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Antibiotic Sensitivity and Effect of Doxcyclin, Tiamulin and Enrofloxacin Against Broiler Chickens with *Ornithobacterium rhinotracheale* (*ORT*) Infection Symptoms

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Abstract: In this syudy, Experimental groups of 10 susceptible 4-week-old chickens were inoculated oculonasally with *Ornithobacterium rhinotracheale (ORT)* bacteria. Antibacterial treatment started 1 day after dual. After infection, the birds were examined and scored for clinical signs daily, weighed at different times, their tracheas and infraorbital sinus swabbed daily. 9 birds were euthanised and examined for macroscopic lesions at necropsy at 6 days post -bacterial inoculation and the reminder at 15. Samples of the trachea, lungs, sinuses, air sacs, liver and heart were collected for bacteriological examination. Recovery from respiratory disease caused by an *ORT* triple infection in 4-week old chicken was over all most successful after doxycycline treatment, irrespective of treatment duration, followed by tiamulin treatment. Compared with the untreated group, clinical signs as well as *ORT* multiplication in the respiratory tract were significantly reduced by both doxycycline and the enrofloxacin treatment, with the doxycycline treatments showing significantly better reduction than the tiamulin treatment. Five- day treatment with tiamulin, compared with the untrated group, did not cause a significant reduction in any of the aforementioned parameters.

Key words: Antibiotic Sensitivity · *Ornithobacterium rhinotracheale* · Broiler Chickens

INTRUDUCTION

Respiratory problems are one of the main disorders leading to economic losses on poultry farms worldwide. They may be induced by various bacterial and viral agents, either alone or in combination [1]. Several bacteria such as *Ornithobacterium rhinotracheale* (ORT), *[E. coli], Mycoplasma spp., Bordetella avium*, *Pasteurella spp. and Chlamydophila psittaci*, have already been proven to make a contribution to the respiratory disease complex in chickens and turkeys [1]. *Ornithobacterium rhinotracheale* is a gram-negative bacterium associated with contagious respiratory disease in poultry [2]. This pathogen, represented by 18 identified serotypes [3], has been isolated from poultry and wild birds all over the world. Infection of the respiratory tract caused by *ORT* is characterized by pneumonia, tracheitis and airsaceulitis [4], but the bacterium can also disseminate to other sites of the body resulting in local pathology such as hepatitis, meningitis and joint-infections [2, 5]. Because of the economic losses due to decreased growth, increased mortality, increased condemnation rates, drops in egg production and decreased hatchability, *ORT* infections are increasingly recognized as a health problem in the poultry industry [6]. ORT can be a primary or secondary etiological agent depending on strain virulence, adverse environmental factors, immune state of the flock and presence of other infectious agents [1]. The problems, however, become more critical when bacterial pathogens are involved. When bacterial organisms are involved in an outbreak of respiratory disease, the only option is treatment with antimicrobials. Hitherto, few studies concerning the actual in vivo efficacy of antimicrobials for the treatment of bacterial infections in poultry have been performed. The normal antibiotic sensitivity test media do not support growth of *ORT*. Furthermore, its slow growth causes abnormally large inhibition zones in the routinely used diffusion test antibiograms, which are difficult if not impossible to interpret. Another and somewhat unusual cause of difficulties in the interpretation of *ORT* sensitivity tests concerns its extraordinarily high rates of acquired resistance against certain antibiotics [7].

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Antibiotics are routinely used in poultry flocks, for the control of bacterial pathogens besides biosecurity measures. Antibiotics are widely used to treat and prevent various bacterial diseases in layer flocks. Indiscriminate use of antibiotics may lead to antibiotic resistance in pathogenic bacteria as well as normal microflora. Very few reports were available on the antibacterial sensitivity of O*RT* Susceptibility of *ORT* isolates to antibiotics is variable depending on the region of isolation. High rates of acquired resistance of the *ORT* strains from poultry and wild birds against certain antibiotics by *in vitro* antibiotic sensitivity test was reported [8]. Establishing the antibiotic sensitivity of the avian respiratory pathogen *ORT* was difficult because of the organism's complex growth requirements and the unusually frequent occurrence of resistance [7]. Studies on antibiotic susceptibility and resistance are very few and hence this study on *in vitro* susceptibility test on *ORT* isolates would be helpful in formulating control measures for-*ORT* infection in poultry. Therefore it is important to evaluate the efficacy of various antimicrobial drugs currently used in poultry and to determine patterns of antibiotic sensitivity. In this study we described the antimicrobial susceptibility profiles of *ORT* strains isolated from broiler chickens.

MATERIALS AND METHODS

In this study Experimental groups of 10 susceptible 4-week-old chickens were inoculated oculonasally with *Ornithobacterium rhinotracheale (ORT)* bacteria. Antibacterial treatment started 1 day after dual. At 2 weeks of age the birds were shown to be free from maternally derived antibodies to ORT by means of an ELISA available commercially (Biochek, The Netherlands) and an in-house serum neutralization test [9], respectively. Bacteria the ORT type strain LMG 9086TM was used, which was originally isolated from a chicken with a respiratory tract infection. The strain was serotyped as type A in an agar gel precipitation test performed by Hafez. The strain was stored at 70°C The organism was retrieved from the frozen suspension and cultured for 48 h at 37on Columbia agar (Oxoid Ltd., Basingstoke, Hampshire, England) with 5% sheep blood in a 5% CO_2 atmosphere. Ten colonies were transferred into 5 ml brain heart infusion broth (BHI) for 24 h at 37°C with agitation. The bacterial challenge inoculum was prepared by washing the cultured bacteria twice in phosphate buffered saline (PBS) followed each time by five minutes of centrifugation at 3000 rpm at 4°C. The resulting pellet was resuspended in PBS to obtain a final concentration of 8.6 log10 colony-forming units (cfu)/ml. Confirmation of the number of cfu/ml was done by inoculating tenfold dilutions in PBS on Columbia agar with 5% sheep blood and counting the number of colonies. The Three antimicrobial compounds were used in this study: enrofloxacin, 0.03 mg/ml, doxcyclin, 2 mg/ml; tiamulin, 1 mg/ml; as determined according.

MIC of the Parent and Re-Isolated ORT Isolates: The MIC values of different ORT isolate retrieved from the tracheal swabs before, during and after antibiotic treatment were determined. More specifically, the enrofloxacin MICs were determined for the isolates from enrofloxacin group A, the doxcyclin MICs were determined for the isolates from doxcyclin group B and finally, the tiamulin MICs for the isolates from tiamulin group C. Determination of the MIC values was performed by sensititre test.

Clinical Analysis: The clinical scores, tracheal swabbing and weight were analysed only from animals that were not euthanised at 5 dpbi. Only the time span between 0 and 9 dpbi was considered in the analysis as it appears from previous experiments [11] that after 9 dpbi most of the response variables, including the clinical score, return to normal. First, the area under the curve of the clinical scores and tracheal swabbing between 0 and 9 dpbi was analysed via a fixed-effects model with the treatment group as fixed effect. Next, the repeated clinical scores between 0 and 9 dpbi were analysed using a mixed proportional odds model with the clinical score as the response variable, animal as random effect and treatment group and time as categorical fixed effects. ORT tracheal swabbing values between 0 and 9 dpbi were analysed using a mixed model with log10cfu/g mucus as response variable, animal as random effect and treatment group and time as categorical fixed effects.

RESULT

Macroscopic findings were comparable in the different experimental groups, with lesions being serous to seromucous exudate in the turbinates and sinuses and hyperaemia of the turbinates and the trachea. These lesions were found in all groups, but in the groups receiving doxcyclin treatment and to a lesser extent in the group receiving tiamulin treatment, the observed lesions were less severe. MIC of the parent and re-isolated ORT isolates compared with the challenge strain, no increase was noted in the enrofloxacin, doxcyclin or tiamulin MIC values for the re-isolated bacteria from the respective

Table 1: Antibiotics dosage per group (mg/kg)

Antibiotics	Enrofloxacin	Tiamulin	Doxcyclin
Dosage	10	20	20

Table 2: The mean daily dose for antibiotics (mg/kg)

Antibiotics	Enrofloxacin	Tiamulin	Doxcyclin
Actual dose	10.2	23.7	23.2
Range	9.7-11.3	18.0-26.3	17.3-24.4

groups. The theoretically consumed dose of antibiotics per group of birds was close to the target dose of 10 mg/kg for enrofloxacin and 20 mg/kg for doxcyclin and tiamulin. The mean actual daily dose (mg/kg) for the total medication period was 10.2 (range: 9.7-11.3) for group enrofloxacin, 23.7 (range 18.0-26.3) for group tiamulin and 23.2 (range 17.3- 24.4) for group doxcyclin. Table 1 and 2.

DISCUSSION

This study is the first to investigate experimentally the clinical efficacy of different antimicrobial therapies for the treatment of ORT infection in chicken. Clinical analysis of the obtained results revealed that under the circumstances used in this study, recovery from respiratory disease caused by ORT infection in 4-week-old turkeys was overall most successful after doxcyclin treatment (3 or 5 days of treatment), followed by tiamulin treatment. Compared with the untreated control group, clinical signs as well as the ORT in the respiratory tract were significantly reduced by both the doxcyclin treatments and the tiamulin treatment, with the doxcyclin treatments showing significantly better reductions than the enrofloxacin treatment. These results are in accordance with a previously executed experiment in which it was proven that enrofloxacin treatment was significantly better than florfenicol for a reduction in clinical signs and bacterial multiplication in APV/ORT - dually infected turkey and that amoxicillin treatment was not efficacious [11]. Data presented by [12], also demonstrated that enrofloxacin treatment was most efficacious for the treatment of colisepticemia in chickens, followed by florfenicol and that amoxicillin was not effective [13]. showed that enrofloxacin was effective for treatment of *E. coli* infection in chickens. Possible explanations for the observed differences between the different antimicrobial productsmay be the different pharmacokinetic and pharmacodynamic properties of the applied antimicrobials, including the apparent volume of distribution at steady state (Vd(ss)), which gives an indication of the diffusion of the antibiotics in the body tissues. Data from different pharmacokinetic studies chickens, demonstrate that the Vd(ss) of amoxicillin, compared with that of florfenicol and enrofloxacin is lower, which means that amoxicillin is less extensively distributed in the extravascular tissues [14-20]. Furthermore, it must be stated that enrofloxacin has long half-life values [21-23] and that the values for florfenicol are generally lower [17, 24]. With the lowest half-life values noted for amoxicillin [25]. It is likely that the bactericidal effect of amoxicillin is being diminished by its less efficient distribution throughout tissues and its relatively fast elimination [26]. Enrofloxacin, however, is rapidly bactericidal at relatively low concentrations [21] and is distributed very well throughout tissues [27], this study which may explain its favourable results. The results of antibiotic sensitivity tests conducted for the *ORT* isolates in the present study correlated with the findings of [28] with respect to tetracycline and erythromycin, but differed regarding the susceptibility towards penicillin. The findings of antibiotic sensitivity tests conducted for the *ORT* isolates in the present study were in accordance with the reports of [29], except towards the antibiotic enrofloxacin. Result of other same studys was expected for sulfadimethoxine and sulfa trimethoprim [30, 31]. Where complete resistance to sulfa methoxine in majority of isolates and an increasing trend of resistance to gentamicin and trimethoprim sulfa was reported.

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