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High prevalence of hemotropic mycoplasmosis among stray cats in Iran

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ABSTRACT

Objective: To understand the prevalence of the hemotropic mycoplasmosis among stray cats.
Methods: In this research, 52 cats (30 queens and 22 toms) of different municipal regions of Tehran, the capital of Iran, were restrained and injected with tranquilizer. Peripheral blood samples were taken in order to detect blood parasites and taken blood smears were transferred to the parasitology laboratory, fixed by methanol and stained by Giemsa.
Results: About 32.7% of samples were positive for *Mycoplasma* spp. and some abnormalities were observed in leukocyte numbers.
Conclusions: There are some unclear aspects of hemotropic mycoplasmosis in Iran. This disease also has zoonotic importance. Therefore, some further researches are necessary to clarify the importance of the disease in different parts of Iran and its potential vectors.

1. Introduction

Feline hemotropic mycoplasmosis (formerly, hemobartonellosis) is caused by different species of wall-less Gram-positive bacteria which belong to genus *Mycoplasma*, class Mollicutes[1]. Three species of *Mycoplasma* have been reported in infected cats [*Mycoplasma haemofelis* (*M. haemofelis*), *Candidatus* Mycoplasma haemominutum (*Candidatus* M. haemominutum) and *Candidatus* Mycoplasma turicensis (*Candidatus* M. turicensis)][2]. *Mycoplasma* spp. can be divided by their size (large variant is classified as *M. haemofelis* and small variant as *Candidatus* M. haemominutum)[3,4]. *Candidatus* M. turicensis was first reported in a Swiss cat but it has not been observed by light microscopy[5].

Blood-sucking arthropods such as fleas are the primary vectors of the hemotropic *Mycoplasma* spp., but *M. haemofelis* was experimentally transmitted by intraperitoneal, intravenous injection and oral administration of infected blood[2,6].

The disease symptoms vary from no observable clinical signs to severe anemia and sometimes death, dependence on infective *Mycoplasma* species and presence of stress or co-infections[2]. The disease which is caused by *M. haemofelis* is divided into preparasitemic, acute, recovery and carrier phases[7]. In cats with severe anemia, depression, tachypnea, weakness, anorexia, weight loss, pale mucous membranes and dehydration can be observed[2,8]. In *Candidatus* M. haemominutum infection, mild fever has been observed in experimentally infected cats[8]. *Candidatus* M. turicensis can cause moderate to severe anemia. Concurrent infections and stress increase the disease severity which is caused by *Candidatus* M. haemominutum and *Candidatus* M. turicensis[2,5].

Diagnosis is usually made by organism observation in blood

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films. PCR can be used for *Mycoplasma* species identification[1,2]. *Mycoplsma* spp. are easily recognized in stained blood films and the organisms appear as small dark blue cocci (0.3–0.8 μm). They can be observed as rods and rings which attach on the surface of erythrocytes[2,9]. *Candidatus* M. haemominutum appears more in small rods and cocci and low numbers of organisms are detected per red blood cell[2].

Hemotropic *Mycoplasma* spp. organisms can potentially be transmitted to human but their zoonotic importance will be discussed later[10].

Stray cats are living and moving in streets, parks and some other public places in Iran. The aim of this study was to understand the prevalence of the hemotropic mycoplasmosis among stray cats.

2. Materials and methods

In this research, based on municipal regions of Tehran, the capital of Iran, 52 cats (30 queens and 22 toms) were restrained in a cage containing sausage and then injected with tranquilizer. Peripheral blood samples were taken in order to detect blood parasites, and taken blood smears were transferred to the parasitology laboratory, fixed by methanol and stained by Giemsa. The infection between toms and queens was analyzed by *Chi*-square test, SPSS software.

3. Results

A total of 17 samples (10 queens, 7 toms) out of 52 were positive in hemotropic mycoplasmosis (32.7%; 20%–45% with 95% confidence interval). There was no significant difference between infected toms and queens ($P > 0.05$). In 14 samples, the separated cocci of *Mycoplasma* spp. were observed but in 3 samples, the bacterial cocci were in chain form (Figure 1). In differential leukocyte counts of positive samples, five groups were described. Group A (5 samples): only band cell increasing was observed. The band cells were counted [5%–10% (2 toms), 15%–20% (1 tom and 1 queen) and > 20% (1 queen)]. Group B (1 queen): neutropenia, band cell increasing (5%–10%), monocytosis and lymphocytosis were recorded. Group C (5 samples): monocytosis with band cell increasing was observed and counted band cells were 5%–10% (3 queens), 15%–20% (1 tom) and > 20% (1 queen). Group D (3 samples): neutropenia, band cell increasing (5%–10%) and monocytosis were observed (2 queens and 1 tom). Group F (3 samples): neutropenia, band cell increasing and lymphocytosis was detected. Band cells were counted as 5%-10% (1 queen, 1 tom) and 10%–15% (1 tom). Anisocytosis was observed in 11 positive samples (Figure 1).

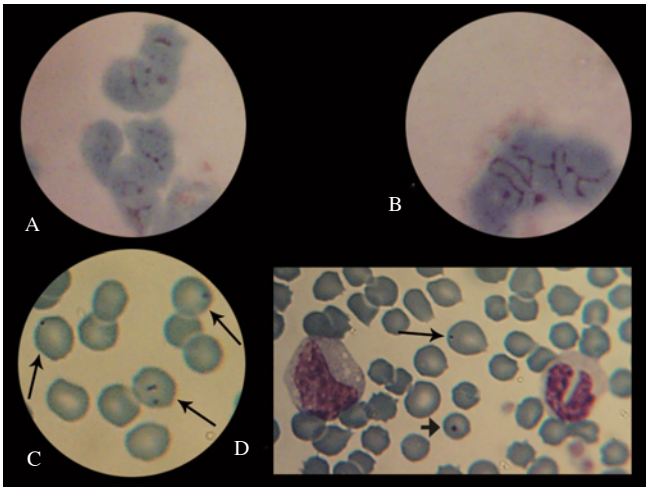

Figure 1. A, B: *Mycoplasma* spp. chains on the reticulocytes, 150×, 200×, respectively, stained by Giemsa; C: Separated cocci of *Mycoplasma* spp. on the reticulocytes (black arrows), 150×, stained by Giemsa; D: *Mycoplasma* spp. on the reticulocyte (long black arrow) and Howell-Jolly body (short black arrow).
A monocyte (left) and a band cell (right) noted the anisocytosis, 100×, stained by Giemsa.

4. Discussion

In Spain, 3.7% of cats had infected by *M. haemofelis*, 9.9% by *Candidatus* M. haemominutum and 0.5% by *Candidatus* M. turicensis. There was positive correlation between hemotropic mycoplasmosis, and outdoor access and male sex[11]. In Canada, subclinical hemotropic mycoplasmosis was detected in 12% of shelter cats and 4% of client-owned cats[12]. In Japan, *M. haemofelis* infection was reported in 67% of domestic cats, *Candidatus* M. haemominutum in 22% and combined infection of *M. haemofelis* and *Candidatus* M. haemominutum in 11% of domestic cats[13]. In Australia, 0.9% of flea-infested cats were positive for *M. haemocanis*, 15.3% for *Candidatus* M. haemominutum and 0.9% for *Candidatus* M. turicensis[14]. In Britain, less than 2% of both groups (ill and healthy cats) were infected with *M. haemofelis*, of which 20% of ill cats and 8% of healthy cats were infected by *Candidatus* M. haemominutum. Male cats were more commonly infected[15]. In the US, about 30% and 14% of anemic and healthy cats, respectively were infected by *M. haemofelis* alone or in combination with *Candidatus* M. haemominutum[16]. In another study in Florida, 8% of samples were infected by *M. haemofelis* and 12% were infected by *Candidatus* M. haemominutum[17]. In one study based on RT-PCR, infection of *M. hamefelis*, *Candidatus* M. haemominutum and *Candidatus* M. turicensis among samples of different countries was estimated 1.6%, 17% and 2.3% in UK, 4.8%, 24% and 10% in Australia and 15%, 38% and 26% in South Africa, respectively[18]. In Iran, 22% of Persian short hair cats were infected by *Mycoplasma* spp., three species of all were detected in the infected blood samples and combined infections were also observed[19]. In this study, hemotropic mycoplasmosis was reported as 32.7% (20%–45% with

95% confidence interval).

Cats with acute infection of *M. haemofelis* have immune-mediated regenerative hemolytic anemia. In the blood films, reticulocytosis, anisocytosis, polychromatophilic macrocysts, nucleated erythrocytes and increased numbers of Howell-Jolly bodies can be observed, but none of them are pathognomonic[2]. In *Candidatus* M. haemominutum infection, mild regenerative anemia is observed[15,20]. Anemia in *Candidatus* M. turicensis infection can be moderate to severe[2]. In one study, *Mycoplasma* spp. infections were observed in regenerative anemic cats, nonregenerative anemic and healthy groups[21].

The cocci form of hemotropic *Mycoplasma* spp. must be differentiated from stain percipitates, Howell-Jolly bodies, basophilic stipplings, siderotic inclusions and *Cytauxzoon felis*[22,23]. The size of *Mycoplasma* spp. are not reliable, because *Candidatus* M. haemominutum has been reported in both small and large size[4,8,24]. Therefore, molecular examinations are necessary for species determination. Different PCR methods can be used now[8,16,25,26]. RT-PCR assays are also available[18,25,27,28]. A recombinant antigen-based ELISA was used to detect *Mycoplasma* serum antibodies and the infective species cannot be determined by ELISA[29].

Differential leukocyte counts are variable in hemotrpic mycoplasmosis[8,30]. In this research, band cell increasing was observed in all positive samples with different percentages. Mature neutropenia, monocytosis and lymphocytosis were also detected as explained in the result section. Mature neutropenia can develop in acute inflammatory conditions when the demand for neutrophils depletes the bone marrow storage pool. Degenerative left shifts are often present in these disorders[9,23]. Band cell increasing in all *Mycoplasma*-infected samples is a sign of left shift. In this research, it is regenerative (with normal number of mature neutrophils) in 10 samples and degenerative (with neutropenia) in 7 samples. Monocytosis may be present in both acute and chronic inflammation[23,31]. Some inflammatory cytokines and endogenous or exogenous glucocorticoid steroids can induce monocytosis and lymphocytosis[23]. Lymphocytosis is sometimes present in chronic inflammation[32]. Lymphocytosis was reported in feline hemotropic mycoplasmosis (bartonellosis), feline leukaemia virus and feline immunodeficiency virus infections[33,34]. In this research, both monocytosis and lymphocytosis were recorded in some *Mycoplasma* positive samples.

Hemotropic mycoplasmosis can be treated by orally administered doxycycline, enrofloxacin, pradofloxacin and marbofloxacin. Glucocorticoids in severely anemic cats can decrease erythrophagocytosis[1,2]. Unfortunately, antimicrobial treatment reduces or eliminates visible parasitemia but the organism does not clear from the infected cat's body[2].

Co-infection of feline hemotropic mycoplasmosis has been reported with feline leukaemia virus, feline immunodeficiency virus and feline retroviruses[4, 11,17,24]. These immunosuppressive infections can increase the mycoplasmosis severity and the parasitemia is recognized more in these immunocompromised cats[9,24]. There are some reports of combined infections of different species of feline hemotropic *Mycoplasma*[11,13,19,21,28,35]. In this research, only peripheral blood smear was available and the infective species of *Mycoplasma* could not be determined.

M. haemofelis was infected an immunocompromised man from Brazil[10]. Other *Mycoplasma* spp. also infect different individuals in the US and China[36,37]. These cases suggested that hemotropic mycoplasmosis can transmit to human[25]. Therefore, more researches are needed to clarify unclear aspects of the disease.

Conflict of interest statement

I declare that I have no conflict of interest.

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