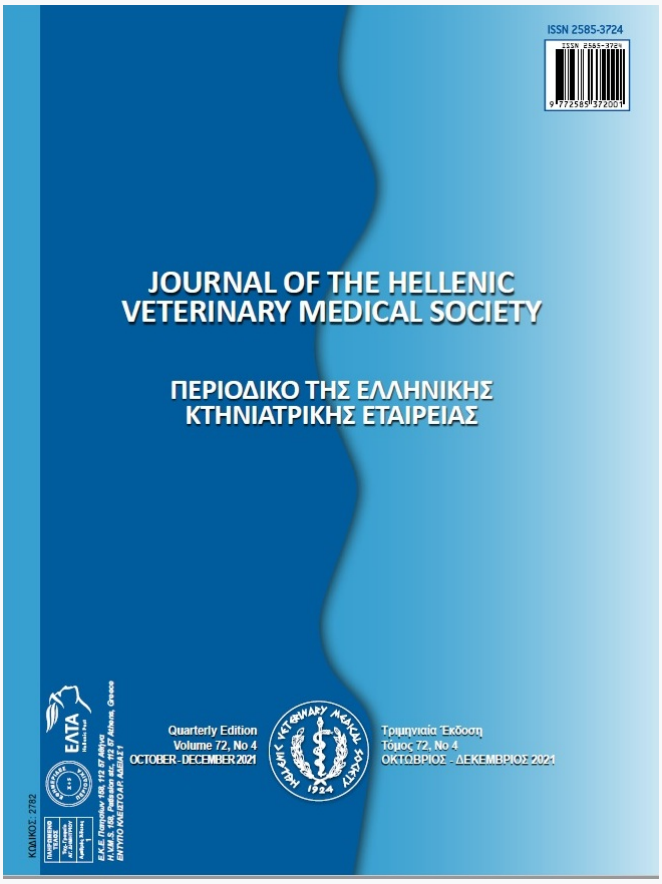
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Clinical and bacteriological analysis of respiratory tract infections in sheltered dogs and determination of antibacterial treatment options

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Research article
Ερευνητικό άρθρο

Clinical and bacteriological analysis of respiratory tract infections in sheltered dogs and determination of antibacterial treatment options*

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ABSTRACT: After canine infectious respiratory disease complex-CIRDC, only bacterial pneumonia or accompanied with CIRDC has a higher proportion in respiratory disease in stray dogs. Management of these respiratory problems in terms of both treatment and prevention in shelters has a big importance for animal welfare. With this purpose, the present study evaluates bacterial pneumonia in terms of clinical, bacteriological and the antibacterial treatment options in 100 sheltered dogs with respiratory tract infection symptoms. In all dogs, status of respiratory disease and treatment efficacy were evaluated by haematological analysis and clinical scores. Haematological analyses showed that all of the dogs suffered leucocytosis before treatment. Health status of all animals before, during and after treatment were evaluated according to nine clinical scores include clinical condition, body temperature, respiratory and heart rates per minutes, nasal discharge, tracheal sensitivity, mucous membranes, coughing, auscultation.For bacteriological analysis and antimicrobial susceptibility tests, Bronchoalveolar lavage-BAL fluids were obtained from all dogs, twice. The bacterial agents isolated in the present study were *Bordetella* spp. (38.98%), *Mycoplasma* spp. (21.19%), *Klebsiella* spp. (16.10%), *E. coli* (5.93%), *S. aureus* (5.08%) and *Pasteurella* spp. (4.24%). Susceptibility tests were performed by using the disc diffusion method for Enrofloxacin (ENR), Trimethoprim/Sulpha (TS), Chloramphenicol (C), Amoxicillin clavulanate (AC), and Erythromycin (E) in all cases. *Bordetella* spp. isolated from 46 cases were found to be most susceptible to ENR (21/46 = 46%), TS (12/46 = 26%), and C (11/46 = 24%). *Mycoplasma* spp. were isolated from 25 cases and were found to be susceptible to C (14/25 = 56%), TS (8/25 = 32%), and ENR (3/25 = 12%). *Klebsiella* spp. were isolated from 19 cases and the antibiotics most effective were ascertained as C (9/19 = 47%), ENR (9/19 = 47%), and TS (1/19 = 5%).The results showed that clinical scores could be useful in the diagnosis and monitoring of respiratory tract diseases in sheltered dogs. Besides, in the light of the findings of presented study, enrofloxacin, chloramphenicol, and trimethoprim/sulpha were proven efficient against to bacterial isolates in sheltered dogs in the treatment of bacterial pneumonia. Antibacterial therapy should be conducted by antibiotic sensitivity test. But, in the cases this is not possible, antibiotic choice may contain enrofloxacin, trimethoprim/sulpha. If it is not forbidden to use for companion animals by administrations, chloramphenicol may also be thought as alternative.

***Keywords*:** Antimicrobial susceptibility, Bacterial pneumonia, Aetiology, Sheltered dog, Treatment

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*The study was summarized from the PhD thesis of the first author.

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INTRODUCTION

One of the most common health problem encountered in dogs which are housed in crowded environments such as shelters and pet shops, is respiratory disease (Kennerman et al., 2000; Maden et al., 2000; Mochizuki et al., 2008; Litster et al., 2011; Ayodhya et al., 2013b). Canine infectious respiratory disease, which is primarily caused by viruses (i.e. distemper virus, canine parainfluenza virus-CPIV) (Mochizuki et al., 2008; Priestnall et al., 2010), can also be of bacterial origin (Kennerman et al., 2000; Ayodhya et al., 2013a). Opportunistic bacteria, including *Bordetella bronchiseptica*, *Mycoplasma* spp., *Klebsiella* spp., *Escherichia coli*, *Pasteurella multocida*, *Streptococcus* spp., *Staphylococcus* spp. and *Pseudomonas* spp., are frequently isolated in cases of bacterial pneumonia (Hawkins, 2005; Gonul et al., 2010; Vieson et al., 2012).

During diagnosis of respiratory diseases, history as well as general and specific physical examinations are used to detect the probable cause and localization of the disease (Peeters et al., 2000; Kuehn, 2005). Bronchoalveolar lavage is the most frequently used for the diagnosis of respiratory diseases (Maden et al., 2001; Silverstein and Drobatz, 2005; Gonul et al., 2010) andis also the safest with the least complications (Maden et al., 2000; Hawkins, 2005; Silverstein and Drobatz, 2005).

The treatment of respiratory diseases requires the control of the infection, the maintenance of the airways in an open state, and the acceleration of the cleaning of the airways (Peeters et al., 2000; Gonul et al., 2010; Vieson et al., 2012). For better efficiency, it is recommended to apply antibacterial treatment based on bacterial culture results (Ettinger and Kantrowitz, 2005; King, 2010a; King, 2010b). In this study, the clinical and bacteriological analysis of bacterial respiratory diseases in sheltered dogs, and the determination of antibacterial treatment options were aimed.

MATERIALS AND METHODS

This study was conducted pursuant to approval numbered with 2011/032 of the Ethics Board of Selçuk University, Faculty of Veterinary Medicine.

Animals

The study materials were 100 dogs in different breeds, age (6 months-7 years) and sex, which were kept at the shelter of the Konya Metropolitan Municipality and showed signs of respiratory disease. The inclusion criteria for dogs showing signs of respiratory disease selection were made on the basis of physical examination, haematology, blood gas analysis and BAL fluid analysis. Dogs with serous eye and nasal discharge and/or sneezing, expectoration, wet/dry cough, signs of abnormal lung auscultation, and general signs of infection such as high fever, weakness, and anorexia were included in the study. Dogs with comorbid diseases other than respiratory tract disease and determined to be treated before were excluded from the study.

All of the dogs with respiratory disease were evaluated according to the results of the treatment as recovered and non-recovered which were defined after 5 days of treatment on the basis of clinical scores and BAL fluid analysis.

Clinical Examination

All of the dogs were physically examined for their general clinical condition, body temperature, heart and respiratory rates, mucous membranes, nasal discharge, tracheal sensitivity, and coughing. Auscultation was also performed. These clinical parameters were scored as shown in Table 1, and were evaluated and recorded on a daily basis throughout the treatment period.

Laboratory Analyses

For to evaluate both the presence of infection in the sick dogs and the state of ventilation of the animals, blood samples were collected from each dog before and after treatment into anticoagulant-coated tubes (BD EDTA K2, BD Diagnostics, USA) for complete blood count (MS™4E, France) and heparin-coated syringe (BD Preset™ Syringe, BD Diagnostics, USA) for venous blood gas analyses (GEM®Premier 3000, USA).

Collection and Examination of Bronchoalveolar Lavage Fluid

For the collection of BAL fluid samples, the animals were anaesthetized with a combination of ketamine hydrochloride (2-4 mg/kg, b.w., i.m., Ketasol® 10%, Interhas, Wels-Austria) and xylazine (1-2 mg/kg, b.w., i.m., Alfazyne® 2%, Ege Vet, Holland). A sterile endotracheal tube, the size of which was selected according to the size of the dog (No: 4.5/7/8 mm, Bıçakçılar®, İstanbul) was inserted into the trachea. Next, a sterile propylene catheter, measuring 2.67 mm x (8 ch) x 500 mm (Feeding tube, Bıçakçılar®,

Table 1. Clinical Scoring

PARAMETER	EVALUATION/SCORES				
	1	2	3	4	5
Clinical Condition	***Normal***	***Mild*** has food and water intake, and environmental relation	***Moderate*** No appetite, has water intake, poor environmental relation	***Severe*** No appetite, water intake too low, depressive	
Mucous Membranes	***Normal***	***Mild*** Less hyperemia	***Moderate*** Diffuse hyperemia	***Severe*** Diffuse dark hyperemia, plumped conjunctival vessels	
Tracheal Sensitivity	***None***	***Available***			
Nasal Discharge	***None***	***Serous***	***Seromucous***	***Mucous***	***Purulent***
Auscultation	***Normal***	***Mild*** Hardened vesicular and bronchial sounds	***Moderate*** Wet rales, crackling and rustling sounds	***Severe*** Dry rales and wheezing / friction sounds	
Cough	***None***	***Mild*** with long intervals	***Moderate*** with short intervals	***Severe*** Continuous	

İstanbul), was passed through the lumen of the endotracheal tube and pushed forward up to the carina region. At this point, 20 cc of saline was injected into the carina and was immediately aspirated. BAL fluid samples were collected twice, before and after treatment.

Microbiological Analyses

The BAL fluid samples taken before treatment were used for the isolation of bacteria and the antimicrobial susceptibility tests. Blood agar, MacConkey agar, Bordet-Gengou agar, Mycoplasma agar and Sabouraud dextrose Agar (SDA) for fungi (OxoidTM, Thermo Fisher Scientific Inc. Basingstoke, England) were used as growth media. The growth media were prepared according to the manufacturers' instructions. After the inoculation of BAL samples, SDA was incubated aerobically at room temperature for 5-7 days while other media except Mycoplasma agar were incubated at 37°C for 24-48 hours. Mycoplasma agar was incubated at 37°C for 24-48 hours in microaerophilic conditions. The colonies that were grown at the end of incubation were identified based on colony morphology, microscopic morphology and biochemical characteristics (Winn et al, 2006). The BAL fluid samples taken after treatment were also inoculated into growth media to check for the presence of bacteria. Biochemical tests were used catalase test, coagulase test, optochin sensitivity test, CAMP test, nitrate test, motility test, haemolysis on blood agar, growing on Mannitol Salt Agar, Bile Esculin Agar for gram positive bacteria and oxidase test, gas production, Methyl Red / Voges-Proskauer (MR/VP) test, urease test, growing on MacConkey agar, Kliger's Iron Agar (KIA), Sulfur Indole motility media for gram negative bacteria. In addition, the germ tube test with human serum performed for the identification of yeasts. Fungi were identified according to microscopic morphology and colony morphology. The antimicrobial susceptibility of the identified bacterial strains was determined using antibiotic discs (OxoidTM, Thermo Fisher Scientific Inc. Basingstoke, England) and the disc diffusion method with Mueller-Hinton agar (OxoidTM, Thermo Fisher Scientific Inc. Basingstoke, England) (Bauer et al, 1966). Results were evaluated according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2015).

Establishment of the Treatment Protocol

The treatment was initiated with an antibacterial drug selected according to the results of the antimicrobial susceptibility test, and a non-steroidal anti-inflammatory drug (NSAID). The antibacterial drug to which the highest susceptibility level was detected was selected for cases, in which a single microorganism was isolated, whilst the antibiotic with the broadest spectrum was selected for cases, in which more than one microorganism was isolated. Depending on the health status of the patient and the course of the disease, supportive medications (i.e. use of expectorants, bronchodilators) were included in the treatment protocol. The appropriate antibacterial drug was selected

according to the BAL fluid culture and antimicrobial susceptibility tests. The dosage and duration for drugs used in the treatment of animals were determined according to manufacturer recommendations described in the drug package insert and administered to rules of shelter management as generally, single-dose use and the period of five days. Ketoconazole was added to the treatment protocol in the cases isolated the fungal agents along with bacteria. The drugs used and their administration doses were determined according to text books (Boothe, 2005; Yazar, 2012; Papich, 2016) and presented in Table 2.

Statistical Analyses

For non-parametric data, Mann-Whitney U test was used and for parametric data, independent *t*-test was used for the evaluation of difference between the groups. The difference between the groups for the treatment results (recovered/non-recovered), the scores of clinical condition, mucous membranes, nasal discharge, tracheal sensitivity, auscultation and cough were analysed with the Mann-Whitney U test (SPSS, 2007). Data related to fever, heart rate and respiration rate were analysed with the independent *t*-test (SPSS, 2007).Within the groups, for the evaluation of difference, daily data collected during the observation of the animals for 5 days was firstly evaluated with the Friedman test. Later, the difference determined by the Friedman test was investigated with Wilcoxon *t-test* for non-parametric data and paired *t-test* (SPSS, 2007) for parametric data. Differences were considered significant when $P<0.05$.

Table 2. Antimicrobial drugs used and their respective dose

ACTIVE INGREDIENT	APPLICATION DOSAGE / WAY
Enrofloxacin (Killoxacin® %5, BaVET, Turkey)	10 mg/kg b.w.,SID, i.m., for five days
Chloramphenicol (Gemysetinsuksinat® 1 gr im/iv lyofilize enjektabl, Deva İlaç, Turkey)	50 mg/kg b.w.,BID, i.m., for five days
Trimethoprim-sulfamethoxazole (Primoksal®, Alke®, Turkey)	25 mg/kg b.w.,SID, i.m., for five days
Amoxicillin-Clavulanic acid (Klavil®, Vilsan, Turkey)	8,75 mg/kg b.w., SID,i.m., for five days
Erythromycin (Apirocin-F®, Teknovet, Turkey)	10 mg/kg b.w.,SID, i.m., for five days

RESULTS

The state of recovery of the sick dogs was assessed on the basis of 9 clinical scores (6 non-parametric and 3 parametric). The clinical scores of the dogs that recovered and did not recover significantly differed for their examination results of general clinical condition ($P<0.05$), the mucous membranes ($P<0.001$) and nasal discharge ($P<0.001$) on the first day of treatment; for pulmonary auscultation findings ($P<0.05$), coughing ($P<0.001$) and respiratory rate ($P<0.05$) on the second day of treatment; for heart rate ($P<0.05$) and tracheal sensitivity ($P<0.001$) on the third day of treatment; and body temperature ($P<0.001$) on the fourth day of treatment (Table 3 and Table 4).

Haematological analyses performed before and after treatment showed that all of the dogs suffered leucocytosis before treatment. While leukocyte counts were observed to normalize in the dogs that recovered upon treatment, the dogs that did not recover continued to suffer from leucocytosis (Table 5). Blood gas analyses demonstrated that pO2 levels, which were low before treatment ($P<0.05$), normalized after treatment in the cases that recovered but remained close to pre-treatment levels in the animals that did not recover (Table 6).

In the microbiological analysis of BAL fluids of all dogs, bacterial agents (n:70), *Aspergillus* spp. (n:3) and in one dog both bacterial agent and *Candida* spp. were isolated (Figure 1).The bacterial agents isolated in the present study were *Bordetella* spp. (38.98%), *Mycoplasma* spp. (21.19%), *Klebsiella* spp. (16.10%), *E. coli* (5.93%), *S. aureus* (5.08%) and *Pasteurella* spp. (4.24%) (Figure 1).

Bordetella spp. isolated from 46 cases were found to be most susceptible to ENR (21/46 = 46%), TS (12/46 = 26%), and C (11/46 = 24%). *Mycoplasma* spp. were isolated from 25 cases and were found to be susceptible to C (14/25 = 56%), TS (8/25 = 32%), and ENR (3/25 = 12%). *Klebsiella* spp. were isolated from 19 cases and the antibiotics they were most susceptible to were ascertained as C (9/19 = 47%), ENR (9/19 = 47%), and TS (1/19 = 5%) (Table7). Treatment with the drugs selected according to the results of the antimicrobial susceptibility tests, and supportive therapy resulted in the clinical recovery of 57 out

of the 74 dogs with bacterial respiratory disease. In ten of these dogs, despite clinical recovery, *Bordetella* spp. and *Klebsiella* spp. were isolated from the BAL fluid samples taken after treatment (Figure 2). The clinical efficacy of drugs used for treatment, on the basis of the antimicrobial susceptibility test results, are given in Table 8. Accordingly, the overall rate of clinical recovery achieved with the treatment of bacterial respiratory disease was determined as 77.02% (74/57), whilst the overall clinical efficacy of drugs used for treatment was ascertained as 63.51% (74/47). Post-treatment evaluation demonstrated that the overall clinical success rates achieved with the drugs used were 76.08% for *Bordetella* spp., 80% for *Mycoplasma* spp. and 73.68% for *Klebsiella* spp. infections.

Table 3. Daily recovery status according to treatment outcomes (non-parametric clinical scores)

			Daily Median of Clinical Scores					
Parameters	Results of treatment	n	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Clinical Status	+	57	3	3	2	2	1	1
	-	17	4	4	4	3	3	3
	P		0.363	0.002	0.001	0.001	0.001	0.001
Mucous Membrane	+	57	4	3	2	2	1	1
	-	17	4	4	4	4	3	3
	P		0.555	0.001	0.001	0.001	0.001	0.001
Tracheal Sensitivity	+	57	2	2	2	1	1	1
	-	17	2	2	2	2	2	2
	P		0.475	0.475	0.866	0.001	0.001	0.001
Nasal discharge	+	57	5	4	4	3	3	2
	-	17	5	5	5	5	5	4
	P		0.105	0.001	0.001	0.001	0.001	0.001
Auscultation	+	57	3	3	2	2	2	2
	-	17	3	3	3	3	3	3
	P		0.886	0.325	0.002	0.001	0.001	0.001
Cough	+	57	3	3	2	2	1	1
	-	17	3	3	3	3	3	3
	P		0.499	0.080	0.001	0.001	0.001	0.001

Clinical condition, mucous membrane, auscultation and cough were scored with in 1 to 4. Nasal discharge was scored with in 1 to 5. And, tracheal sensitivity was scored as none (1) or available (2) (Table 1). +: Recovered, -: Non-recovered, *P*: Significance value of between groups, recovered or non-recovered.

Table 4. Daily recovery status according to treatment outcomes (parametric clinical scores)

			Daily Means of Clinical Scores ($\bar{x}$) ± SEM					
CS	Results of treatment	n	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
T (°C)	+	57	39.177 ± 0.106	39.053 ± 0.084	38.849 ± 0.070	38.667 ± 0.068	38.454 ± 0.047	38.204 ± 0.040
	-	17	39.088 ± 0.166	39.164 ± 0.188	39.000 ± 0.211	38.965 ± 0.191	39.141 ± 0.185	39.159 ± 0.155
	P		0.680	0.545	0.383	0.070	0.001	0.001
PL	+	57	92.684 ± 2.870	94.263 ± 2.324	94.877 ± 1.864	92.386 ± 1,485	93.737 ± 1.288	92.070 ± 0.974
	-	17	97.177 ± 6.515	98.412 ± 5.058	100.235 ± 5.477	100.706 ± 5.156	101.529 ± 4.388	99.706 ± 3.607
	P		0.481	0.416	0.240	0.036	0.023	0.005
R	+	57	26.825 ± 1.345	25.737 ±1.039	25.018 ± 0.919	23.737 ± 0.735	23.807 ± 0.612	22.228 ± 0.524
	-	17	27.118 ± 2.602	28.824 ± 2.456	30.059 ± 2.396	31.118 ± 2.14	32.647 ± 2.174	35.824 ± 2.187
	P		0.918	0.188	0.020	0.001	0.001	0.001

+: Recovered, -: Non-recovered, T: Body temperature, PL: Heart Rate/min, R: Breath/min, $\bar{x}$: Mean, SEM: Standard error of means, *P*: Significance value of between groups, recovered or non-recovered.

Table 5. Comparative haematology analysis before and after treatment

Parameter	Treatment result	n	BEFORE ($\bar{x}$) ± SEM	*P*	AFTER ($\bar{x}$) ± SEM	*P*
WBC (x10³/mm³)	-	17	23.132±1.931	0.912	21.582±0.777	0.001
	+	57	22.874±1.135		14.311±0.432	
RBC (x10⁶/ mm³)	-	17	6.248±0.308	0.935	5.988±0.291	0.019
	+	57	6.220±0.163		6.761±0.154	
HCT (%)	-	17	38.171±1.815	0.794	37.506±1.776	0.046
	+	57	37.619±1.011		41.679±0.991	
HB (g/dl)	-	17	12.914±0.768	0.673	12.865±0.650	0.241
	+	57	12.612±0.315		13.563±0.260	
MCV (fL)	-	17	59.594±1.133	0.963	59.276±0.723	0.003
	+	57	59.509±0.940		62.209±0.489	
MCH (Pg)	-	17	20.182±0.538	0.676	20.612±0.503	0.203
	+	57	20.653±0.588		21.907±0.529	
MCHC (g/dL)	-	17	33.471±0.585	0.613	33.506±0.858	0.251
	+	57	34.386±0.964		35.588±0.945	
RDW (%)	-	17	11.453±0.440	0.132	12.853±0.527	0.004
	+	57	10.775±0.205		11.482±0.203	

-: Non-recovered, +: Recovered, $\bar{x}$: Mean, SEM: Standard error of means, *P*: Significance value of between groups, recovered or non-recovered.

Table 6. Comparative venous blood gas analysis before and after treatment

Parameter	Results of treatment	n	BEFORE ($\bar{x}$) ± SEM	*P*	AFTER ($\bar{x}$) ± SEM	*P*
pH	-	17	7.325±0.014	0.593	7.292±0.010	0.001
	+	57	7.315±0.010		7.363±0.004	
pCO2 (mmHg)	-	17	43.294±1.419	0.178	48.941±1.273	0.001
	+	57	41.088±0.778		38.281±0.520	
pO2 (mmHg)	-	17	40.941±2.240	0.026	52.647±1.768	0.001
	+	57	48.509±1.688		59.000±0.964	
HCO3 (mmol/L)	-	17	22.388±1.180	0.242	19.588±0.985	0.001
	+	57	20.998±0.539		24.058±0.254	
BEecf (mmol/L)	-	17	-3.482±1.346	0.240	-7.412±1.171	0.001
	+	57	-5.153±0.658		-1.118±0.263	

-: Non-recovered, +: Recovered, $\bar{x}$: Mean, SEM: Standard error of means, *P*: Significance value of between groups, recovered or non-recovered.

Table 7. Results of antimicrobial susceptibility tests

Agents	n	TS	ENR	C	E	AC
Mycoplasma spp.	74/25	25/8	25/3	25/14		
Bordetella spp.	74/46	46/12	46/21	46/11	46/1	46/1
Pasteurella spp.	74/5		5/5			
Klebsiella spp.	74/19	19/1	19/9	19/9		
E. coli	74/7		7/3	7/4		
Staphylococcus spp.	74/2		2/1			2/1
Streptococcus spp.	74/2			2/2		
S. aureus	74/6	6/1	6/1	6/1	6/2	6/1
*Staphylococcus*coagulase -	74/1		1/1			
Trueperella pyogenes	74/1					1/1

TS: Trimethoprim sulfamethoxazole, ENR: Enrofloxacin, C: Chloramphenicol, E: Erythromycin, AC: Amoxicillin / clavulanate.

Table 8. Percentage results of clinical efficacy after antimicrobial treatment

	ENR			C			TS			AC			E		
	+	-	%	+	-	%	+	-	%	+	-	%	+	-	%
Mycoplasma spp.	2	1	66.66	12	2	85.71	6	2	75						
Bordotella spp.	13	8	61.90	10	1	90.90	11	1	91.66	0	1	0	1	0	100
Pasteurella spp.	5	0	100												
Klebsiella spp.	6	3	66.66	7	2	77.77	1	0	100						
E. coli	0	3	0	4	0	100									
Staphylococcus spp.	1	0	100							1	0	100			
Streptococcus spp.				2	0	100									
S. aureus	1	0	100	1	0	100	1	0	100	1	0	100	2	0	100
*Staphylococcus*coagulase -	1	0	100												
T. pyogenes										1	0	100			

+: Effective, -: Non-effective, TS: Trimethoprim sulfamethoxazole, ENR: Enrofloxacin, C: Chloramphenicol, E: Erythromycin, AC: Amoxicillin / clavulanate. %: Percentage of clinical efficacy of drugs chosen according to results of antimicrobial susceptibility tests.

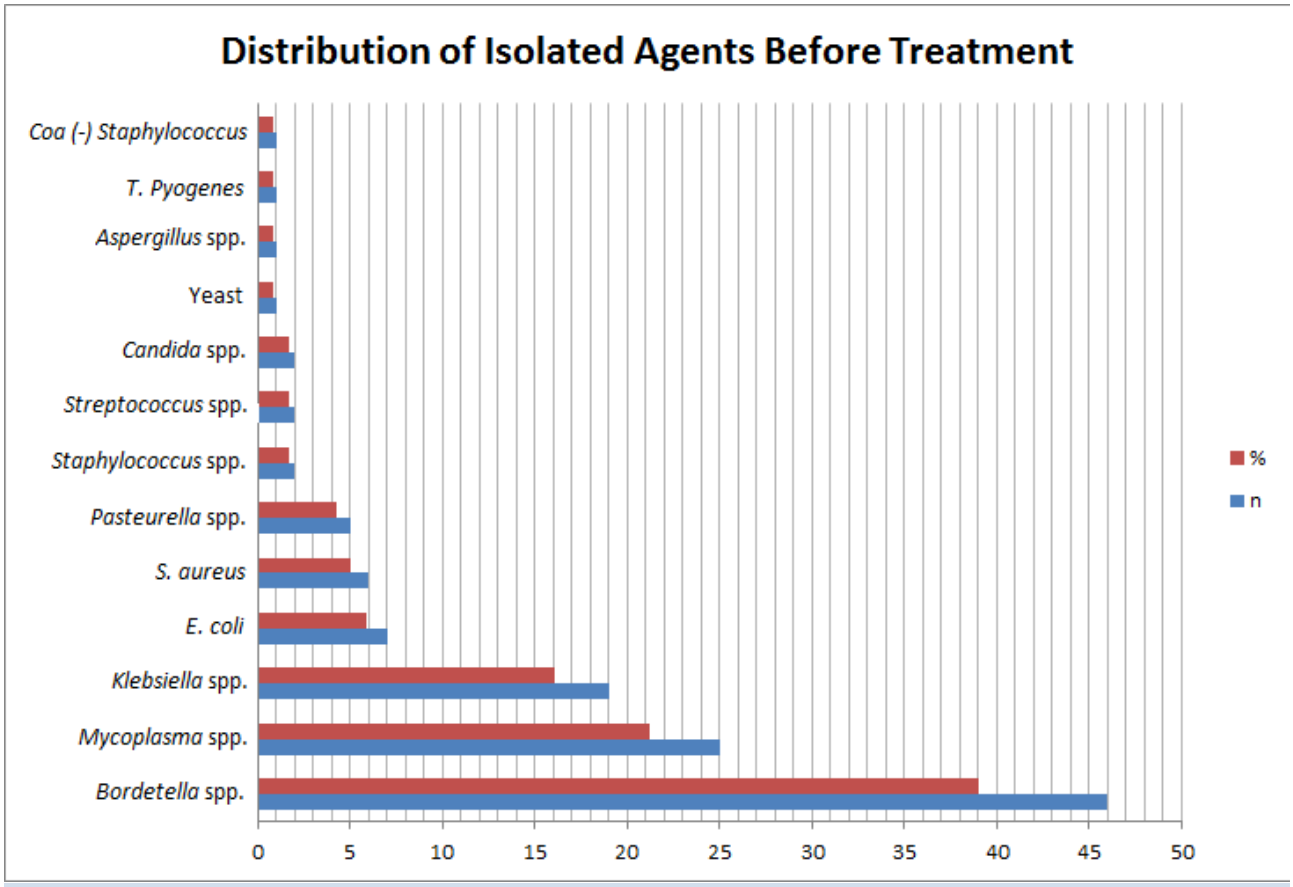


Figure 1.

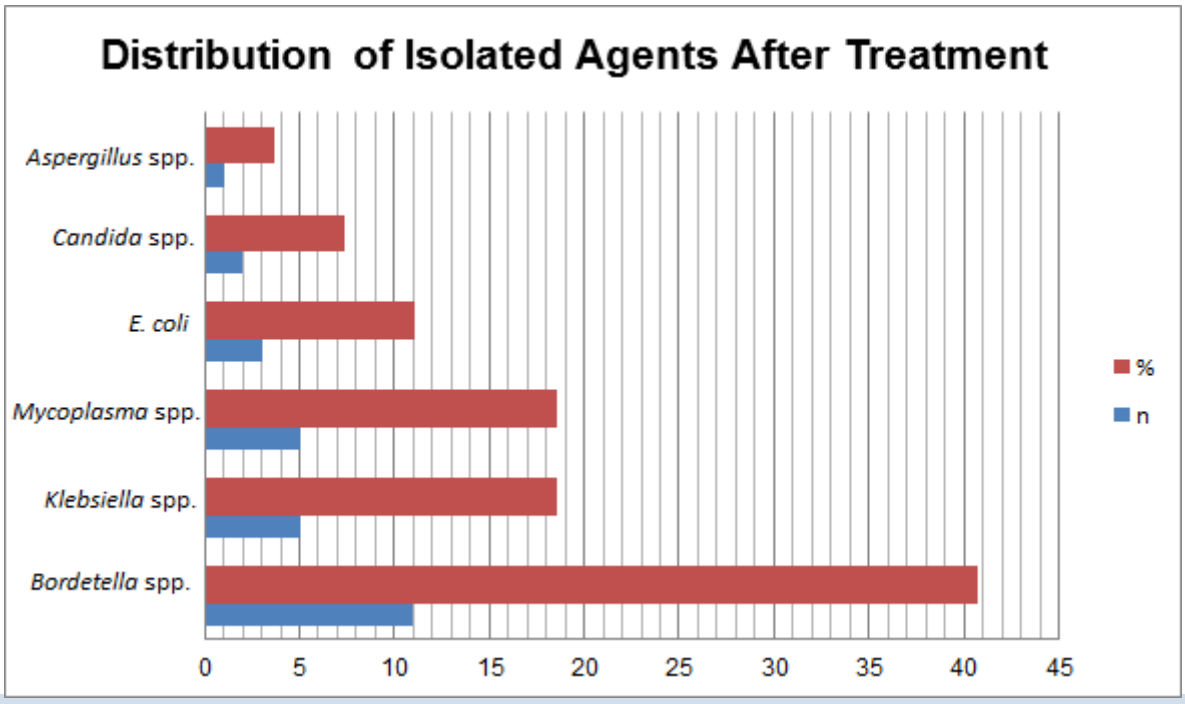


Figure 2.

DISCUSSION

General clinical symptoms such as lethargy and anorexia, which were accompanied with coughing, fever, nasal discharge and tracheal sensitivity, may be seen in the dogs suffering from respiratory disease (Maden et al., 2000; Maden et al., 2001; Chalker et al., 2003a). Clinical scoring for animals in the respiratory infection has been made between 1-5 according to clinical findings such as coughing, nasal discharge, depression and/or anorexia, and symptoms of lower respiratory tract infection (Chalker et al., 2004). In a study, clinical findings were scored based on the severity of respiratory disease as mild (dry cough, serous nasal discharge, normal appetite, no depression), moderate (dry or moist cough, mucous nasal discharge, mild dyspnoea, anorexia, mild fever of 39.05 ± 0.055), and severe (dry or moist cough, mucopurulent nasal discharge, severe dyspnoea, dyspepsia, fever of 40.25 ± 0.056) (Ayodhya et al., 2013a). Also, clinical scoring was made based on the characterization of ocular and nasal discharge, and according to the existence of coughing, sneezing, dyspnea, depression, and body temperature (Jirjis et al. 2010). In our study clinical scores as described before (Table 1) were used for the evaluation of treatment success and monitoring the prognosis of dogs. The results demonstrated that clinical scores contribute to the assessment of treatment and the prognosis in animals suffering from respiratory disease, and suggest that particularly general clinical condition, mucous membrane, nasal discharge, auscultation and body temperature scores, which showed significant differences as from the start of treatment, could be used for the monitoring of dogs with respiratory disease. Also, literature knowledge shows that considering the clinical score assessments in this area, there are differences between the studies (Chalker et al., 2003a; Chalker et al., 2003b; Jirjis et al. 2010; Weiser, 2012; Ayodhya et al., 2013a).

A complete blood count is a useful diagnostic tool that is considered not specific, they point out the existence of inflammation for animals showing signs of respiratory disease (Priestnall and Erles, 2011; Weiser, 2012). The cases of bacterial pneumonia are characterized by inflammatory leucogram results (Dear, 2014). In previous researches carried out in dogs with respiratory infection, total leucocyte numbers were observed to increase in parallel with the severity of the disease (Maden et al., 2000; Zeugswetter et al., 2007; Ayodhya et al., 2013b), high WBC counts were determined to suggest active inflammation (Maden *et al.*, 2000) and increased leucocyte concentrations were considered to be associated with the bacterial respiratory infection (Priestnall and Erles, 2011; Ayodhya *et al.*, 2013b). In the present study, on the basis of the haematological data of the dogs diagnosed with respiratory disease, the presence of leucocytosis

was interpreted as an indicator of active inflammation and bacterial infection. The decrease observed in this parameter after treatment in the dogs that recovered demonstrated the success of the treatment applied. The erythrocyte profile determined in the present study was found to fall within the reference range (Rizzi et al., 2010; Khan et al., 2011), and similar findings have been reported in previous research on respiratory diseases (Maden et al., 2000; Ayodhya et al., 2013b). Blood gas analyses not only aid in determining the severity and prognosis of the disease, but also provide data on the first interventions that need to be made to the patient (serum therapy, oxygenation, electrolyte administration etc.) (Irizarry and Reiss, 2009; Gonzalez and Waddell, 2016). Venous blood gases provide data on the acid-base state and ventilation (Irizarry and Reiss, 2009; Waddell, 2013). With insight of the blood gas analysis results of the study presented, the alleviation or elimination of the respiratory inflammatory disorder with treatment was considered to be an indicator of the improvement of ventilation in the dogs that recovered. Thus, it is suggested that the venous blood gas analyses can be used for respiratory diseases.

Reports indicate that several infectious agents (viral, bacterial, parasitic, fungal, etc.) are involved in canine lower respiratory tract diseases (Kennerman et al., 2000; Vieson et al., 2012; Ayodhya et al., 2013a), and most of these diseases are reported to be of bacterial origin (Kennerman et al., 2000; Peeters et al., 2000; Ayodhya et al., 2013a; Ayodhya et al., 2013b; Lavan and Knesl, 2015). Johnson et al. (2013)reported that the agents most frequently isolated from dogs with lower respiratory tract infection were *Mycoplasma* spp. (30%), *B. bronchiseptica* (22%), *Pasteurella* spp. (21%) and 20% *Enterobacteriaceae* (*E. coli* 17%, *K. pneumoniae* 2%, *Proteus* spp. 2%). Battersby (2014) indicated that *B. bronchiseptica*, *Mycoplasma* spp. and *Streptococcus* spp. are frequently isolated from cases of infectious tracheobronchitis. Meyer and Rawton (2010) reported beta-haemolytic streptococci, *S. intermedius*, and *Klebsiella* spp. to be commonly isolated from sick dogs. In another study carried out in dogs with respiratory disease, the most common infectious agents were determined as *Pasteurella* spp. (25%), *B. bronchiseptica* (11%), *E. coli* (11%), and *K. pneumoniae* (4%) (Epstein *et al.*, 2010). In their study on sheltered dogs with respiratory disease, Chalker et al. (2003b) reported to have isolated *B. bronchiseptica* from the post-mortem pulmonary fluid at a rate of 47%, and indicated that this agent was isolated at a rate of 39% from clinically healthy sheltered dogs. In another study conducted by the same researchers in sheltered dogs with respiratory disease (Chalker *et al.*, 2004), *M. cynos* was isolated at rates of 9.7% and 23.9% from the tracheal wash fluid of healthy and sick dogs, respectively, and at rates of 9.7% and 21.7% from the bronchial lavage samples of healthy and sick dogs, respectively. Thus, these researchers suggested that this species could be involved in the aetiology of canine respiratory diseases. Maden et al. (2000) reported to have isolated *B. bronchiseptica* and *E. coli* (24%), *Pasteurella* spp., coagulase (+) *Staphylococcus* spp. and *Corynebacterium* spp. (12%); *Mannheimia haemolytica* and *Enterobacter* spp. (8%), and *S. aureus*, *Streptococcus* spp., *Pseudomonas* spp., *Bacillus* spp., *Proteus* spp. and *Klebsiella* spp. (4%) from BAL fluid cultures. Durgut et al. (2003) indicated that the infectious agents isolated from transtracheal aspiration and pharyngeal swab samples were *Pasteurella* spp. (16/54, 29.62%), *K. pneumoniae* (12/54, 22.22%), *E. coli* (8/54, 14.81%), beta-haemolytic streptococci (7/54, 12.96%), enteric bacteria (6/54, 11.11%), and coagulase-positive staphylococci (5/54, 9.25%). In their study in dogs with lower respiratory tract disease, Gonul et al. (2010) isolated *E. coli* (4/30, 13.33%), *Staphylococcus epidermidis*, *K. pneumoniae*, *Mycoplasma* spp. and *Enterobacter cloacae* (2/30, 6.66%) from BAL fluid samples. On the basis of data obtained in the present study and previous research, the most common bacteria isolated from sheltered dogs with respiratory disease are *B. Bronchiseptica* (Maden et al., 2000; Epstein et al., 2010; Johnson et al., 2013), *Mycoplasma* spp. (Chalker et al., 2004; Sumner et al., 2011; Johnson et al., 2013) and *E. coli* (Epstein et al., 2010; Sumner et al., 2011; Ayodhya et al., 2013a). In the present study, the three most common bacteria isolated from sheltered dogs with respiratory disease both before and after treatment were *Bordetella spp., Mycoplasma spp*., and *Klebsiella spp*. (Figures 1, 2).

The comparison of the results of the present study and previous research demonstrate that while the first two most common bacteria isolated by Johnson et al. (2013) are the same, the third most common bacteria differs in some studies (Epstein et al., 2010; Ayodhya et al., 2013a). While Sumner et al. (2011) identified other agents as the first two most common bacteria, the third most common bacteria they isolated (*Klebsiella* spp., 20%) is in agreement with the results of the present study (16.10%). The prevalence determined for *Bordetella* spp. in the present study (38.98%) is

close to that reported in Chalker et al. (2003b) (47%) and higher than Johnson et al. (2013) (22%). The prevalence determined for *Mycoplasma* spp. in the present study (21.19%) is close to (21.7%) (Chalker et al., 2003b) and lower than (30%) (Johnson et al., 2013). The results obtained in the present study for the isolation of *Klebsiella* spp. (16.10%) are close to (20%) (Sumner et al., 2011) and higher than (2%) (Johnson et al., 2013). As regards *E. coli*, the prevalence detected in the present study (5.93%) is lower than the isolation rates previously reported, such as (75%) (Sumner et al., 2011) and (17%) (Johnson et al., 2013). Rycroft et al. (2007) isolated *Mycoplasma* spp. at a rate higher (46%) than that detected in the present study (21.19%). The results of the previous research referred to above and the present study demonstrate that bacterial agents and their prevalence vary in different regions. It should also be taken into consideration that the prevalence of respiratory diseases and the bacterial species involved in the aetiology of these diseases may be influenced by environmental factors and living conditions.

It is emphasized that the selection of the treatment method for bacterial infections should be based on causative agent isolation and antimicrobial susceptibility test results using diagnostic samples (Vieson et al., 2012). It is suggested that, depending on in vivo factors (host, causative agent and pharmaceutical), the clinical efficacy of antibiotics may differ from in vitro susceptibility test results (Carbone et al., 2001). However it was stated that empiric antimicrobial therapy should be based on the most likely agent to be present. Broad-spectrum antimicrobials are more suitable for empirical use if a bacterial infection is doubtful of to be secondary to an underlying viral infection (Reagan and Sykes, 2020). Reports indicate that the options for the empirical treatment of infectious tracheobronchitis could be fluoroquinolones, chloramphenicol (Murphy *et al.*, 2012), trimethoprim/sulphonamides and tetracyclines (Battersby, 2014). In a previous study carried out with *B. bronchiseptica* cultures, susceptibility levels to several antibiotics including enrofloxacin (100%), sulfadiazine (81%), and trimethoprim (73%) were detected (Speakman *et al.*, 2000). According to the results of antimicrobial susceptibility tests, *Klebsiella* spp. are reported to be susceptible to cefotaxime (100%), and enrofloxacin (90%) (Kruth, 2006). Grobbel et al. (2007) determined that *Klebsiella* spp. were resistant to ampicillin (53-67%), sulfamethoxazole (19-29%), trimethoprim/sulfamethoxazole (19-24%), and enrofloxacin (29%). Durgut *et al.* (2003) ascertained that cultures of transtracheal aspiration and pharyngeal swab samples were susceptible to amikacin, gentamycin, amoxicillin/clavulanic acid, chloramphenicol, tetracycline and ticarcillin/clavulanic acid. Johnson et al. (2013) detected that most of the *B. bronchiseptica* strains they isolated were resistant to some antimicrobial agents (such as cefazolin, ceftiofur, ceftizoxime, marbofloxacin) and thus, indicated that a specific recommendation for the treatment of cases confirmed or suspected to be caused by *Bordetella* strains could not be made. In the same study, *Bordetella* strains were found to be susceptible to chloramphenicol (>90%), enrofloxacin (<70%), and trimethoprim/sulfamethoxazole (<55%). *In vitro* antibacterial efficacy against *Bordetella* spp. has been reported as 48-100% for ENR (Speakman et al., 2000; Carbone et al., 2001; Epstein et al. 2010; Johnson et al., 2013), 16-100% for TS (Speakman et al., 2000; Johnson et al., 2013), and 57-95% for C(Epstein et al. 2010; Johnson et al., 2013). In vitro antibacterial efficacy against *Klebsiella* spp. has been reported as 48-90% for ENR (Kruth, 2006; Epstein et al. 2010; Johnson et al., 2013), 57-90% for C (Epstein et al., 2010; Johnson et al., 2013), and 76-100% for TS (Grobbel et al., 2007; Johnson et al., 2013). The in vitro antibacterial efficacy of ENR and C against *Mycoplasma* spp. has been reported as 47.05% and 5%, respectively (Chandler and Lappin, 2002). In view of the results of previous field studies and the present study, it is obvious that over time, bacteria develop resistance to antibiotics under the influence of various factors, including among others environmental factors, geographical conditions, the immunological status of the host, the virulence of bacterial agents (cell wall structure, ability to form biofilms etc.), and erroneous dosage.

This study has some limitations such as inability to evaluate the presence of viral agents in the aetiology of the canine respiratory disease complex, not performing radiographic examinations in the shelter conditions, and not knowing the detailed disease history in stray dogs. The findings and treatment results of our study, only bacterial and fungal agents of respiratory tract diseases in shelter dogs are related and have been made according to limited shelter conditions. Although the results of this study provide useful information for veterinary medicine practice on the aetiology and the treatment options in shelter dogs with respiratory disease, it was also evaluated that detailed studies including viral agents, supportive treatments, dosage regimen, and treatment period are needed.

CONCLUSION

In conclusion, it was determined that in sheltered dogs, bacterial lower respiratory tract infections are mostly caused by *Bordetella* spp., *Mycoplasma* spp. and *Klebsiella* spp. in this study. The drug selection and the treatment should be made on the basis of BAL fluid bacterial culture and antimicrobial susceptibility tests (ENR, TS and C against *Bordetella* spp.; C, TS, and ENR against *Mycoplasma* spp.; C, ENR, and TS against *Klebsiella* spp.). It is suggested that in the event of emergency cases and outbreaks of respiratory diseases until the antimicrobial susceptibility tests are completed, by taking into consideration etiological agents, the treatment protocol can be arranged with these antimicrobial drugs.

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CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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