



Mycoplasma from the upper respiratory tract and conjunctival infections in household dogs

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Article information

Article history:

Received September 14, 2022
Accepted November 24, 2022
Available online December 04, 2022

Keywords:

Conjunctival infections
Household dogs
Isolation
Mycoplasma
Upper respiratory tract infections

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Abstract

The study was performed to isolate and detect *Mycoplasma* as a causative agent of the upper respiratory tract and conjunctival infections in household dogs in Mosul city. One hundred household dogs of different ages, sex, and breed were subjected to the study, including 60 dogs suffering from moderate to severe respiratory infections and 40 healthy dogs in Mosul city from 1/2/2022 to 15/6/2022. Three hundred swabs were collected, including nasal swabs 100, oropharyngeal swabs 100, and conjunctival swabs 100. The swabs were cultured in *Mycoplasma* media and incubated at 37°C with 5% CO₂ in a candle jar for 4-14 days. Light and the dissecting microscope examined the growing colonies microscopically under low magnification. The colonies were also stained with modified Dienes stain. The results of the current study indicated that respiratory infections were predominant in young male household dogs compared with conjunctival infections in all examined dogs. The conjunctival infections were obvious only in Husky and Belgium dogs. The high isolation rate of *Mycoplasma* was from upper respiratory tract infections in diseased female dogs more than one-year age 100%. In contrast, the conjunctival infections were more dominant and had a higher isolation rate in less than one-year males. The current study revealed a high *Mycoplasma* isolation rate in respiratory swabs of Terrier and German dogs, while the conjunctival swabs were positive for mycoplasmal culture in Husky and Belgium dogs 100%. In conclusion, mycoplasmal infections were more dominant in upper respiratory infections in female German and Terrier dogs.

DOI: [10.33899/ijvs.2022.135824.2525](https://doi.org/10.33899/ijvs.2022.135824.2525), ©Authors, 2022, College of Veterinary Medicine, University of Mosul.
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Introduction

Mycoplasmas are in the class *Mollicutes*, are small prokaryotic cells have the ability to self-replication. They require enriched media for growth and although most *Mycoplasmas* are facultative anaerobes, some grow optimally in an atmosphere of 5-10% CO₂. Many species are important veterinary pathogens and settled the mucous membrane of respiratory, genital tract as well as red blood cells causing respiratory infection, mastitis, conjunctivitis, arthritis and infrequently abortion (1-4). The Dog (*Canis lupus familiaris*) is a domestic mammal of the family *Canidae* (order *Carnivora*). It is a subspecies of the gray wolf

(*Canis lupus*) and is linked to foxes and jackals. The dog is one of the two most abundant and most popular domestic animals in the world (the cat is the another). For more than 12,000 years it has lived with humans as a hunting companion, protector, object of scorn or adoration, and friend (5-8). The first notarized occurrence of *Mycoplasma* in dogs was in 1934 (9). Dogs were sensitive to multiple *Mycoplasma* species, despite of not all *Mycoplasma* infection caused clinical disease (10,11). *Mycoplasma* in dogs is believed to form portion of the normal bacterial flora of the upper airways (12,13), but resent study assessing the microbiome of healthy dogs showed that *Mycoplasma* occurred in reducing frequently from the nasal cavity to the

lower airways. This could indicate that colonization of the lower respiratory tract by *Mycoplasma* may result from the upper airways (14-19). Moreover, the task of *Mycoplasma* as an important etiological cause of canine infectious respiratory disease complex (CIRDC), recently isolated *Mycoplasma cynos* and *Mycoplasma canis* from CIRDC in dogs (20-25).

The existing study aimed to isolate and identify *Mycoplasma* from the upper respiratory tract and conjunctival infections of household dogs using the Conventional cultural methods and describe its isolation rate.

Materials and methods

Ethical approve

The committee of scientific ethics gave the ethical approval to conduct this scientific work in the College of Veterinary Medicine according to the approval certificate numbered UM.VET.2022.018.

Samples

Three hundred swabs were taken from nasal 100, oropharyngeal 100, and conjunctiva 100 of both diseased that showed clinical signs of respiratory infections, and healthy household dogs of different breeds, ages and sex from many veterinary clinics in Mosul city during the period of 1/2/2022 to 15/6/2022. The swabs were placed in a *Mycoplasma* broth medium and aseptically carried immediately to the laboratory of Microbiology, College of Veterinary Medicine, University of Mosul.

Mycoplasma broth medium

3.7 gm of brain heart infusion broth suspended in 100 ml of distilled water, then boiled and autoclaved at 121°C for 15min. The medium was cooled at 45°C and added the prepared supplement to the medium (3,4). The yeast extract with a serum prepared as 0.5 gm of yeast extract powder suspended in 40 ml of distilled water, boiled, then cooled and accurate pH=8, and filtered. For prepared yeast extract-serum, added 20 ml of filtered bovine serum to 10 ml of filtered yeast extract in percentage as 2:1, which were added 100 ml of prepared medium. 3 ml of filtered penicillin solution which contain 100,000 un/ml at pH=7.8 added to 100 ml of the prepared medium for inhibiting the growth of contaminated gram-positive bacteria. 3 ml of filtered sulfadiazine-trimethoprim solution was added to 100 ml of the prepared medium to inhibit the growth of contaminated Gram-negative bacteria. 1 ml of nystatin solution was added to 100 ml of the prepared medium to inhibit the growth of yeast. 1 ml of clotrimazole solution was added to 100 ml of the prepared medium to inhibit fungi.

Mycoplasma agar medium

4.9 gm of brain heart infusion agar suspended in 100 ml of distilled water, boiled and autoclaved at 121°C for 15 min.

then cooled at 45°C and added the supplement as in *Mycoplasma* broth medium (3,4).

Culture

The swabs were placed in *Mycoplasma* broth medium and transported immediately to the laboratory of Microbiology, and incubated at 37°C with 5% CO₂ for 4-14 days in a candle jar. When turbidity appeared, an inoculum of each broth was cultured on *Mycoplasma* agar medium and incubated at 37°C with 5% CO₂ for 7-14 days in a candle jar. The culture was checked daily for the first 7 days and weekly thereafter.

Staining with modified dienes stain

The stock solution prepared from Methylene blue 2.5 gm, Azure II 1.25 gm, Maltose 10 gm, Na₂CO₃ 0.25 gm, distal water 100 ml. while the working solution prepared from diluting stock solution in distal water by adding three volumes of distal water to one volume of stock solution. For staining the colonies, submerged the agar dish surface which contain the colonies of *Mycoplasma* in 1ml of working solution of Dienes stain, washed with distal water then decolorized with 1ml of 95% of ethyl alcohol for 1min., removed the excess alcohol, washed with distal water, and examined the color of colonies of *Mycoplasma* (4).

Results

Colony morphology

The growing colonies had an umbonate appearance when illuminated obliquely and small, circle colonies with center surrounded by white to colorless halo identical to a fried egg appearance in transmitted light. According to the colonies s appearance, there were two types of colony morphology on agar plate, in the first type the colonies were transparent (Figure 1a), while the other type the colonies appeared as opaque brown color (Figure 1b).

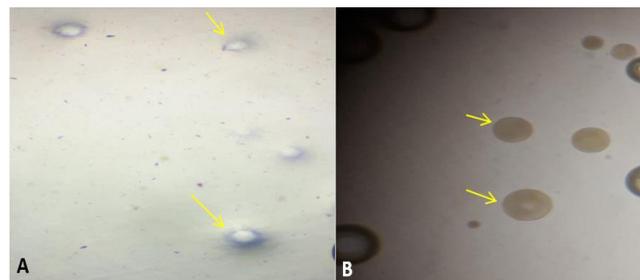


Figure 1: Two types of colony morphology.

Dienes stain

The results of staining using the modified Dienes stain on *Mycoplasma* colonies grown on mycoplasma agar, which showed light blue center circumscribed with dark blue halo (Figure 2).

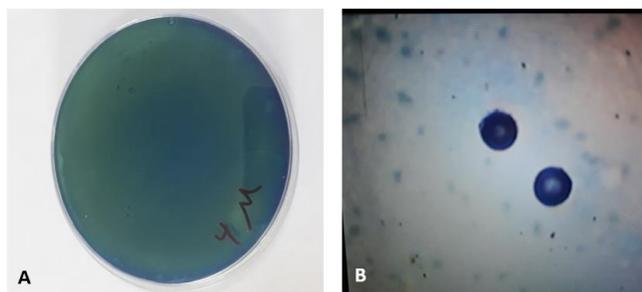


Figure 2: a: staining petri dish b: staining colonies

Infection and isolation rates

Mycoplasma isolation rate from total swabs were 56.6%, while high rate indicated from nasal swabs 68%, followed by oropharyngeal swabs and conjunctival swabs 58 and 44% respectively. The results of infections indicated that respiratory infections were predominant in household dogs in comparison with conjunctival infections in all examined dogs. The young ages were more susceptible to both respiratory and conjunctival infections 58.3 and 16.7% respectively. According to the sex, upper respiratory and conjunctival infections appeared more frequently in males 66.6 and 20% respectively. The conjunctival infections were obvious only in Husky 16.7% and Belgium dogs 25% (Table 1).

The results of *Mycoplasma* isolation rate indicated that the high isolation rate of *Mycoplasma* was from upper respiratory tract infections in diseased dogs more than one-year age 100%, while it was the opposite situation with

conjunctival infections 66.6%, these according to age. But according to sex, the respiratory swabs from diseased female dogs were more perfect for *Mycoplasma* isolation than in males since all female swabs 100% were positive for *Mycoplasma* culturing, whereas the conjunctival infections were more dominant and higher isolation rate in male swabs 50%. According to the breed, the existing study revealed a high *Mycoplasma* isolation rate in respiratory swabs of Terrier 100% and German dogs 93.75%, while the conjunctival swabs were positive for *Mycoplasma* culture in Husky and Belgium dogs 100% for each one, also the healthy dogs indicated positive culture of *Mycoplasma* but in very low rate (Table 2).

Table 1: Respiratory and conjunctival infections in diseased household dogs

Characters	Diseased dogs (n=60)	
	Conjunctival	Upper respirator
Age	<1 year	10/60(16.7%)
	>1 year	9/60(15%)
Sex	Male	12/60(20%)
	Female	3/60(5%)
Breed	Husky	2/12(16.7%)
	German	0/32(0%)
	Terrier	0/5(0%)
	Belgium	2/8 (25%)
	Wolf dog	0/2(0%)
	Kangal	0/1(0%)

Table 2: The isolation rate of *Mycoplasma* from diseased and healthy household dogs

Characters	Diseased dogs		Healthy dogs
	Conjunctival	Upper respiratory	
Age	<1 year	10/10(100%)	5/26(19.2%)
	>1 year	6/9(66.6%)	2/14(14.3%)
Sex	Male	6/12(50%)	7/28(25%)
	Female	1/3(33.3%)	1/12(8.3%)
Breed	Husky	2/2(100%)	1/4(25%)
	German	0/32(0%)	4/22(18.2%)
	Terrier	0/5(0%)	0/2(0%)
	Belgium	2/2(100%)	1/8(12.5%)
	Wolf dog	0/2(0%)	0/2(0%)
	Kangal	0/1(0%)	0/1(0%)

Discussion

Dogs are sensitive to infection by several *Mycoplasma* species, and can be identified from the respiratory tract of healthy and diseased dogs despite not all *Mycoplasma* infections causing clinical disease (10,26-28). The growing colonies in the existing study were identical of *Mycoplasma* when grown on *Mycoplasma* agar, and revealed the fried egg

appearance as reported in scientific references (1,3), which confirmed that the in vitro culture was the standard method for isolation and identification of *Mycoplasma*, however the slow growing of these bacteria make this method time-consuming even for particular laboratories (3,28,29).

Mycoplasma isolation rate from total swabs in our study 56.6% which was far away from data recorded by Randolph *et al.*, (30) who recorded 27% and Barreto *et al.* (31) who

recorded 29.96%. The differences may be attributed to the way of samples collection, conditions used for isolation, case history and samples collection period (3). In addition to many risk factors related to dogs themselves, such as multi-dog householding, interest in dog training, and doggy day attention (32). The higher isolation rate of *Mycoplasma* was from nasal swabs 68% followed by oropharyngeal swabs 58% and conjunctival swabs 44%, whereas different results were confirmed by previous studies Barreto *et al.* (31) and Schulz *et al.* (33) who indicated the highest isolation rate from oropharyngeal swabs 42.86%, and agreed with him in the lowest rate of isolation from conjunctival swabs 18.30%. The high isolation rate from oropharyngeal swabs supports that *Mycoplasma* are normal commensals of the upper respiratory tract and when stressful conditions occurred, the *Mycoplasma* convert to pathogens and the clinical respiratory cases appear (34,35), and what proves that is the low isolation rates from the same swab types of normal healthy dogs (31). The high upper respiratory tract infections in current study was recorded in the young (less than one - year aged) dogs 58.3%, While they occurred less frequently in the more-one year dogs and more frequent in diseased males 66.6% than diseased females 8.3% and mainly in Terrier and German dogs 100%, although the *Mycoplasma* isolation rate was higher from the swabs of the more-one year female dogs. These results were agreed with Chalker *et al.* (36) who confirmed that there was a considerable relation between *Mycoplasma cynos* infection and young age <1 year, but so differed from Chan *et al.* (37) who recorded high isolation rate of *Mycoplasma* from upper respiratory tract infections in age near 1.5year, also they recorded high isolation rate of *Mycoplasma* from upper respiratory tract infections in female more than male, and in Toy dog breed more than another breed. in common, the likelihood of upper respiratory tract infections is improved in dogs that are <1 year of age, immunocompromised, or susceptible to another respiratory tract infections. Perhaps the discrepancies resulted from diverse diagnostic procedures, sample populations, or inclusion standards (37). A high isolation rate of *Mycoplasma* from conjunctival infections were recorded in diseased Husky and Belgium 100%, while other researchers recorded low isolation rate of *Mycoplasma* from conjunctival infections (31,38-40), may be due to these are more common breeding of these species recently to be used for a variety of purposes.

Conclusion

The results of our study indicated high isolation rate of *Mycoplasma* in diseased household dogs with upper respiratory tract and conjunctival infections by cultural method, and also recorded *Mycoplasma* with high isolation rate in diseased Terrier and German with upper respiratory tract infection, and in diseased Husky and Belgium with conjunctival infections.

Acknowledgments

The author would like to thank the dean of College Veterinary Medicine, the head of Department of Microbiology, University of Mosul.

Conflict of interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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المفطورات من إصابات المجرى التنفسي العلوي وملتحمة العين في الكلاب المنزلية

صبا عبد الرحيم حسين و محمد علي حمد

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الخلاصة

أجريت الدراسة لعزل وتحديد المفطورات كمسبب لإصابات المجرى التنفسي العلوي وملتحمة العين في الكلاب المنزلية في مدينة الموصل. شملت الدراسة 100 كلب منزلي بمختلف الأعمار والأنواع ومن كلا الجنسين، تضمنت 60 كلب يعاني من أعراض تنفسية متوسطة إلى شديدة و 40 كلب سليم في مدينة الموصل والفترة من 2022/2/1 إلى 2022/6/15. جمعت 300 مسحة ضمت 100 مسحة من الأنف، 100 مسحة من البلعوم الفموي و 100 مسحة من ملتحمة العين. زرعت المسحات على أوساط المفطورات وحضنت بدرجة حرارة 37 درجة مئوية بوجود 5% من ثاني أكسيد الكربون في ناقوس الشمعة لمدة 4-14 يوم. فحصت المستعمرات النامية مجهريا تحت القوة الصغرى بالمجهر الضوئي والتشريحي. صبغت المستعمرات بعدها بصبغة دنس المحورة. أكدت نتائج الدراسة الحالية إن الإصابات التنفسية كانت أكثر سيادة في ذكور الكلاب المنزلية الصغيرة العمر بالمقارنة مع إصابات الملتحمة في جميع الكلاب المفحوصة. إصابات الملتحمة كانت أكثر وضوحا في كلاب الهاسكي والبلجيكي. نسبة عزل المفطورات العالية من إصابات المجرى التنفسي العلوي كانت في إناث الكلاب المريضة بعمر أكثر من سنة 100%، بينما إصابات الملتحمة كانت الأعلى نسبة وسيادة في ذكور الكلاب المريضة بعمر أقل من سنة. كما كشفت الدراسة الحالية أن نسبة عزل المفطورات العالية كانت من المسحات التنفسية لكلاب التيرر والألماني، بينما مسحات الملتحمة كانت موجبة لزراع المفطورات في الكلاب نوع هاسكي والبلجيكي 100%، وفي استنتاج لهذه الدراسة، أن خمجات المفطورات كانت الأكثر سيادة في إصابات المجرى التنفسي العلوي لإناث كلاب الألماني والتيرر.