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Original Article

Bacteriological and therapeutic studies on *Mannheimia haemolytica* and *Pasteurella multocida* in bovine respiratory disease at dairy cattle farms in Tehran

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ABSTRACT

In this study, we performed a bacteriological investigation on bovine respiratory infections. Additionally, the study evaluates the efficacy of florfenicol 30 µg produced by Rooyan Darou Company (test group) as compared to a similar drug produced by Aboraihan Company (control group). Sampling was carried out on 30 calves at two farms with 30-55% incidence rate of respiratory disorders. Nasopharyngeal swabs were collected for culture, biochemical activity, and antibiograme testing from all the calves before commencing the treatment. The calves of the test group received florfenicol 30 µg (20 mg/kg, two intramuscular [IM] injections with a 48-hour interval). Similarly, the control group received the same drug dose produced by Aboraihan Company. All the calves were examined clinically before treatment, and ultimately, clinical index score was recorded for each calf based on Observation Gradings on days two, four, and seven after the treatment. The results of culture analysis and routine bacteriological techniques indicated that *Mannheimia haemolytica* and *Pasteurella multocida* were isolated as dominant bacteria, and the antibiogram test emphasized sensitivity to florfenical 30 µg. The results indicated a significant reduction in the mean total value index in both groups after the treatment ($P \leq 0.05$), and changes of the total value index showed no significant differences between the two groups ($P \geq 0.05$), indicating that both antibiotics have the same effect on resolving clinical signs and the treatment of respiratory infection.

Keywords: Bovine respiratory disease, Florfenicol, Rooyan Darou, *M. haemolytica*, *P. multocida*

Etudes bactériologique et thérapeutique sur les infections respiratoires aux *Mannheimia haemolytica* et *Pasteurella multocida* dans les élevages de vaches laitières de Téhéran

Résumé: Cette étude bactériologique avait pour but la détection des infections respiratoires bovines ainsi que la comparaison de l'efficacité de deux traitements antibiotiques à base de florfenicol 30 µg produits par les compagnies Rooyan Darou (groupe d'essai) et Aboraihan (groupe control). Les prélèvements ont été effectués sur 30 veaux originaires de deux élevages différents avec des taux d'incidence des troubles respiratoires allant de 30 à 55%. Des prélèvements nasopharyngés ont été effectués avant le traitement sur tous les veaux afin de mener des tests de culture, d'activité biochimique et d'antibiogrammes. Les veaux constituant le groupe d'essai ont reçu du florfenicol 30 µg (20 mg/kg, deux injections intramusculaires [IM] à 48 h d'intervalle). Réciproquement, le groupe control a reçu une dose similaire de cette antibiotiqueproduit par une autre compagnie (Aboraihan Co.). Tous les veaux ont été soumis à des examens cliniques juste avant le traitement et

un indice de scores cliniques a était établi à partir des observations répertoriées aux deuxièmes, quatrièmes et septièmes jours suivant le traitement. Les résultats des tests de culture et des techniques d'identification bactériologique conventionnelles ont démontré que *Mannheimia haemolytica* et *Pasteurella multocida* constituaient la majorité des isolats bactériens. Les analyses antibiogrammes ontainsi révélé une bonne sensibilité des bactéries isolées vis-à-vis du florfenicol 30 µg. De plus, une réduction significative dans la valeur moyenne de l'index des symptômes a été observéeaprès traitement (P≤0,05), sans différence significative entre les deux groupes (P≥0.05). Ceci indiquait donc que les deux antibiotiques montraient un effet similaire dans le traitement des signes cliniques des infections respiratoires bovines.
Mots clés: Maladie respiratoire bovine, Florfenicol, Rooyan Darou, *M. haemolytica*, *P. multocida*

INTRODUCTION

Mannheimia haemolytica, formerly known as *Pasteurella* (*P. haemolytica*), is the primary etiological agent of pneumonic pasteurellosis (one of the most important respiratory diseases in the cattle and sheep). *M. haemolytica* is a commensal of cattle, sheep, and other ruminants, which can cause bovine and ovine pneumonic pasteurellosis, which is responsible for considerable economic losses to the cattle, sheep, and other livestock industries in many parts of world (Frank and C., 1983; Bowland and Shewen, 2000). *Pasteurella* spp., mycoplasma, parainfluenza virus type-3, bovine respiratory syncytial virus, and secondary bacterial infections are involved in the occurrence of bovine diseases (Radostits et al., 2000). Bovine respiratory diseases can cause significant losses to the dairy industry. Enzootic pneumonia occurs most commonly in housed dairy calves from 2 weeks to 5 months of age, which are being raised as herd replacements. Pneumonia can be responsible for up to 30% of all calf mortality in dairy herds from birth to 16 weeks of age. Florfenicol is a fluorinated synthetic analog of thiamphenicol, and as an antibiotic, it is effective against a wide range of gram-negative bacteria, such as *M. haemolytica*, *Psteurell multocida*, *Haemophilus somnus*, *E. coli*, *Klebsiella*, *Enterococcus*, *Shigella*, *Fusobacterium*, *Proteus*, and *Neisseria,* and gram-positive bacteria, including *Corynebacterium pyogenes*, *Streptococcus agalactia*e, *Streptococcus uberis*, *Staphylococcus aureus*, and *Clostridium* spp. Indeed, to confirm the effectiveness of an antibiotic the results of the drug administration are evaluated in a clinical trial setting with particular diseases occurring naturally (Anadon et al., 2008; Catry et al., 2008; Sidhu et al., 2009). Herein, we carried out a bacteriological investigation on the respiratory infections in calves between the ages of 2 and 4 months old. Additionally, the study evaluates the efficacy of florfenicol 30 µg produced by Rooyan Darou Company (test group) as compared to a similar drug produced by Aboraihan Company (control group).

MATERIALS AND METHODS

With regard to the incidence and prevalence of bovine respiratory diseases among dairy herds, this field evaluation used multicenter, longitudinal observations conducted in two dairy farms in Tehran with 30-55% prevalence of pneumonia (Zakeri Company in Eslamshahr and Behgol Co. in Herjab-e-Kurdan). A total of 30 Holstein calves aged between 2 and 4 months were included in the study. The calves having visual signs of pneumonia, including fever, loss of appetite, cough, and discharge, were entered into the study. The calves were randomly assigned to receive either florfenicol 30 µg (20 mg/kg, two intramuscular injections with a 48-hour interval, n=15) produced by Rooyan Darou Company or florfenicol 30 µg (20 mg/kg, two intramuscular injections with a 48-hour interval, n=15) produced by Aboraihan Company. All the calves were examined clinically before treatment (day zero), then clinical index scoring (CIS) was recorded for each calf. This examination continued at

pre-defined intervals (second, fourth, and seventh days after the treatment). In each examination and for each calf, CIS was recorded based on Observations Grading. CIS was calculated as:

Total measured value index /total value index= CIS

Nasopharyngeal sampling from calves for microbial culture and antibiogram test was conducted before the treatment on day zero. To this end, cotton-tipped, 15 cm long, sterile swabs were used. To avoid picking contamination from the external nares, the swab was carefully inserted into each nostril, and then placed back into its jacket. The swabs were stored in an icebox and taken to the laboratory. The swabs were removed from the transport media and inoculated onto bovine blood agar containing antibiotics (Becton-Dickinson) and MacConkey agar. The plates were incubated at 37°C in 5-10% carbon dioxide atmosphere and were inspected after 24 and 48 h of incubation. Based on morphology, the suspected colonies were chosen for further identification by using gram staining and biochemical reactions. There was no limitation on the selection and retention of types and the number of colonies per plate. Bacterial isolates identified as *Pasteurella multocida* and *M. haemolytica* were evaluated for the presence of hemolysis on blood agar and differential characteristics of biochemical activity such as oxidase, ornithine decarboxylase, indole, urease, and growth on MacConkey (Qinne et al., 2013; Mahon and Manuselis, 2000). The clinical information related to the calves was transformed to calculate CIS in standard form (Table 1). In both groups, the difference between mean of the total value index before and after the treatment was analyzed by using Wilcoxon Signed Rank and Mann-Whitney U test (to compare the difference between the two groups in terms of mean CIS). P-value less than 0.05 was considered statistically significant.

RESULTS

The results of culture and isolation of bacteria from the samples taken from the calves with respiratory disease indicated that *Mannheimia haemolytica* and *P. multocida* were the dominant pathogenic bacteria, and the antibiogram test emphasized the susceptibility of the mentioned isolates to florfenicol.

Table 1. The clinical index scoring form for respiratory diseases

Clinical parameter	Observation grading	Value index
Behavioral status	Normal	2
	Depressed	4
	In agony	8
Appetite	Normal	2
	Weak	4
	No appetite	8
Rectal temperature	<39.5	4
	<40.5; >39.5	8
	>40.5	12
The respiratory rate per minute	<35	3
	35-50	6
	50<	9
Type of breathing	Normal	1
	Irregular	2
Cough	Yes	2
	No	1
Nasal discharge	Yes	2
	No	1
Total	--	79

The results of identification by culture and biochemical reactions are showed in Table 3. The results of total value index before and after the

treatment are presented in Table 2. Based on our findings, the means of total value index for the test group on days 0, 2, 4, and 7 were 30, 24, 20, and 16, respectively. The means of the total value index for the control group on days 0, 2, 4, and 7 were 34, 28, 28, and 20, respectively (Table 2). For the test group, the means of total value index on day zero (before the treatment) and on the seventh post-treatment day (florfenicol 30 µg produced by Rooyan Darou Co.) were 30 and 16, respectively, and for the other group (florfenicol 30 µg produced by Aboraihan Co.), these values were 34 and 20, respectively. These results reflected that both drugs can lower clinical signs. Similarly, the increasing mean of total value index in both groups indicates an acceptable therapeutic effect on respiratory disease of calves.

DISCUSSION

The present study demonstrated that *M. haemolytica* and *P. multocida* isolates are predominant over the other genera in the upper respiratory tract of diseased calves. Frequencies of *Mannheimia* and *Pasteurella* strains are very variable depending on the source of isolation (Angen et al., 2002). These results are in general agreement with those reported by Kaoud et al. (2010), Haji Hajikolaei et al. (2010), and Odendaal and Henton (1995), all of whom reported the preponderance of *M. haemolytica* and *P. multocida* in cattle respiratory tract. In both groups, the Wilcoxon Signed Rank reflected a significant downward trend of total value index on observation days (day 0 to 7th day after the treatment; P<0.05; Table 2). Mann-Whitney U test demonstrated no significant difference in the value indexes on days zero and seven between the test and control groups (Table 2), that is, both florfencol 30 µg drugs produced by Aboraihan Company and Rooyan Darou Company have the same effect in resolving clinical symptoms and in the treatment of the calves with respiratory infection; this finding should be considered by veterinary clinicians. In one study, subcutaneous injection of florfenicol (40 mg/kg) for the treatment of metaphylactic in herds with normal prevalence of bovine respiratory infections aged under three months indicated better efficacy of the drug compared with tilmicosin (12.5 mg/kg administered orally twice a day for five days) or compared to doxycycline (12.5 mg/kg administered orally twice a day for five days) (Catry et al., 2008). Another study reported from Western Canada showed higher therapeutic effect of intramuscular injection of florfenicol (20 mg/kg) twice with an interval of 48 hours compared with once subcutaneous injection of tilmicosin (10 mg/kg) to calves with undifferentiated respiratory infections (Hoar et al., 1998).

Table 2. Arranged distribution of total value index on day zero (before the treatment) and on seventh post-treatment day for both groups of calves with respiratory tract infection in farms of Behgol in Harjab-e-Kurdan Complex and Zakeri in Eslamshahr.

Total value index pre- and post-treatment							
Test group (florfenicel 30% produced by Rooyan Darou Co.)			**Control group (florfenicel 30% produced by Aboraihan Co.)**			**Changing total value index duration**	
Calf No.	*Zero day*	*7day*	*Calf No.*	*Day zero*	*Day seven*	*Test group*	*Control group*
1011	34	17	1010	39	24	-17	-15
908	34	17	907	39	23	-17	-16
902	34	17	899	38	21	-17	-17
1203	34	16	1205	36	20	-18	-16
816	32	16	819	35	20	-16	-15
910	31	16	912	35	20	-15	-15
1203	30	16	1208	35	20	-14	-15
118	30	16	119	34	20	-14	-14
203	30	16	204	32	20	-14	-12
214	30	16	212	32	20	-14	-12
303	29	16	301	32	20	-13	-12
1015	29	16	1013	30	20	-13	-10
1019	28	16	1016	30	19	-12	-11
314	28	16	325	29	19	-12	-10
912	28	16	911	29	19	-12	-10
Mean range	28-34	16-17	Mean Range	29-39	19-24	Wilcoxon Rank Sum Test	
		16		34	20		
						NS	
Wilcoxon Signed Rank Test			Wilcoxon Signed Rank Test				
		S	S				
P<0.05			P<0.05				

a: S=statistically significant b: NS= statistically non-significant

Results of another study of a single intravenous injection of florfenicol (20 mg/kg) to six calves with an average weight of 154 kg, showed the mean elimination half-life of 18.3 hours, the mean

distribution volume of 0.95 lit/kg, and mean cleaning of 0.22 liters/kg per hour (de Craene et al., 1997).

Table 3. Differential characteristics of *M. haemolytica* and *P. multocida*.

Species	β-hemolysis	Oxidase	Ornithine decarboxylase	Indole	Urease	Growth on MacConkev
Mannheimia haemolytica	+	+	V	+	+	V
Pasteurella multocida	_	+	+	+	+	–

+: 90% or more of the strains are positive within 1-2 days; -: 10% or less of the strains are positive within 14 days; V: 20-80% of the strains are positive**.**

In a clinical study, a single subcutaneous injection of florfenicol (40 mg/kg) was found to be twice as effective as the intramuscular injection of florfenicol (20 mg/kg) at an interval of 48 hours for the treatment of bovine respiratory diseases. In the three countries of Belgium, Spain and France, in a study of 210 calves under six weeks of age, affected by the normal enzootic pneumonia, the bacteria of *Pasteurella multocida*, *Homolytic mannheima*, *Mycoplasma bovis*, and *Haemophilus somnus* were separated. Therapeutic effects of florfenicol were compared by using it together with flunixin meglumine. The results showed a reduction of clinical signs, especially fever, at six hours after the treatment in the combination group of florfenicol and flunixin (Thiry et al., 2014). Therapeutic effects of florfenicol and tulathromycin on the treatment of calves with respiratory infection were compared and no significant difference was found between the two drugs (Jolyce and Janice, 2008). Our findings support the hypothesis that *M. haemolytica* and *P. multocida* are the dominant pathogens in the upper respiratory tract in bovine respiratory disease. The results of this study exhibited the effect of the injectable form of florfenicol 30 µg, produced by Rooyan Darou pharmaceutical company, on the treatment of calves with respiratory infection, which is in line with the results of other studies emphasizing the importance of this antibiotic, which is produced in Iran, as an analog of chloramphenicol with a broader spectrum effect, more stability against enzyme deactivation, and higher effectiveness against several resistant bacterial strains.

Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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