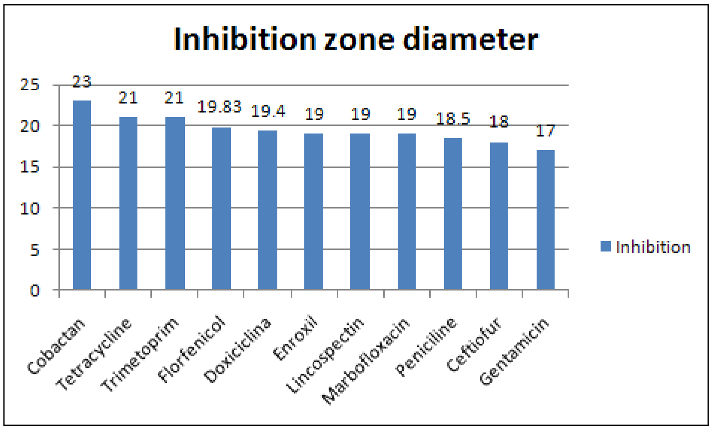

Fig 2. Average inhibition zone diameter for antibiotics in susceptibility of samples obtained through transtracheal lavage

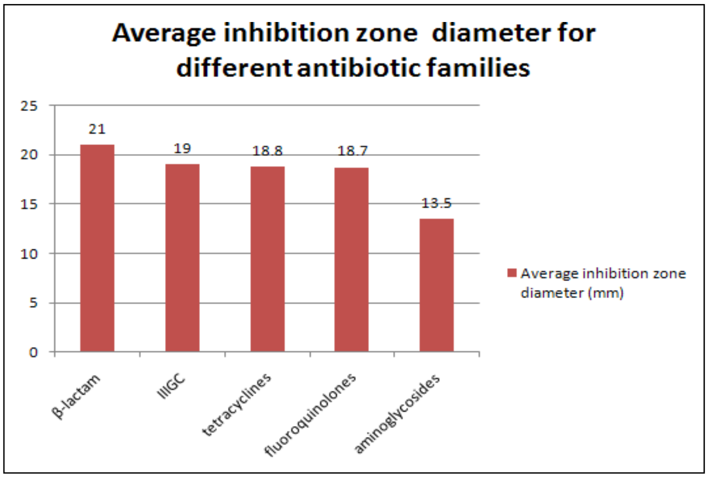

Fig. 3. Average inhibition zone diameter for different families of antibiotics in susceptibility of samples obtained through bronchoalveolar lavage

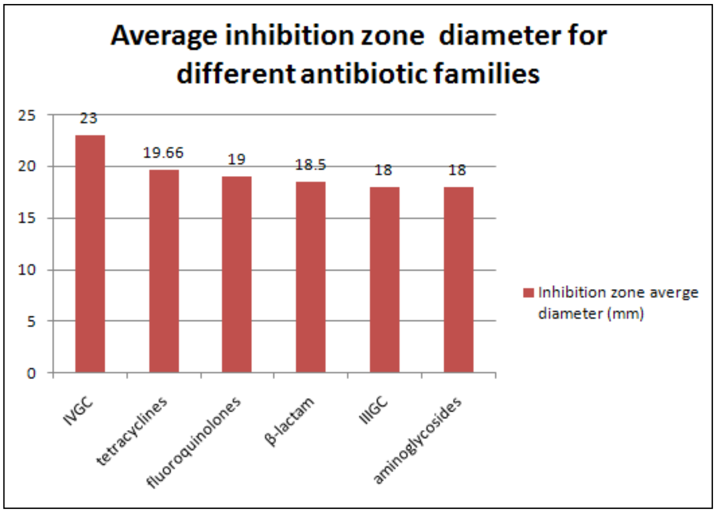

Fig. 4. Average inhibition zone diameter for different families of antibiotics in susceptibility of samples obtained through transtracheal lavage

Average inhibition zone diameter (by families of antibiotics) for the samples obtained through bronchoalveolar lavage had a relatively wide distribution (13.5-21 mm) which would seem to indicate a substantial difference in effective concentration of the active substance. (Approximately 2.4 times the concentration of the less effective antibiotic required to obtain inhibition). In other words it would take a greatly increased concentration of aminoglycosides to obtain the same therapeutic effects as β-lactamines.

Similarly the concentration of third generation cephalosporin's required to obtain the same therapeutic effects to that of β-lactamines would be 1.22. It would take a concentration 1.25 times higher for tetracyclines to obtain the therapeutic effects of β-lactamines, already a substantially higher dose.

The concentration of fluorquinolones required to have a similar efficacy to β-lactamines would be 1.26 times that of the later antibiotic.

The samples obtained by means of a transtracheal lavage were shown to also have a relatively large variety of average inhibition zone diameters (when sorted by antibiotic family they were tested against):

The best inhibition zone diameter were fourth generation cephalosporins who showed an average of 23 mm, placing them at the top of the charts as the best antibiotic family to use for treatment of respiratory infections in equines. (When the sample was obtained by means of transtracheal lavage)

In second place for this method of sampling were the tetracyclines that averaged an inhibition zone diameter of 19.66 mm. While this seems to be a good result, because of the concentration variation it still means that it takes a concentration of tetracyclines about 1.37 times higher than that of fourth generation cephalosporins to have a comparable pharmacological result. Fluorquinolones showed an average inhibition zone diameter of 19 mm thus it becomes apparent that a higher concentration (1.46 times) is required to obtain the same therapeutic effect as fourth generation cephalosporins when it comes to treating bacterial respiratory infections in equines.

The concentration of β-lactamines required for a comparable result to fourth generation cephalosporins is in excess of 1.5 times the concentration of said antibiotics.

Third generation cephalosporins and aminoglycosides showed similar average inhibition zone diameters of 18 mm. The concentration of these antibiotics needs to be 1.63 times higher than fourth generation cephalosporins to obtain inhibition.

Conclusions

1 Among the samples obtained by means of a bronchoalveolar lavage there were no microorganisms with resistance to florfenicol, enrofloxacin, and amoxicillin.

2 However, the best antibiotics (with the highest average inhibition zone diameter) for treating said samples were (in order): penicillin (36 mm), ciprofloxacine (25 mm), enrofloxacin (22.5 mm) and tetracycline (20 mm). As such, these antibiotics require lower doses for clinical effectiveness.

3 The best antibiotics (be average inhibition zone diameter) for treating the pathogens in samples obtained by means of a transtracheal lavage were: cobactan (23 mm), tetracycline (21 mm), trimetoprim (21 mm) and florfenicol (19.83 mm).

4 Average inhibition zone diameter (by families of antibiotics) for the samples obtained through bronchoalveolar lavage had a relatively wide distribution: β-lactamines> third generation cefalosporins> tetracyclines> fluorquinolones>aminoglycosides.

5 The samples obtained by means of a transtracheal lavage were shown to also have a relatively large variety of average inhibition zone diameters (when sorted by antibiotic family they were tested against): fourth generation cephalosporins> tetracyclines>fluoroquinolones> β-lactamines> third generation cephalosporins and aminoglycosides.

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