



## RESEARCH ARTICLE

# Response to doxycycline and oxytetracycline treatments in cats infected with *Mycoplasma* spp. and analysis of haemato-clinical findings and risk factors

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### ABSTRACT

This study was planned to determine the efficacy of doxycycline and oxytetracycline in the treatment of feline *Mycoplasma* spp. infections and to analyze risk factors as well as clinical and haematological findings. Cats accepted to the clinic with complaints such as weakness, loss of appetite and fever were routinely examined. Clinical history of animal was demanded from the owner. An interview and a short questionnaire were conducted to owner to evaluate the risk factors associated with possible *Mycoplasma* status of animal. During the interview, the age, sex, breed, housing, flea infestation, worm control, external parasite control, other accompanying infections and vaccination status of cats were asked and the data recorded. Peripheral blood smears were stained with the DIFF-QUICK. Fifty positive cats were included in the study, 16 of which were treated with doxycycline (orally, 5 mg/kg twice a day) while other 34 with oxytetracycline (intramuscular injection, 10 mg/kg once a day). It was determined that all (100%) cats with *Mycoplasma* spp. infection had a flea infestation. Majority of cats (94%) did not receive the regular antiparasitic application and only 6 (12%) of them had complete vaccinations. *Mycoplasma* spp. infection was associated with younger age and outdoor access. Among the feline breed, striped cat (74%) was more prone to infection. The most common clinical signs encountered in cats were weakness (94%) and anorexia (84%). Haematological parameters revealed thrombocytopenia (86%), and anaemia (40%) in infected cats. At the end of the treatment, recovery rates for doxycycline and oxytetracycline groups were 87.5% and 67.6%, respectively. General status of these cats indicated significant improvement on the 4<sup>th</sup> day of treatment, and clinical symptoms are restored on the 7<sup>th</sup> day. Mycoplasmosis is an important infection for cats having anaemia, thrombocytopenia and leukocytosis. Flea infestations are risk factor for contamination of the disease. Based on our results, we recommend doxycycline treatment (5 mg/kg) for up to 21 days for elimination of *Mycoplasma* spp. in the blood of infected cats.

**Keywords:** Cats; *Mycoplasma* spp; findings; risk factors; treatment.

### INTRODUCTION

Feline *Mycoplasma* are small, gram-negative and wall-less epicellular erythrocytic bacteria. These agents are called hemotropic *Mycoplasmas* and can cause feline infectious anaemia, in which the main clinical manifestation is anaemia (Barker & Tasker, 2013; Rosenqvist *et al.*, 2016; Munhoz *et al.*, 2018). The feline *Mycoplasma* group consists of four distinct species; *Mycoplasma haemofelis* (Mhf), *Candidatus Mycoplasma haemominutum* (CMhm) and *Candidatus Mycoplasma turicensis* (CMt) (Foley & Pedersen, 2001; Willi *et al.*, 2005; Rosenqvist *et al.*, 2016) and as reported recently *Candidatus Mycoplasma haematoparvum*-like (CMhp) (Vergara *et al.*, 2016). Infections with these four species differ in their pathogenicity

with Mhf causing severe symptoms such as anaemia, depression and fever (Rosenqvist *et al.*, 2016; Vergara *et al.*, 2016). Mhf leads to severe macrocytic normochromic anaemia and induces acute hemolysis associated with anorexia, lethargy and death (Foley *et al.*, 1998; Westfall *et al.*, 2001). Especially in the acute phase of Mhf infections, massive bacteriemia within the erythrocytes causes severe haemolytic anaemia (Munhoz *et al.*, 2018). Infections with the other *Mycoplasma* species may cause a drop in erythrocyte parameters, but these cats do not usually become clinically anaemic unless their health is compromised or they are immunosuppressed (Tasker *et al.*, 2009; Vergara *et al.*, 2016).

There are still many unknown points about how feline hemoplasmas are transmitted. Although the transmission

mechanism is not yet fully understood, direct transmission through aggressive interactions or blood transfusion has also been hypothesized as potential infection sources (Díaz-Regañón *et al.*, 2018; Munhoz *et al.*, 2018). Under experimental conditions, cat hemoplasmas have been transmitted by infected blood subcutaneously, intravenously, or intraperitoneally (Museux *et al.*, 2009). In the natural environment, transmission between cats is mainly through blood-sucking arthropods. It is likely that the primary vectors are hematophagous arthropods, such as ticks and fleas (Munhoz *et al.*, 2018). As a result of grooming behavior, the cats may be exposed to ticks, fleas and other arthropods, increasing the likelihood of vector-borne infections.

In addition to other transmission routes, bite abscesses is a risk factor for *Mycoplasma* infection (Grindem *et al.*, 1990). Detection of CMt and CMhm in the saliva of cats suggests that *Mycoplasma* infection might be transmitted via the saliva during cat bites (Willi *et al.*, 2006; Vergara *et al.*, 2016).

Feline *Mycoplasmas* are widely distributed in pet cats throughout the world, however, the prevalence varies geographically. These variations may be linked to climate differences since close correlation has been evidenced for the prevalence of *Mycoplasma* in warmer climates. This higher prevalence is suggested to be due to the presence of a higher number of blood-sucking arthropods, which are suspected to be vectors for *Mycoplasmas* (Lobetti & Tasker, 2004; Rosenqvist *et al.*, 2016). Arthropod infestation rates are highly variable from year to year and this depends on location, whether animals are in rural or urban areas, lifestyle, for example, outdoor access, whether they live in multi-pet households, and the frequency of effective insecticidal treatments. Meanwhile, increasing levels of pet ownership and growing urbanization, along with factors such as climate change and globalisation, may, over time, affect the endemicity and prevalence of insect vectors and change the dynamics of pathogen transmission (Abdullah *et al.*, 2019).

Multiplication of the microorganisms can be associated with a drastic decrease in haematocrit level thus, cats need antibiotic treatment to overcome the hemolytic crisis (Willi *et al.*, 2010; Baumann *et al.*, 2013). Effective antibiotic treatment is, therefore, of great clinical importance. On the other hand, effective treatment of *Mycoplasma* infection is particularly important in immunocompromised cats, blood donors, and animals in multicat environments with *Mycoplasma* naïve cats (Novacco *et al.*, 2018). Tetracyclines, particularly doxycycline, have been commonly used to treat feline *Mycoplasma* infection, and enrofloxacin has been proposed as an alternative treatment (Tasker *et al.*, 2004).

Antibiotic treatments have been shown to reduce blood organism loads; however, these are not effective to consistently eliminate microorganisms and may cause improved hematological abnormalities (Tasker *et al.*, 2006; Willi *et al.*, 2007; Novacco *et al.*, 2018).

Turkey's climate is similar to the typical temperate climate of some other Mediterranean countries. This, combined with the geographical location and other factors, favors the development of many arthropod vectors, including ticks, fleas, and mosquitoes. Therefore, the probability of *Mycoplasma* infection in cats in Turkey would be very high. However, there are very limited studies conducted in Turkey regarding to *Mycoplasma* infection in cats (Akkan *et al.*, 2005; Ural *et al.*, 2009; Atalay *et al.*, 2015; Cetinkaya *et al.*, 2016), and most of them are case report (Kurtdede & Ural, 2004; Ural *et al.*, 2008; Aslan *et al.*, 2010; Aslan *et al.*, 2015).

Cetinkaya *et al.* (2016) studied the prevalence of *Mycoplasma* infection in blood samples of 384 cats in İstanbul by polymerase chain reaction (PCR). The authors found that

19.3% of cats were positive for one of *Mycoplasma* species, and the total prevalence of *Mhf*, *CMhm*, and *CMt* infections was 9.9%, 17.7%, and 0.8%, respectively. In another study performed in Kayseri province of Turkey, the prevalence as examined by PCR was 9.52% in blood samples of 84 cats (Atalay *et al.*, 2015).

To our knowledge, there is no detailed study concerning the risk factors and hematological findings in *Mycoplasma* infections in cats in Turkey. Moreover, until now, no treatment studies have been performed for *Mycoplasma* comprising the large number of cats. As well, no long-term follow-up study related to natural infections has been published.

In this study, we aimed to develop and evaluate an antibiotic treatment protocol using doxycycline and oxytetracycline in cats infected with *Mycoplasma* spp. Additionally, we also purposed to (i) evaluate hematological parameters, (ii) analyze clinical findings (iii) determine risk factors in cats infected with *Mycoplasma*.

## MATERIALS AND METHODS

### *Inclusion of cat in the study, clinical examination, and determination of risk factors*

The study was conducted between October 2017 and October 2018 in a veterinary clinic in the Bayrampaşa district of İstanbul/Turkey. A total of 150 suspected cats were examined for *Mycoplasma* spp. and 50 positive cats were enrolled by taking into account their clinical and microscopic examination status rather than sex, breed, or age features.

In the routine clinical examination, cats having complaints such as weakness, loss of appetite, and fever were considered, their clinical history was obtained from the owner. Besides, to evaluate risk factors associated with *Mycoplasma* positivity, a semi-structured interview was conducted for each sampled cat's owner. The data recorded included: age, sex, breed, housing, presence of fleas, worm and external parasite control, vaccination to other diseases, and concomitant infections.

### *Blood sampling and haematology*

Five ml blood samples were collected aseptically in EDTA containing vacutainer tubes by cephalic venipuncture. Haematological examination such as red blood cell (RBC), white blood cell (WBC), platelets (PLT) counts; hemoglobin concentration (HB); packed red cell volume (PCV); mean corpuscular volume (MCV); mean corpuscular hemoglobin concentration (MCHC) and others were performed using the Abacus Junior Vet haematology cell counter (Diatron MI Ltd, Hungary). Possible effects of infections on blood parameters were examined before the treatment. Cats with hematocrit value (HCT) below 30% were evaluated as anemic.

### *Preparation and examination of blood smear*

Another part of EDTA-treated samples was then used for the preparation of blood smear. Thin blood smears were prepared and stained with DIFF-QUICK. After staining, blood smears were examined using light microscopy (BX51, Olympus, Tokyo, Japan) under 1,000 X magnification.

### *Treatments*

Cats in group Doxy were treated twice a day by oral (every 12 h) doxycycline at a dose rate of 5 mg/kg (100 mg in each capsule). Cats in the Oxy group, oxytetracycline was administered intramuscularly (i.m.) at a dose rate of 10 mg/kg per day (30 mg/ml). In both groups, treatment was lasted on 21<sup>st</sup> day. In addition, methylprednisolone (40 mg 1 ampoule) was administered at a dose rate of 2 mg/kg (per

12 h, i.m.) as a supportive treatment. Furthermore, 100 ml isotonic sodium chloride (0.9%) (i.v.), 50 ml 5% dextrose ringer lactate (i.v.), 50 mg/kg dose of iron and vitamin B complex (Multivitamin mineral combination) were given once a day.

#### Follow-up of recovery

On the 8<sup>th</sup> day of the treatment, blood parameters were monitored, and the presence of *Mycoplasmas* spp. was investigated. At the end of the treatment period (on the 22<sup>nd</sup> day), those cats which were carried to clinics were examined clinically for their recovering status. Five ml blood samples were collected, smears were prepared, and *Mycoplasmas* spp. were monitored under a microscope. The owners who did not carry their cats to clinics were contacted by phone and requested about the course of the disease and status of animals.

#### Statistical analysis

Descriptive statistics were used to report the results of haematology and variable occurrences. For this purpose, data were analyzed using the Minitab 17 statistical Package.

## RESULTS

A total of 50 cats infected with *Mycoplasma* spp. were examined for clinical findings and haematological parameters. Moreover, different risk factors such as age, breed, sex, ectoparasite control, and any others were evaluated. Most importantly, the effectiveness of doxycycline and oxytetracycline in the treatment has been investigated.

Analysis of risk factors in cats infected with *Mycoplasma* spp. is shown in Table 1. This study revealed that the most commonly infected cat breed is the striped cat (74%, n:37) while the least infected breeds are Ankara (2%, n:1) and Iranian cats (2%, n:1). Among those cats, 27 (54%) were female, and 23 (46%) were below two years of age. As can be seen in Table 1, only three of the 50 cats were treated against internal parasites and ectoparasites, while the remaining 94% did not receive any treatment. Similarly, 88% of cats have never been vaccinated against other infections. Fourteen (28%) of the cats were living in garden conditions, while 16 (32%) of the cats were living in the house and only roaming outside for exercise. Flea infestation was detected in all cats that were clinically examined (100%).

Apparent clinical signs among 50 *Mycoplasma* spp. infected cats were presented in Table 2. All of the cats were referred to the clinic with at least one of the following clinical signs; vomiting, anorexia, dehydration, respiratory disorder, and neurological symptoms. The rectal temperature ranged from 33-40.7°C; thirty cats exhibited increased fever (above 39.6°C) and six cat hypothermia (33-37.8°C). The most commonly observed clinical signs were weakness (94%), anorexia (82%), fever (60%), respiratory disorders (28%), pale mucous membranes (26%), and dehydration (24%). Anaemia (46%) and thrombocytopenia (86%) were the most commonly detected haematological findings. In addition, leucocytosis was observed in 15 cats (30%), while leukopenia and pancytopenia were determined only in 9 (18%) and 5 (10%) cats, respectively.

Hemogram parameters of cats infected with *Mycoplasma* spp. prior to treatment are presented in Table 3. The CBC (Complete Blood Count) showed that MPV (Mean Platelet Volume), HCT (Haematocrit), PCT (Platelet Crit), and MCH (Mean Cell Haemoglobin) were lower compared to the normal reference interval. HCT ranged from 7.36 (%) to 55.80 (%) (mean: 27.17±1.62) on initial presentation. On the other hand, WBC

**Table 1.** Analysis of risk factors in *Mycoplasma* spp. positive cats

Risk factor	<i>Mycoplasma</i> spp. positive (n:50)	
	n	%
<b>Sex</b>		
Female	27	54
Male	23	46
<b>Age (year)</b>		
1-2	23	46
2.5-5	19	38
6-10	8	16
<b>Breed</b>		
Ankara	1	2
Iranian	1	2
Yellow cat	2	4
Scottish	2	4
Siamese	2	4
Striped cat	37	74
Three color	2	4
Crossbred	3	6
<b>Vaccination (against any disease)</b>		
Available	6	12
Not available	44	88
<b>Internal parasite control</b>		
Regularly applied	3	6
Not applied regularly	47	94
<b>Ectoparasite control</b>		
Regularly applied	3	6
Not applied regularly	47	94
<b>Life style</b>		
Home	16	32
Outdoor access	14	28
Home + Outdoor access	20	40
<b>Flea infestation</b>		
Infested	50	100
Not infested	0	0

**Table 2.** Clinical findings and hematological parameters in *Mycoplasma* spp. positive cats

	<i>Mycoplasma</i> spp. positive (n:50)	
	n	%
<b>Clinical findings</b>		
Hyperthermia	30	60
Hypothermia	6	12
Pale mucose membranes	13	26
Anorexia	41	82
Dehydration	12	24
Diarrhoea	9	18
Vomiting	12	24
Weakness	47	94
Respiratory disorders	14	28
Neurological symptoms	3	6
<b>Haematological parameters</b>		
Leucocytosis (WBC > 19.5 x10 <sup>9</sup> /L)	15	30
Leukopenia (WBC < 5.5 x10 <sup>9</sup> /L)	9	18
Pancytopenia (The Lowest value of leucocytes, erythrocytes and thrombocytes are based on)	5	10
Thrombocytopenia (PLT: < 150 x10 <sup>9</sup> /L)	43	86
Anaemia (RBC: < 6 x10 <sup>12</sup> /L)	20	40

**Table 3.** Hemogram values before and after the treatment in *Mycoplasma* spp. positive cats with normal reference values

Parameter	Before treatment						After treatment	Normal reference values
	Mean±SEM	Min.	Max.	Q1	Median	Q3	Mean±SEM	
WBC (x10 <sup>9</sup> /L)	16.47±1.82	0.49	49	7.05	13.20	22.48	11.35±0.95	5.50-19.5
LYM (x10 <sup>9</sup> /L)	3.79±0.56	0.36	21.1	1.09	2.25	4.93	4.34±0.25	1.50-7
MONO (x10 <sup>9</sup> /L)	1.01±0.16	0.03	3.89	0.17	0.49	1.53	0.79±0.09	1-4
GRA (x10 <sup>9</sup> /L)	11.61±1.53	0.06	35.50	2.96	8.88	15.80	6.31±2.03	2.50-14
RBC (x10 <sup>12</sup> /L)	6.55±0.42	1.37	12.70	4.01	5.96	8.95	6.78±0.50	6-10
HGB (g/dL)	8.53±0.53	2.40	17.80	5.60	8.10	11.52	11.2±1.30	9.5-15
PLT (x10 <sup>9</sup> /L)	160.90±19.30	6	531	50.8	131.5	224.80	217.5±90.50	150-600
PCT (%)	0.24±0.03	0.01	1.25	0.07	0.15	0.34	0.40±0.06	0.9-7
MPV (fL)	11.97±0.29	7.20	17.60	10.37	11.8	13.17	17.45±2.45	12.0-17
HCT (%)	27.17±1.62	7.36	55.80	18.33	25.95	36.13	37.75±2.65	29.0-45
MCV (fL)	43.08±1.41	32	102	39	41	44.25	61.50±0.50	41-55
MCH (pg)	13.17±0.24	9.80	17.60	11.95	12.80	17.60	18.0±0.70	13.3-17.5
MCHC (g/dL)	31.23±0.47	13.50	38.30	29.77	31.75	32.92	29.40±1.40	31-36

(White Blood Cell), LYM (Lymphocytes), GRA (Granulocytes), and MCV (Mean Cell Volume) values were found within the normal reference range. Although MONO (Monocytes), RBC (Red Blood Cells), PLT (Platelets), and MCHC (Mean Cell Haemoglobin Concentration) values were within the normal range, they were at the lowest limit. Light microscopy images of blood smear showed coccoid-shaped small basophilic epierythrocytic structures around erythrocytes, individually attached and often more than one per erythrocyte or freely on smears (Figure 1), anisocytosis, polychromasia and regeneration with reticulocytes and metarubricytes. The smear also contained lymphoblasts.

Over this twenty-one-day antibiotic and supportive treatment period, 13 cats (26%) died. 9 (69.2%) of them were accompanied by viral, bacterial, or metabolic disease secondary to *Mycoplasma* spp. infections. 14 (87.5%) doxycycline-treated and 23 (67.6%) oxytetracycline treated cats were recovered. Animals' general conditions progressed after the 4<sup>th</sup> day, the clinical symptoms improved after the 7<sup>th</sup> day. Clinically complete recovery was observed on the 21<sup>st</sup> day of treatment.

On the other hand, microscopic examinations performed on the 8<sup>th</sup> day of the treatment revealed that the number of intraerythrocytic inclusions decreased but did not disappear completely. A mild lymphocytosis was noticed on the blood smear. Three weeks after treatment (on the 22<sup>nd</sup> day), the cat appeared in excellent health, appetite was normal, and bacteriemia was negative by blood smear microscopic examination (Figure 2).

WBC (mean 16.47 ± 1.82 x 10<sup>9</sup> / L) values which were very high at the beginning of the infection decreased to normal levels (mean 11.20 ± 1.30 x 10<sup>9</sup> / L) on the 8<sup>th</sup> day of infection and PLT which was very low at the beginning (mean 160.90 ± 19.30 x 10<sup>9</sup> / L) and HCT (27.17% ± 1.62) values increased to normal levels [PLT (mean 217.5 ± 90.50 x 10<sup>9</sup> / L) and HCT (37.75% ± 2.65)].

## DISCUSSION

Over recent years in Turkey, the cat population has shown a marked increase because of the ease of handling and feeding and no need for much space. Therefore, it is important to investigate the diseases affecting cats and the risks of transmission to humans.

The first case of *Mycoplasma* (Formerly known as *Haemobartonella*) in Turkey had been detected by Tuzer et al.

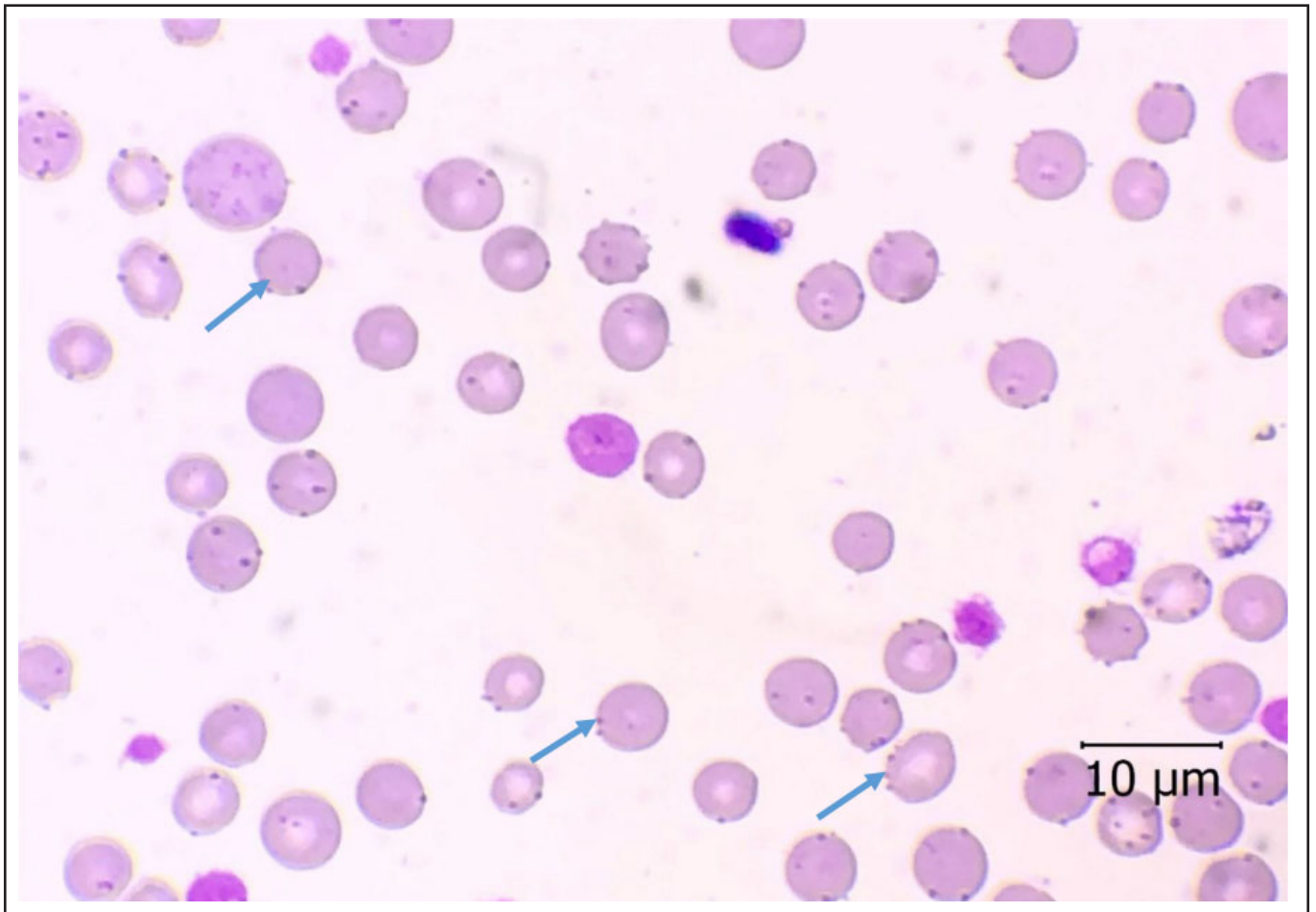
(1993) in a cat in Istanbul. Although some studies have been conducted in the following years, most of them are case reports (Kurtdele & Ural, 2004; Ural et al., 2008; Aslan et al., 2010; Aslan et al., 2015). Therefore, the number of studies examining the prevalence of the disease is very limited (Akkan et al., 2005; Ural et al., 2009; Atalay et al., 2015; Cetinkaya et al., 2016). Ural et al., found the overall prevalence of the disease as 18.9% by PCR method in samples they collected from four different cities (Bursa, İzmir, Ankara, Antalya) in 2009. With the cytological diagnosis method, the prevalence of hemotropic *Mycoplasma* was found to be 14.88% in Van (Akkan et al., 2005). Atalay et al. (2015) determined the prevalence of *Mycoplasma* infections as 9.52% in their study in Kayseri province.

In order to better understand the epidemiology of infectious diseases, it is important to determine the prevalence of the disease and associated risk factors. Concerning clinical, haematological, and risk factor analysis of cat mycoplasmosis, little is documented in the veterinary literature, especially in Turkey. In this study, to compare these features and assess risk factors for mycoplasmosis in cats, we enrolled only positive animals in which most of them were infected with ticks and fleas.

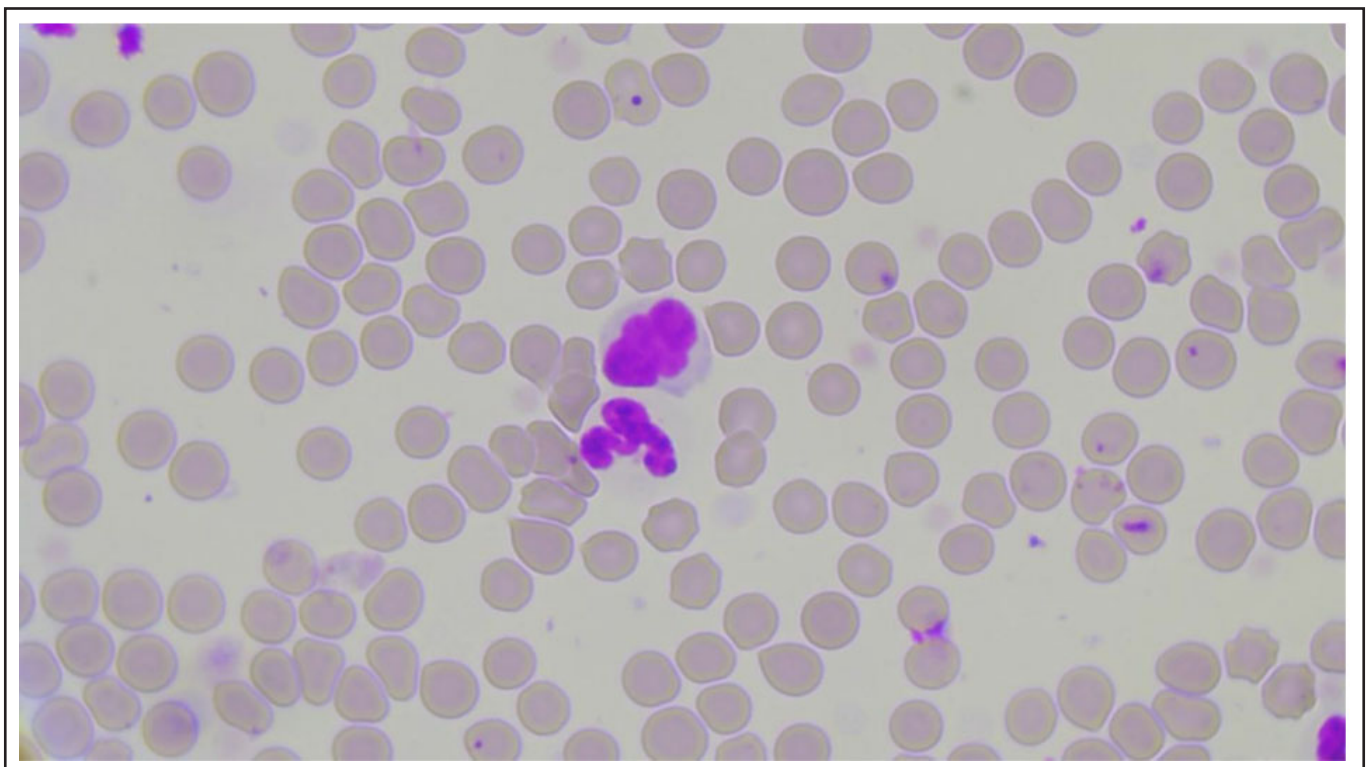
The criteria used for animal selection have a significant influence on the probable results, therefore, caution is needed in comparing the results of studies. In the current study, cats were selected not for their sex, breed, and age, but only sick animals on clinical and microscopic examination were recruited.

As reported in many previous studies (Macieira et al., 2008; Aquino et al., 2014; Santos et al., 2014; Vergara et al., 2016), male sex is one of the most critical risks factors for *Mycoplasma* infections. Vergara et al. (2016) reported that the association with male cats might be because of a higher likelihood of being infected due to their behavior patterns such as roaming, biting, and fighting. In contrast, Grindem et al. (1990) determined that cats' gender in the same household was not an important factor in terms of infections. By contrast with Vergara et al. (2016), in this study, female cats were found to be more infected than male cats. Feline haemoplasmosis has a very variable course of the disease, which is influenced by the immune status of the host. In relation to this, we think that changing hormonal balance during oestrus and pregnancy and partial suppression of the immune system during birth constitutes an infection risk for female cats.





**Figure 1.** Bacteria (*Mycoplasma* spp.) on the erythrocyte surface (arrows) in smears prepared from the blood of infected cats (x 1000).



**Figure 2.** Blood smear prepared from a recovered cat on the 21<sup>st</sup> day of doxycycline treatment (x 1000).

Another important risk factor associated with *Mycoplasma* infections is age; older cats are reported to be more susceptible (Sykes et al., 2007; Bauer et al., 2008; Tanahara et al., 2010). In our study, positivity was more frequent in older than two years of cats (54%) as opposed to juvenile (46%) ones. This may be due to increased exposure to infection throughout life or a weakening of the immune system by virtue of diseases that often develop in older cats, such as FeLV (Feline Leukemia Virus), FIV (Feline Immunodeficiency Virus) carcinoma. This would reflect a chronic clinical picture due to their longterm carrier state. Some studies indicated that these older cats have an increased risk of exposure over time, producing a chronic asymptomatic carrier state (Wergara et al., 2016).

Grindem et al. (1990) determined that many different factors such as FeLV infection, anaemia, bite abscesses are the risk factors for *Mycoplasma* infection but not the breed (Atalay et al., 2015). Although in some studies conducted in Turkey, searchers used the cats of different breeds, there is no result about the susceptibility of these breeds to infection (Ural et al., 2009; Atalay et al., 2015; Cetinkaya et al., 2016). Cats of the same breed are constantly mated with each other so that their characteristics are not lost in cats, and kittens are born without genetic diversity. Genetic deterioration can predispose cats to many different diseases. The fact that striped cats were more frequently infected (74%) with *Mycoplasma* spp., genetic predisposition may have a role in infection; however, more detailed studies are needed on cats' genetic basis.

The immunological status of the cat, the use of drugs that suppress the immune system, and other infectious diseases such as FIV are the important factors in the clinical feature in *Mycoplasma* infections. Vergara et al. (2016) reported that FIV-positive cats had a 3.77 times higher risk of being infected by mycoplasmas. However, contradictory results have been reported on whether concomitant FIV and FeLV infections favor the chronic carrier state or worsen the clinical course (Sykes et al., 2007; Peters et al., 2008; Gentilini et al., 2009). In the current study, it was determined that more than half (64%) of the cats had an infectious agent such as FIV, FIP (Feline Infectious Peritonitis), coronavirus, herpesvirus, and etc.

Vaccination against other diseases and parasitic control measures are of great importance in *Mycoplasma* infections in cats. The natural route of transmission of *Mycoplasma* infection between cats in the field has not yet been determined (Barker & Tasker, 2013), but blood-sucking arthropods such as ticks and fleas may play a role in hemotropic *Mycoplasma* transmission (Vergara et al., 2016). Some observational studies support the possibility of transmission by arthropods (Lappin et al., 2006; Kamrani et al., 2008), which could partly explain our results. In experimental studies, it has been shown that the agents are transmitted to *Ctenocephalides felis* from cats infected with *Candidatus M. haemominutum*. In addition, *Candidatus M. haemominutum* DNA was found in the feces, larvae, and eggs of these fleas (Woods et al., 2005). In our study, only three out of fifty cats whose blood was examined for *Mycoplasma* were regularly treated for external parasite control. Interestingly, all of the cats in this study were determined to be infested with fleas. Adult cat fleas are semi-obligate parasites on their hosts. However, horizontal transmission of fleas consequently the pathogens from one host to another may occur when there is close contact with infested bedding, lairs, or through predation. Therefore, we suggested that fleas may play a role in the transmission of *Mycoplasma* infections.

Torkan et al. (2013) and Atalay et al. (2015) emphasized that the infection risk could be increased depending on the cats' outdoor access. In the different study it has been reported that *Mycoplasma* infection was higher in stray cats (Kamrani et al., 2008; Spada et al., 2014; Duarte et al., 2015) and client-owned cats associated with the street (Willi et al., 2006; Roura et al., 2010; Martínez Díaz et al., 2013; Santos et al., 2014), which had more risk of infection through direct transmission associated with fighting or by exposure to bloodsucking arthropods. Similarly, the majority of the cats (68%) in our study had outdoor access, which means that they had a higher chance of being infected due to their living style.

Clinical signs and the laboratory data of cats experimentally infected with *Mycoplasma* have been documented (Atalay et al., 2015; Aslan, 2016; Vergara et al., 2016; Weingart et al., 2016). However, there are few published data about the natural course of infection in cats (Weingart et al., 2016). The current study describes the natural course of *Mycoplasma* infection in 50 cats. Stress and concurrent disease are predisposing factors for the clinical manifestation of feline mycoplasmosis and the course of infection. Clinical signs of disease depend on the degree of anaemia, the stage of infection, and the immune status of infected cats. Infection does not necessarily result in clinical disease and in some cases, healthy cats can also be positive for *Mycoplasma* DNA in the blood (Willi et al., 2006; Peters et al., 2008; Roura et al., 2010) and so PCR assay results do not always correlate well with clinical illness (Abdullah et al., 2019). After the incubation period lasting 2-3 weeks in experimental infections, clinical findings did not occur in some patients, while anaemia, depression, weight loss, icterus, high fever, and enlarged spleen were found in some patients (Aslan, 2016).

The most commonly represented clinical signs in this study showed similarity to those previously reported in cat mycoplasmosis (Atalay et al., 2015; Aslan, 2016; Vergara et al., 2016; Weingart et al., 2016). Weakness, anorexia, fever, respiratory disorder and pale mucous membranes were more frequent clinical signs in our cases. Icterus was rarely observed unless severe acute hemolysis develops. Fever is a common clinical finding in mycoplasmosis, which especially in cats with a strong immune system, maybe the only prominent clinical symptom. In contrast, hypothermia may occur when cats were about to die. In the current study, rectal temperatures ranged from 33-40.7°C whereby thirty cats exhibited increased temperatures (above 39.6°C) and six cats exhibited hypothermia (33-37.8°C).

Thrombocytopenia (86%) and anaemia (40%) were the most common haematological signs in the current study. Our results are in line with the results of Raimundo et al. (2016), who determined the most common hematological findings as anaemia and thrombocytopenia. Thrombocytopenia may be due to the destruction and consumption of platelets, increased hepatic or splenic platelet sequestration, decreased platelet production following bone marrow hypoplasia, and production of antiplatelet antibodies. Several studies have indicated that *Mycoplasma* infection is a predisposing condition for anaemia (Sykes et al., 2007; Roura et al., 2010; Vergara et al., 2016). Several mechanisms such as the mononuclear phagocytic system, suppression of erythropoiesis at the bone marrow and cell lysis mediated by the complement system may lead to anaemia (Weingart et al., 2016).

Laboratory findings detected in *Mycoplasma* infections depend on a wide range of factors. Therefore, concurrent diseases or previous infections, *Mycoplasma* species, and the stage (acuteness or chronicity) of *Mycoplasma* infection could considerably change these findings.

Controversial results have been obtained in many studies conducted on *Mycoplasma* infections of cats. Tasker et al. (2018) reported a low mean PCV value in their study. In the same study, although haemoglobin (Hb) concentrations and red blood cell counts (RBCC) displayed similar changes, mean cell volume (MCV) remained within the reference range. On the other hand, Diaz Reganon et al. (2018) determined low red blood cell (RBC) counts and low haemoglobin (HGB) concentration associated with infection by any *Mycoplasma* species in cats. Consistent with the results of Diaz Reganon et al. (2018) in our study, red blood cell (RBC) counts and haemoglobin (HB) concentrations were found very low. In agreement with our results, several studies have indicated that *Mycoplasma* infection is a predisposing condition for anaemia and *Mycoplasma* positive cats are more likely anemic than negative cats (Sykes et al., 2007; Roura et al., 2010; Obara et al., 2011).

WBC counts were often quite variable during *Mycoplasma* infection. The increase of monocyte counts could be related to the inflammatory response, as it was determined in previous studies (Sykes et al., 2008; Novacco et al., 2012; Vergara et al., 2016). Contrary to these authors, the present study revealed that WBC, LYM, MONO, GRA values could be within the normal reference range. Meanwhile, the current study showed some remarkable laboratory findings, such as low MPV, HCT, PCT, and MCH values compared to normal reference intervals. These low values indicate that the bone marrow was suppressed during mycoplasma infections, and the platelets and other blood cells were not produced in sufficient quantities. In addition, we postulate that erythrocyte production and life span are negatively affected, and erythrocytes are destroyed rapidly due to infection in sick animals. Apart from these, disruption of biochemical mechanisms, interruption of iron absorption and metabolism may result in a decreased hemoglobin amount.

The primary aim of this study was to determine the efficacy of doxycycline and oxytetracycline in cats infected with *Mycoplasma* spp. There are just a few studies reporting antibiotic treatment in *Mycoplasma* infection in cats in Turkey (Kurtdele & Ural, 2004; Akkan et al., 2005; Aslan et al., 2010; Aslan et al., 2015). Except for one of these studies (Akkan et al., 2005), all the others are case reports (Kurtdele & Kural, 2004; Aslan et al., 2010; Aslan et al., 2015). Indeed, in these studies, only one antibiotic was tested for a period of maximum 15 days. Here, we compared the effects of two antibiotics on 50 positive cats for 21 days. Therefore, this study, which was conducted on a large number of animals and followed for a long time, differs from the other studies conducted in Turkey.

In feline mycoplasmosis, multiplication of the organisms can be associated with drastic and rapid decreases of hematocrit value, and cats need antibiotic treatment to overcome the hemolytic crisis (Novacco et al., 2018). Therefore, effective antibiotic treatment is of great clinical importance. *Mycoplasma* species are susceptible to fluoroquinolones and tetracyclines, which are most often reported in treatment studies. However, other antibiotics classes, such as imidocarb, azithromycin (Novacco et al., 2018), and fluoroquinolones (Willi et al., 2007), also have been tested. The tetracyclines are bacteriostatic antibiotics that interfere with bacterial protein synthesis. Doxycycline is known for its broad-spectrum efficacy and higher intracellular concentra-

tions than other tetracyclines (Kompore et al., 2013). Therefore, doxycycline is the first preferred and generally recommended drug to treat feline *Mycoplasma* infections (Novacco et al., 2018). For these reasons, we chose to use doxycycline and tetracycline as the first line of treatment in this study.

In our study, 14 out of 16 cats in the Doxy group and 23 out of 36 cats in the Oxy group recovered at the end of the treatment process. In agreement with Novacco et al. (2018), we found that 21 days of doxycycline successfully decreased the *Mycoplasma* spp. blood loads in all cats. In this clinical trial, although doxycycline (87.5%) treatment produced superior results compared to tetracycline (67.6%) in *Mycoplasma* infected cats, the clinical course in both groups was almost similar.

Long-term doxycycline treatment is suggested for *Mycoplasma* infections. Some authors recommended treatment for up to 6-8 weeks to increase the chance of eliminating infections (Novacco et al., 2018). However, due to the rapid emergence of resistant bacteria occurring worldwide and possible side effects, the extent of antibiotics use should be carefully considered. Treatment time of infected cats should therefore be determined only if clinically indicated and with the consultation of a veterinary clinician and cat owner. In this study, daily doses of medication were given to infected cats, and the treatment continued for 21 days. Better results were obtained, especially with doxycycline. Improvements in clinical signs such as a decline in anaemia, jaundice, weakness, diarrhea, and vomiting occurred during the first week of the study.

The tablet form of the doxycycline has ulcerogenic properties in cats, and esophagitis and esophageal strictures were reported subsequent to oral doxycycline treatment (Willi et al., 2007). Therefore, this drug should be used with caution; preferably water or food should be taken just after administration. In our study, no significant adverse effect of doxycycline was observed even though it was used in tablet form. This could be due to food given to animals together with the drug, which likely reduces esophageal ulceration risk.

Complete elimination of *Mycoplasma* spp. maybe critical in some cases; i) cats living in multicat environments together with *Mycoplasma* naïve cats: acute primary infection may lead to severe hemolytic anaemia, ii) immunodeficient cats, iii) cats used as blood donors, and also iv) cats living with immunocompromised humans (Novacco et al., 2018). Appropriate antibiotic treatment may improve mycoplasma associated hematological abnormalities, however, a standard optimal antibiotic treatment regime has not been yet established (Willi et al., 2007; Novacco et al., 2018).

Microscopic examinations performed on the eighth day of the treatment revealed that the number of intraerythrocytic inclusions decreased but did not disappear completely. Three weeks after the beginning of the treatment (22<sup>nd</sup> day), bacteriemia was negative by blood smear microscopic examination. However, the presence of *Mycoplasmas* in recovered cats should have been investigated using a more sensitive method such as PCR. But it was not possible to investigate the presence of *Mycoplasma* with PCR, as the cost of the analysis was too much for cat owners.

In conclusion, the treatment protocol used in the current study is effective in reducing *Mycoplasma* spp. in cats having clinical signs. Hence, we suggest the oral use of doxycycline up to 21 days at a dose rate of 5 mg/kg as the first choice for treating *Mycoplasma* spp. infections in cats suffering from severe clinical signs and anaemia. Furthermore, data obtained from this study revealed that *Mycoplasma* spp. infections should be taken into consideration in cats with



anaemia, thrombocytopenia and leukocytosis, and flea infestations. Outdoor access of animals is an important risk factor for the disease. Finally, the veterinary clinician should be aware that early detection and treatment is critical since the therapeutic intervention may reduce the risk of development of severe anaemia and the need for blood transfusions.

#### Conflict of interest

The authors declare there is no conflict of interest. All procedures on animals were conducted in accordance with animal welfare standards by a certified veterinarian.

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