Antimicrobial Susceptibility Profile of Microbial Pathogens Isolated From Calves With Respiratory Diseases

George Cosmin NADĂŞ*, Flore CHIRILĂ, Cosmina BOUARI, Nicodim FIŢ

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, 3-5 Mănăştur Street, 400372, Romania
*corresponding author: gnadas@usamvcluj.ro

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Abstract

Respiratory disease in calves is an actual problem, a major cause of economic losses due to mortality, growth delay and improper development. These conditions are frequent in calves due to the weaning stress, transport and environmental changes. The aim of this study was the isolation of bacteria from 30 calves with respiratory disorders and their antibiotic susceptibility testing. Samples were collected from calves with respiratory disorders (nasal discharge) aged 6 to 9 weeks in 2 series, using sterile swabs. Samples were initially inoculated on blood agar and MacConkey agar following the characteristics of the colonies and microscopic examination that enabled the identification of bacterial species. Isolated strains were used to flood Mueller-Hinton agar to carry out sensitivity testing. The antibiotics tested were represented by: Amoxicillin with clavulanic acid, Gentamicin, Florfenicol, Enrofloxacin, Marbofloxacin, Penicillin G, Cefquinone, Tulathromycin, Ceftiofur, Tylosin and Cephalotin.

Genus *Streptococcus* have been identified in 23 samples, followed by *Staphylococcus* identified in 14 samples, and *Bacillus* spp., in 10 nasal swabs; The most common bacteria associations were represented by *Streptococcus-Staphylococcus*, *Streptococcus-Staphylococcus-Bacillus*, and *Streptococcus-E.coli*. The most efficient antibiotic was Cefquinome (Cobactan), followed by Penicillin G and Amoxicillin with clavulanic acid (Amoxiclav); the least effective antibiotics were Florfenicol and Tulathromycin.

The study carried out on nasal discharge samples collected from calves with respiratory disorders and their antimicrobial profile testing led to the following conclusions: 1) Low susceptibility to Florfenicol is caused by previous treatments when this molecule was excessively used and without prior sensitivity testing. 2) Cefquinome may represent an emergency therapeutic antibiotic for respiratory infections in calves, but the administration should always be preceded by susceptibility testing of the isolates.

Keywords: *calves, respiratory diseases, nasal discharge, Streptococcus spp*.

INTRODUCTION

Pneumonia of infectious nature is a major problem due to large economic losses encountered in both the European countries and the US. Losses due to respiratory infections in cattle range between 37-52%. In the US are estimated to be between 250 million to 1 billion dollars. Bacteria, viruses, mycoplasma, fungi and parasites play an important role in the etiology and pathogenesis of lung infections particularly in calves. It is well known that a primary role in these disorders includes viruses: bovine respiratory syncytial virus, parainfluenza 3 virus, bovine herpesvirus type 1 and mucosal disease/viral diarrhea virus. Bacteria such as *Manheimia haemolytica*, *Pseudomonas aeruginosa* were isolated in 45% and

25% of cases in a study of 137 calves, and in other cases *E. coli, Klebsiella spp., Staphylococcus aureus, Serratia* spp. and *Neisseria* spp. were isolated.

Another study on 27 calves with respiratory illnesses reported as the main agent *Mannheimia haemolytica* (25%) in most of the calves, followed by *Klebsiella pneumoniae* (20%), *Trueperella pyogenes* (15%), 13 species of hemolytic streptococci (10%), *Staphylococcus* spp. (5%), *E. coli* (5%), *Penicillium* spp. (5%), *Aspergillus* spp. (5%), and fungi (10%) (Aslan, 2002).

The incidence of respiratory disease in calves is most common in those aged 5 to 7 weeks. Most cases are recorded in the autumn seasons (Svensson *et al.*, 2006) and are associated with poor ventilation and poor hygiene conditions at the shelters (Callan and Garry, 2002). Thus, bovine respiratory diseases have a huge economic impact. The costs are due to the loss of animals and increase growth and decrease costly treatments.

In the present study, predisposing causes are multiple. The first factor to be considered is the fact that calves came from different farms or households, transport stress, weaning, environment changes; all have a significant impact on the immune system. The second important factor is the growing technology that has been applied for calving and weaning. Rearing calves in pens provide a convenient environment for weather changes. The problem is that in such conditions, the hot air may contain harmful gases: ammonia, hydrogen sulfide, dust and microorganisms etc. Ammonia and dust particles may reach the alveoli causing irritation and inflammation. Dust particles often contain microbes, using them as a vehicle until they find the optimal conditions for multiplication in the lung parenchyma.

The aim of this study was the isolation of bacteria from 30 calves with respiratory disorders and their antibiotics susceptibility testing. The main objectives of this study are represented by:

- Isolation and identification of bacterial species isolated from nasal discharge samples in calves with respiratory disorders;
- Determination of antibiotic susceptibility of these strains to establish a specific and appropriate treatment to prevent the selection of resistant strains;
- Correlation of "in vitro" and "in vivo" efficiency of antibiotics after the administration for a period of 7 days.

MATERIALS AND METHODS

The researches in this study were performed in the laboratory of Microbiology at the Faculty of Veterinary Medicine in Cluj-Napoca, during April 2015 - January 2016 and the material was represented by nasal discharge samples from calves aged 6 and 9 weeks housed in Iernut, Mureş county. Respiratory diseases were clinically represented by rhinitis, muco-purulent nasal discharge; subsequent clinical picture is characterized by fever, loss of appetite, listlessness and dyspnea.

Samples were individually collected in two series: in April 2015 nasal secretions from 10 calves were collected using sterile tampons with Amies transport medium, and in January 2016, 20 samples using sterile swabs were sampled. Each swab was labeled with the sample number, specifying individual registration number and sampling date.

The first test was the cultural examination using the swabs and streaked by quadrant method in blood agar Petri dishes and MacConkey agar for each sample. After the inoculation and overnight incubation at 37 °C for 24 hours, the identification of colonies was performed for each sample and the culture medium, on the basis of the microscopic (Gram smears stained), and cultural examination. Diffusimethrical method was used for testing the sensitivity of bacteria to several antibiotics Mueller-Hinton is the culture media used for this determination. The antibiotics tested were represented by: Amoxicillin+clavulanic acid, gentamicin, fluorfenicol, enrofloxacin, marbofloxacin, penicillin G, cefquinome, tulathromycin and ceftiofur. The plates were incubated in a thermostat at a temperature of 37°C for 24 hours.

RESULTS AND DISCUSSIONS

The results of microscopic and cultural examination shows that the most frequently isolated bacteria was Streptococcus, identified in 23 samples, followed by Staphylococcus in 14 samples and Bacillus in 10 samples. Regarding the bacterial associations, they were most often represented by Streptococcus-Staphylococcus in 13 samples, followed by the association of Streptococcus-Staph-

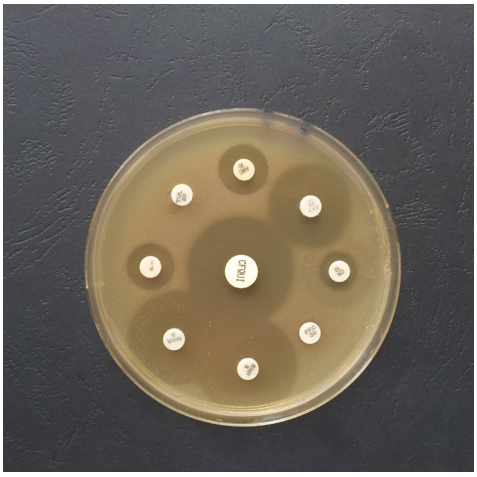

Fig. 1. The susceptibility testing for sample nr. 7

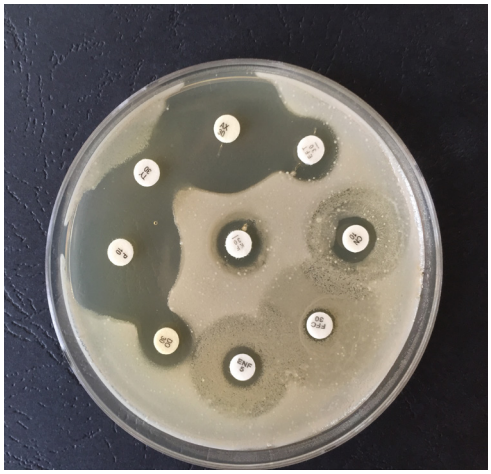

Fig. 2. The susceptibility testing for sample nr. 26

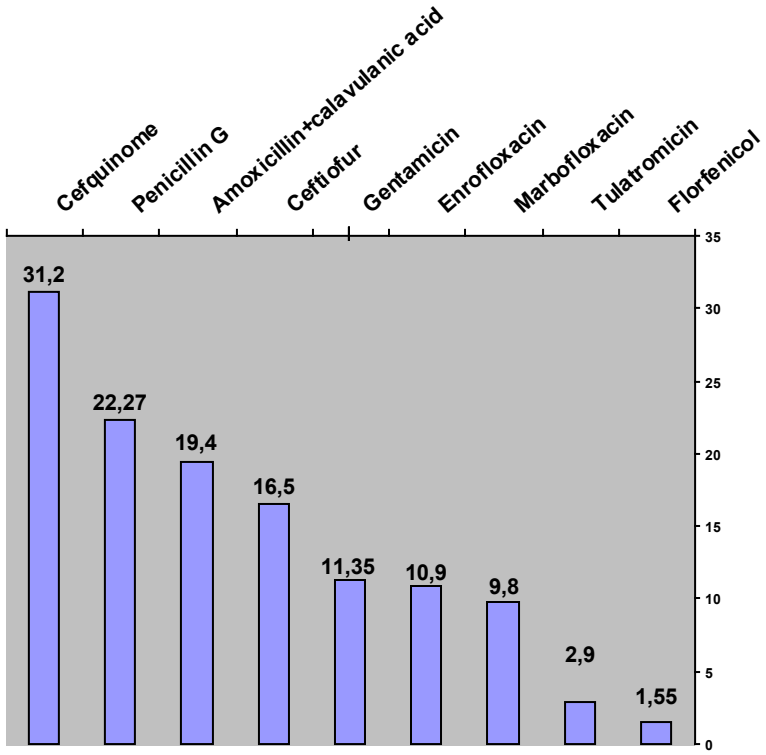

Fig. 3. The average of inhibition area diameter for the tested samples

ylococcus-Bacillus in 9 samples, Streptococcus-E. coli in 4 samples and in one case association Bacillus –Micrococcus-Streptococcus-Klebsiella.

Data for antibiotic susceptibility testing have shown that the most effective antibiotic was the cefquinome (Cobactan) with an average diameter of inhibition area of 31.2 mm followed by penicillin G with 22.27 mm and amoxicillin with clavulanic acid with 19.4 mm. In terms of resistance, the less effective antibiotics were the florfenicol with the average of 1.55 mm and Tulathromycin with 2.9 mm.

Florfenicol resistance of the tested strains is most probably the result of improper treatment by overdosing performed by the owner - treatment was only aministered for 3 days. In a recent study on 188 staphylococci strains isolated from animals resistant to florefenicol, 11 of them have de-

veloped one or both genes cfr and fexA for which the 9 cases was demonstrated transmission via plasmids (Kehrenberg, Schwarz, 2006). Similar resistance is possible to be encountered in our study.

After the treatment performed using the *in vitro* susceptibility using Cefquinome 1 mg/kg (Cobactan) good results were obtained after 1-2 days of treatment. Treatment was established for 7 days, followed by complete healing of the treated individuals.

CONCLUSIONS

The study carried out on nasal discharge samples from calves with respiratory disorders and their antimicrobial susceptibility testing led to the following conclusions:

1. the most frequently isolated bacteria was Streptococcus identified in 23 samples followed by Staphylococcus identified in 14 samples and Bacillus in 10 samples.

2. the most common bacterial associations were represented by Streptococcus-Staphylococcus, followed by the combination of Streptococcus-Staphylococcus-Bacillus.

3. the largest diameters of the inhibition areas were observed for Cefquinome, followed by penicillin G and amoxicillin with clavulanic acid. The least efficient were florfenicol and tulathromycin.

4. Florfenicol decreased susceptibility is due to previous treatments where this product was used excessively and without prior sensitivity testing ''in vitro''.

5. Cefquinome could represent an emergency therapeutic solution for respiratory infections in calves, but antibiotic administration should always be preceded by susceptibility testing of the isolates.

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