P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2023.011

Flea-borne *Mycobacterium* and *Mycoplasma* in Small Ruminants on the Northern West Coast of Egypt

Samah Yassin[1]*, Dalia Mahmoud[2], Amani Hafez[1], Mohamed Salama[2] and Safaa Barghash[1]

[1]Animal & Poultry Health Department, Animal Production and Poultry Division, Desert Research Center, El-Naam, Cairo, Egypt; [2]Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt
***Corresponding author:** samahelsayed77776@yahoo.com

Article History: 22-736 Received: 27-Oct-22 Revised: 05-Dec-22 Accepted: 19-Dec-22

ABSTRACT

Many pathogens that cause newly emerging and re-emerging zoonotic diseases are globally hosted by fleas. There is a dearth of recorded information on flea-borne animal pathogens in Egypt. The present study aimed for the first time to investigate some bacterial pathogens that could be transmitted by fleas infesting small ruminants in the Northern West of Egypt using conventional PCRs and sequence analyses. Of 249 sheep selected in different localities in the study area, 223 fleas belonging to *Ctenocephalides* were pooled in tubes containing pleuropneumonia-like organisms (PPLOs) broth after being determined by all sexes of *Ctenocephalides felis* and *C. canis* and then crushed thoroughly. The fleas' samples were screened molecularly for *Mycobacterium* and *Mycoplasma*. Results revealed *Mycobacterium* is only present in two sexes of *C. canis* collected from the Marsa Matruh location. *Mycoplasma* spp. was detected in both males and females of *C. felis* and *C. canis* from Maryout in the West of Alexandria Governorate. Whereas *Mycobacterium* was recorded in the GenBank database as *Mycobacterium bovis* under accession number ON076426, the three mycoplasmas identified under accession numbers ON076423 (*M. agalactiae*), ON076424 (*M. arginini*), and ON076425 (*M. ovipneumoniae*). We concluded that *Mycoplasma* spp. and *Mycobacterium bovis* could be transmitted by fleas infesting sheep in Egypt.

Key words: Egypt, *Ctenocephalides*, *Mycoplasma*, *Mycobacterium Bovis*, Sheep, PCR.

INTRODUCTION

The geographic and host ranges of numerous vector-borne pathogens and associated diseases have changed because of climate change and the degradation of wild ecosystems in recent decades (Bitam et al. 2010). One of the most significant arthropod vectors of medical and veterinary concern is fleas (Insecta, Siphonaptera) (Nguyen et al. 2020). Because they persist in the environment for a long period, *Ctenocephalides* species play a vital role as vectors of many infections by the regurgitation of blood meals or the fecal channel, by infected feces pellets (Dobler and Pfeffer 2011).

Worldwide, fleas can lead to the emergence and re-emergence of zoonotic illnesses, in epidemic form as hosts for a wide range of understudied pathogens except for *Yersinia pestis* (Beugnet and Marie 2009). Host specificity is essential for studying the transmission of disease agents. Fleas are rarely specific at the host species level because hosts are similar in their ecologies and are likely to share flea species as they feed on any available animal and thus have the potential of hosting similar pathogens. Of those, *C. felis* (cat flea) is much more frequent than *C. canis* (dog flea) (Bitam et al. 2010).

The dairy sector is worried about the bacteria *Mycobacterium bovis.* It is anaerobic bacterium, a Gram-positive, acid-fast, and tuberculosis-causing agent (Torres-Gonzalez et al. 2016; Desouky et al. 2023). It can infect a wide range of hosts, including people when they consume unpasteurized milk, and cattle are now primarily a pulmonary disease. The principal route for transmission is likely to be through aerosol dissemination, goats, bison, and deer (Smith et al. 2004). *Mycoplasma* is a bacterial species belonging to the Mollicutes, which has a small genome size and a lack of a cell wall (Nicholas et al. 2008; Bosila et al. 2021). It can cause pneumonia, conjunctivitis, arthritis, and mastitis (Kumar et al. 2013). Many diseases and veterinary problems are related to different *Mycoplasma* species in mammals and birds (Glen et al. 2012; Wu et al. 2021).

Cite This Article as: Yassin S, Mahmoud D, Hafez A, Salama M and Safaa Barghash S, 2023. Flea-borne *Mycobacterium* and *Mycoplasma* in small ruminants on the Northern West Coast of Egypt. International Journal of Veterinary Science 12(4): 586-592. https://doi.org/10.47278/journal.ijvs/2023.011

Goats and sheep, commonly known as "poor man's cows," are essential to the economies of many countries, including Egypt, because they are used for the production of milk, wool, and meat sheep account for over 10% of Egypt's meat production (Hakim et al. 2014). *Mycoplasma* infections cause high rates of illness and death in sheep and goats in Africa, including Egypt, Europe, and India (Sandip et al. 2014), resulting in serious economic losses for ruminant farmers worldwide. Data literature on flea-borne pathogens (FBPs) in animals is limited and remains scarce in Egypt. The aim of this investigation was to identify and genotype certain possible bacterial pathogens (*Mycobacterium* and *Mycoplasma* spp.) in fleas-infested sheep in Egypt's Northern West coastal zone.

MATERIALS AND METHODS

Ethical Approval

The current study was conducted in compliance with internationally accepted principles of the European (EU) Directive 2010/63/EU for animal experiments and the National Institutes of Health guide (NIH Publications No. 8023 revised 1978). All the methods for the use of animals were adopted in agreement with the ethics guidelines of the Ministry of Agriculture and Reclamation, and Desert Research Center in Egypt. This article does not contain any studies with human participants performed by any of the authors.

Study Area

The present study lasted from April 2020 to June 2020 on the Northern West Coast of Egypt, including Matruh and Alexandria Governorate. Two prime locations were selected where fleas were collected, i.e., 1) Marsa Matruh, the Capital of Matruh Governorate, and 2) Maryout, where the biggest Station of the Desert Research Center is, which lies 35Km Southwest of Alexandria Governorate.

Fleas Sample Collection

A total of 223 fleas have randomly collected from 249 sheep with reported data on *Mycoplasma* but no data or history of tuberculosis. The main bulk was healthy animals without clinical signs; others showed fever, anemia, weight loss, abortion, and diarrhea. Fleas were collected directly from their skin or by flea combs, placed in an icebox and submitted immediately to the laboratory. They were determined to species level using a stereoscopic microscope SZ-PT-40 (Olympus, Japan), according to Skuratowicz key (Skuratowicz 1967)**.** Then, they subdivided into pooled samples of the same species and sexes of *C. felis* or *C. canis.* After that, fleas were conserved on pleuropneumonia-like organisms (PPLOs) broth before being directed to PCR.

Extraction of DNA from Flea Samples

For genomic DNAs, flea samples were mechanically homogenized and subjected to short centrifugation at 5,000rpm. DNAs were extracted from the supernatants using a commercially available DNeasy Blood and Tissue Kit (QIAGEN Inc. Germany). Each sample was well blended using a vortex in 180µL of buffer ATL and 20µL of proteinase K. To guarantee total tissue lysis, these were spun down and incubated at 56°C overnight. DNA samples were kept at -20°C pending PCR testing while additional extraction procedures were carried out according to the manufacturer's instructions.

Identification of *Mycobacterium* and *Mycoplasma*

For infections with *Mycobacterium* and *Mycoplasma*, the extracted DNAs were examined. As shown in Table 1, conventional PCR was carried out in a single step for each pathogen using forward and reverse specific primers: 16S rRNA to detect 520bp for *Mycoplasma* and 16S rRNA to detect 582bp for *Mycobacterium* (Invitrogen, Life Technologies). Each 25µL reaction volume contained 12.5µL of the Emerald Amp GT PCR master mix (2x premix), 1µL of each forward and reverse primer (20pmol), 5µL of the template DNA, and 5.5µL of PCR-grade water. The thermal cycling technique included a first denaturation step at 94°C for 5min, then 35 cycles of 94°C for 30s, 62°C for 40s, and 72°C for 45s for *Mycobacterium*, and 35 cycles utilizing a thermocycler at 94°C for 30s, 60°C for 40s, and 72°C for 45s for *Mycoplasma* (Biorad T100 thermal cycler, Biorad, Watford, UK). Amplified DNA was electrophoresed in a 2% agarose gel that had been pre-stained with 0.05g/mL ethidium bromide before being examined under an ultraviolet light source. The defined bands on the gel for the marker with a 100bp molecular weight (Fermentas, Germany) and positive samples were detected as being 582bp and 520bp in size.

Sequencing and Phylogenetic Analysis

Positive amplicons of the expected sizes for each pathogen identified in the fleas were purified and subjected to a one-way sequence analysis using ABI 3130 automated DNA Sequencer (Applied Biosystems, Thermo Fisher Scientific, Foster City, CA, USA) using the same forward and reverse primers for PCR. BLAST analysis was used to determine the closest similarities in the GenBank database (Altschul et al. 1990). Partial sequences of the 16S rRNA genes were matched with sequences of *Mycobacterium* or *Mycoplasma* retrieved from GenBank, using the Clustal/W multiple sequence alignment program version, 1.83 of the MegAlign module of Lasergene DNAStar software Pairwise (Thompson et al. 1994). The maximum likelihood phylogenetic trees were generated using the Mega 6.0 software (Tamura et al. 2013) with 100 bootstrap replicates for phylogenetic analyses.

Table 1: Target genes and oligonucleotide primers for *Mycobacterium* and *Mycoplasma*

Agent	Gene	Sequence	Size (bp)	References
Mycobacterium	*16S rRNA*	CACATGCAAGTCGAACGGAAAGG GCCCGTATCGCCCGCACGCTCACA	582	Tevere et al. (1996)
Mycoplasma	*16S rRNA*	CGCCTGAGTAGTACGTTCGC GCGGTGTGTACAAGACCCGA	520	Uphoff et al. (2012)

RESULTS

Morphology and Classification of Fleas

Based on the shape, location, and arrangement of setae, spines, and ctenidia throughout the body, all fleas collected from sheep raised on the Northern West coast of Egypt were recognized under the microscope as 138 *C. felis* (97 females and 41 males) and 85 *C. canis* (62 females and 23 males) based on the very complex genitalia's anatomy to determine the gender.

Identification and Genotypes of *Mycobacterium* and *Mycoplasma*

The current study showed for the first-time cases of *Mycobacterium* and *Mycoplasma* in *C. felis* and *C. anis* in Egypt (Fig. 1). It recorded the existence of the two pathogens (Table 2). *Mycobacterium* detected in males and females of *C. canis* in Matruh Governorate identified as *Mycobacterium bovis* under accession number ON076426. The three mycoplasmas were isolated from *C. felis* and *C. canis* from the Maryout location. The sequences of the three fragments of *Mycoplasma* identified in the current investigation were isolated from DNAs of the two species and sexes of fleas, such as *M. agalactia* (from all sexes of *C. felis*), *M. arginine* (from females of *C. canis*), and *M. ovipneumoniae* (from males *C. canis*). Such representative sequences of detected mycoplasmas are expressed under the accession numbers in the GenBank database: ON076423 (*M. agalactiae*), ON076424 (*M. arginini*), and ON076425 (*M. ovipneumoniae*).

Phylogenetic and Gene-sequencing Analysis

The sequence of *Mycobacterium bovis* got from this study was 90.2–100%, and *Mycoplasma* was identical from 82.8-100% to previously submitted sequences in GenBank (Borgers et al. 2019; Modipane et al. 2019; Oliveira et al. 2022). The divergence table of the 16S rRNA gene showed a divergence from 0.0 to 7.7 for *Mycobacterium* and from 0.0 to 17.3 for *Mycoplasma* between submitted and other isolates preserved in the GenBank database (Supplementary Tables 1, 2). The fragments of 582-bp for *Mycobacterium* and 520-bp for *Mycoplasma* targeted the 16S rRNA gene in *C. felis* and *C. canis* DNAs in the present study, besides other 16S rRNA sequences retrieved from GenBank, were used to establish a phylogenetic tree. The results showed that the phylogenetic trees confirmed a genetic diversity within *Mycoplasma* species and were distributed into three sub-clusters. Whereas *Mycobacterium bovis* isolated from fleas in the present study is identical to that separates from *Tapirus terrestris*, *Homo sapiens*, animal organs, and fleas in Belgium, Brazil, South Africa, and France (Fig. 2 and 3).

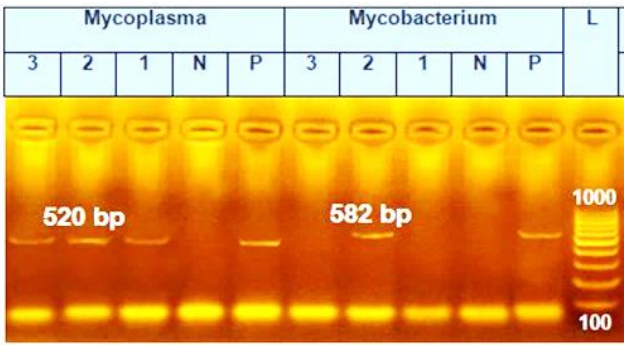

Fig. 1: Agarose gel electrophoresis on 2% showed positive results in lane 2 for *Mycobacterium* and lanes 1, 2, and 3 for *Mycoplasma* targeted *16S rRNA* gene. Positive samples amplified producing 582bp and 520bp, respectively. Lanes P and N correspond to positive and negative controls, respectively.

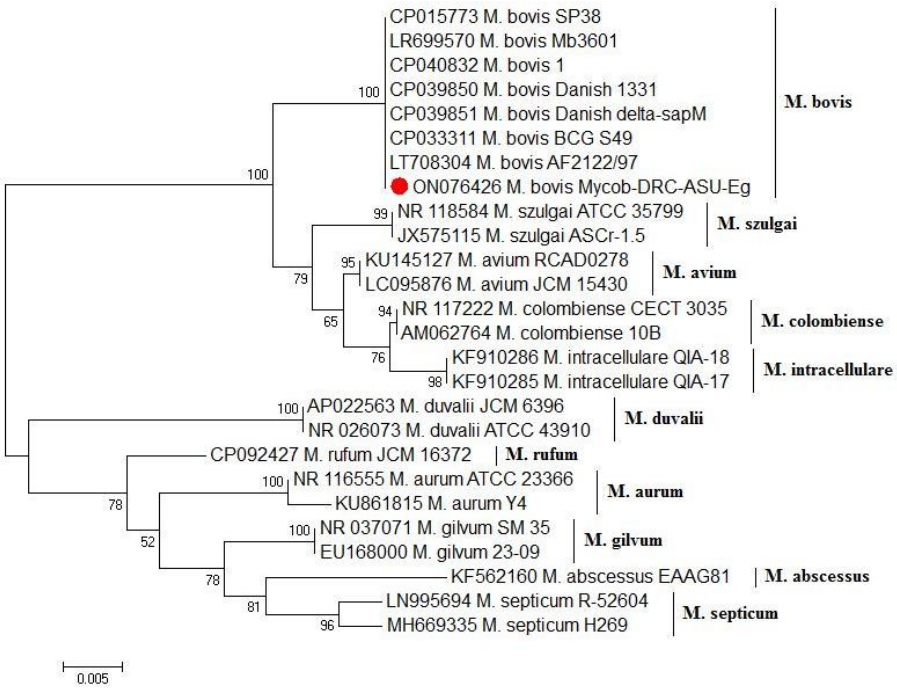

Fig. 2: A phylogenetic tree was constructed in MEGA 6.0 using the neighbor-joining method for analysis of the *16SrRNA* gene for *Mycobacterium bovis*. The sequence got in this study is shown in a red circle.

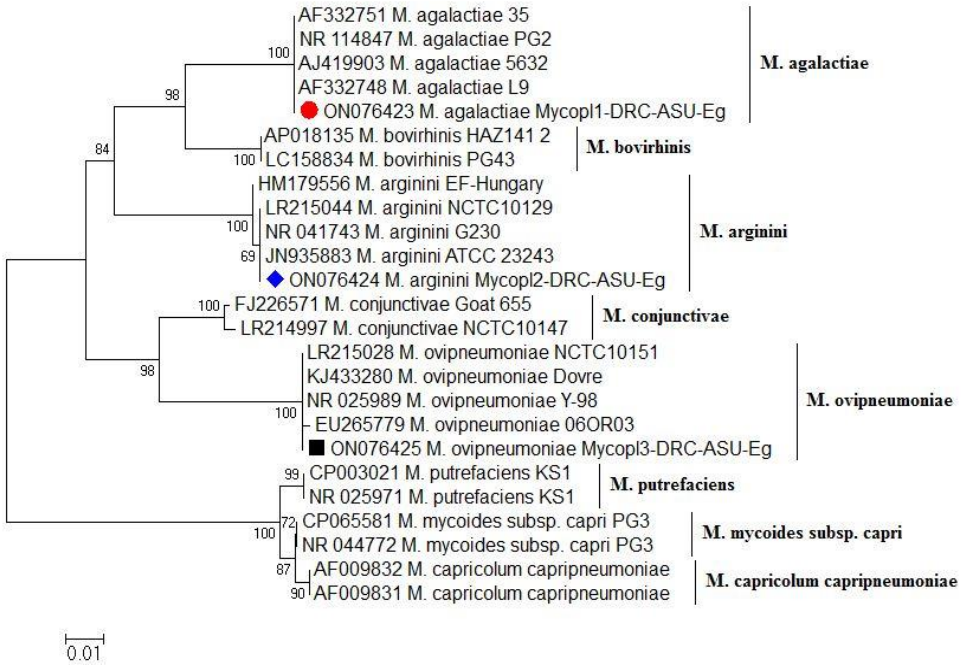

Fig. 3: A phylogenetic tree was constructed in MEGA 6.0 using the neighbor-joining method for analysis of the *16S rRNA* gene for *Mycoplasma spp*. The accession numbers got in this study are colored.

Table 2: GenBank database included identity and accession numbers of DNA isolates from fleas in the study areas

Location	Fleas			Submitted isolates	Accession No.
	Species	Sex	Host		
Marsa Matruh	*C. canis*	♀+ ♂	Sheep	*Mycobacterium bovis*	ON076426 *Mycob-DRC-ASU-Eg*
Maryout	*C. felis*	♀+♂	Sheep	*M. agalactiae*	ON076423 *Mycopl1-DRC-ASU-Eg*
Maryout	*C. canis*	♀	Sheep	*M. arginini*	ON076424 *Mycopl2-DRC-ASU-Eg*
Maryout	*C. canis*	♂	Sheep	*M. ovipneumoniae*	ON076425 *Mycopl3-DRC-ASU-Eg*

DISCUSSION

One of the most hematophagous arthropod vectors for the spread of numerous infections is the flea (Abdullah et al. 2019; Do et al. 2021). It has a widespread distribution; however, there isn't a thorough analysis of the frequency and presence of fleas infesting small ruminants in the research area in the Northern West of Egypt. Animal reproductive failure and economic losses are frequently caused by infectious abortion, particularly in camels in the research locations. *Mycoplasma bovis* was the principal cause of abortion, which was recovered from aborted animals, their placenta and their aborted fetus (Abo-Elnaga et al. 2012; Wassif and Balata 2020; Hussain et al. 2022). It was also detected in tick-infested camels (Barghash et al. 2012), but no previous history of *Mycobacterium* infection. Thus, the present study evaluated the prevalent flea species identified (*C. felis* and *C. canis*) to provide evidence that fleas could be able to transmit *Mycobacterium* and *Mycoplasma* or not.

Flea-borne bacterial diseases are significant medical diseases in humans. Of those, although plague and murine typhus have been recognized for centuries, novel flea-transmitted diseases, including *Rickettsia felis* and *Bartonella henselae* have emerged in recent years (Dobler and Pfeffer 2011) but have had little impact on *Mycobacterium bovis* in some developing countries. It is likely to be frequent but under-reported because of reliance on laboratory techniques that do not identify the different species within the *Mycobacterium tuberculosis* complex (Torres-Gonzalez et al. 2016). In other countries, there has been an alarming increase in the incidence of bovine tuberculosis. Recent reports highlighted the importance of understanding the epidemiology of mycoplasmas and described that close contact with cattle and small ruminants is an associated factor in developing the disease (Majoor et al. 2011; Gallivan et al. 2015). Among the various *Mycoplasma* species, *C. felis* was listed as a possible vector for *M. Candidatus* and *Candidatus M. haemominutum* (Shaw et al. 2004; Woods et al. 2005).

In Egypt, scientists have found *M. arginini*, *M. ovipneumoniae*, and *M. agalactiae*, among other *Mycoplasma* species (Ammar et al. 2008). *M. arginine* is frequently isolated from pneumonic sheep and goats in Egypt (Abdel Halium et al. 2019). Although *Mycoplasma bovis* has been described in ticks, camels, and small ruminants in the deserts of Egypt (Barghash et al. 2016; Mahmoud et al. 2019; Wassif and Balata 2020), no information exists about the association between *Mycobacterium*, *Mycoplasma*, and fleas in our geographic location. The present study revealed *Mycobacterium bovis* and *Mycoplasma* in *C. canis* and *C. felis* DNAs in Egypt for the first time and made a case for the potential involvement of fleas in the spread of the two pathogens.

Mycoplasma can cause pneumonia, conjunctivitis, arthritis, and mastitis (Kumar et al. 2013). Pneumonia in small ruminants is frequently associated with

Mycoplasma species such as *Mycoplasma ovipneumoniae*, *M. arginini*, *M. capri*, *M. capripneumoniae*, and *M. capricolum* (Chinedu et al. 2016). *M. ovipneumoniae* and *M. arginini* are often found in typical pneumonia in sheep and goats or with lung consolidation (Kumar et al. 2013; Fernández et al. 2016). *M. arginini* has been found in places like the genital organs, eyes, and ears (Olusola et al. 2015). Its isolation from diseased cases in humans raises the possibility that *M. arginini* may have zoonotic importance (Mayumi et al. 2017). *M. agalactiae* is the main cause of contagious agalactia and is an OIE-listed disease characterized by the MAKePS syndrome (Valsala et al. 2017). Even though it is detected in pneumonic cases more often linked to mastitis and abortion.

In the present study, fleas-infested sheep harboured *Mycobacterium bovis* and mycoplasmas, with sequence significant data identities of >90%-100% and >82%-100%, respectively, compared to archived genes. According to phylogenetic tree analysis, the present isolation of *Mycobacterium bovis* (ON076426) is present in one clade with seven preserved strains in GenBank with 100% identity and the highest bootstrap value. Of the identical strains were CP039850 and CP039851 recorded from Belgium (Borgers et al. 2019), CP040832 (Brazil) (Oliveira et al. 2022), and CP033311 (South Africa) (Modipane et al. 2019). They were isolated respectively from *Tapirus terrestris*, *Homo sapiens*, animal organs, and fleas.

The present study also showed genetic diversity within *Mycoplasma* species clustered in three clades. One contained the first sequence of ON076423 (*M. agalactiae*), which is 100% identical to AF332751, AF332748 (Sweden) (Konigsson et al. 2002), and NR_114847 (USA) (Pilo et al. 2003). The second sequence of ON076424 (*M. arginini*) in the second cluster is 100% identical to LR215044 (United Kingdom), NR_041743 (Sweden) (Pettersson et al. 2000), and JN935883 (USA) (Volokhov et al. 2012). Whereas the third sequence of ON076425 (*M. ovipneumoniae*) in a separate cluster is 100% identical to LR215028 (United Kingdom), KJ433280 (Norway) (Handeland et al. 2014), NR_025989 (Pettersson et al. 1996), and EU265779 (western North America) (Besser et al. 2008) isolated from different sheep.

Conclusion

This is the first molecular detection of *Mycobacterium* and mycoplasmas in both *C. canis* and *C. felis*-infested small ruminants settled in the Northern West coastal zone of Egypt. Identified sequenced isolates were assigned in the GenBank database under accessions ON076426 (*Mycobacterium bovis*), ON076423 (*M. agalactiae*), ON076424 (*M. arginini*), and ON076425 (*M. ovipneumoniae*).

Acknowledgment

The researchers gratefully acknowledge the financial and scientific support from the Desert Research Center (DRC), and the Faculty of Science, Ain Shams University (ASU), Cairo, Egypt. We would also like to thank the workers in the study area for their help during this study.

Authors' Contributions

MS, DM, and SM designed the study and revised the manuscript. SY collected and identified fleas, and also corresponds to the paper. SB carried out the molecular analysis. AH prepared PPLOs broth. SB, AH, and SY wrote the manuscript. The final manuscript was read and approved by all authors.

Conflict of Interest

There are no conflicts of interest, according to the authors.

REFERENCES

Abdel Halium MMA, Salib FA, Marouf SA and Massieh ESA, 2019. Isolation and molecular characterization of *Mycoplasma* spp. in sheep and goats in Egypt. Veterinary World 12(5): 664-670. https://doi.org/10.14202/vetworld.2019.664-670

Abdullah S, Helps C, Tasker S, Newbury H, and Wall R, 2019. Pathogens in fleas collected from cats and dogs: Distribution and prevalence in the UK. Parasites &Vectors 12: 1-10. https://doi.org/10.1186/s13071-019-3326-x

Abo-Elnaga TR, Gouda AS, Kamel YM, Mahmoud MA and Osman WA, 2012. Application of the polymerase chain reaction for identification of *Mycoplasma* isolated from aborted she camel. Global Veterinaria 8 (4): 386-392.

Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ, 1990. Basic local alignment search tool. Molecular Biology 215 (3): 403-410.

Ammar AM, Eissa SI, El-Hamid MIA, Ahmed HA and ElAziz AEE, 2008. Advanced study on Mycoplasmas isolated from sheep. Zagazig Veterinary Journal 36: 128-137.

Barghash SM, Hafez AA, Darwish AM and El-Naga TRA, 2016. Molecular detection of pathogens in ticks infesting camels in Matrouh Governorate, Egypt. Bacteriology & Parasitology 7: 259. https://doi.org/10.4172/2155-9597.1000269

Besser TE, Cassirer EF, Potter KA, VanderSchalie J, Fischer A, Knowles DP, Herndon DR, Rurangirwa FR, Weiser GC and Srikumaran S, 2008. Association of *Mycoplasma ovipneumo*niae infection with population-limiting respiratory disease in free-ranging Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*). Clinical Microbiology 46 (2): 423-430.

Beugnet F and Marie JL, 2009. Emerging arthropod-borne diseases of companion animals in Europe. Veterinary Parasitology 163: 298-305. https://doi.org/%2010.1016/j.vetpar.2009.03.028

Bitam I, Dittmar K, Parola P, Whiting MF and Raoult D, 2010. Fleas and flea-borne diseases. International Journal of Infectious Diseases 14: e667-e676. https://doi.org/10.1016/j.ijid.2009.11.011

Borgers K, Ou JY, Zheng PX, Tiels P, Van Hecke A, Plets E, Michielsen G, Festjens N, Callewaert N and Lin YC, 2019. Genome and comparative genome analysis for the WHO reference strain for *Mycobacterium bovis* BCG Danish, the present tuberculosis vaccine. BMC Genomics 20: 561. https://doi.org/10.1186/s12864-019-5909-5

Bosila MA, Mekky HM, Fedawy HS, Elbayomi KM and Amer MM, 2021. Histopathological lesion of arthritis in *Mycoplasma synoviae* naturally infected breeder chicken in Egypt. International Journal of Veterinary Science 10: 72-74. https://doi.org/10.47278/journal.ijvs/2020.006

Chinedu AA, Kennedy FC, Stephen IO and Jude SR, 2016. Pathogenicity of local isolates of *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* in experimental

West African dwarf goats. Advanced Veterinary and Animal Research 3(3): 242-251.

Desouky SM, Elgedawy AA, Abdel-Moein KA and Samir A, 2023. Bacteriological and molecular studies on *Mycobacterium bovis* in cattle, with special reference to its antimicrobial resistance. International Journal of Veterinary Science 12(1): 54-59. https://doi.org/10.47278/journal.ijvs/2022.155a

Do T, Inpankaew T, Duong DH and Bui KL, 2021. First molecular evidence of pathogens in fleas collected from dogs in Northern Vietnam. Pathogens 10(9): 1185. https://doi.org/10.3390/pathogens10091185

Dobler G and Pfeffer M, 2011. Fleas as parasites of the family *Canidae*. Parasites & Vectors 4: 139. https://doi.org/10.1186/1756-3305-4-139

Fernández S, Galapero J, Rey J, Pérez CJ, Ramos A, Rosales R, Ayling R, Alonso JM and Gómez L, 2016. Investigations into the seasonal presence of *Mycoplasma* species in fattening lambs. Veterinary Journal 212: 80-82. https://doi.org/10.1016/j.tvjl.2016.04.006

Gallivan M, Shah N and Flood J, 2015. Epidemiology of human *Mycobacterium bovis* disease, California, USA, 2003–2011. Emerging infectious diseases 21: 435-443. https://doi.org/10.3201/eid2103.141539

Glen CW, Mark LD, Frances EC and Alton CSW, 2012. Detection of *Mycoplasma ovipneumoniae* and *M. arginini* in bighorn sheep using enrichment culture coupled with genus and species-specific polymerase chain reaction. Wildlife Diseases 48(2): 449-453.

Hakim AS, Bakry MA, Nagwa SSA and Mona SZ, 2014. Role of molecular techniques in the characterization of bacteria causing pneumonia in small ruminants. Life Science Journal 11(6): 147-153.

Handeland K, Tengs T, Kokotovic B, Vikoren T, Ayling RD, Bergsjo B, Sigurethardottir OG and Bretten T, 2014. *Mycoplasma ovipneumoniae*-A Primary cause of severe pneumonia epizootics in the Norwegian Muskox (*Ovibos moschatus*) population. PLoS ONE 9 (9): E106116. https://doi.org/10.1371/journal.pone.0106116

Hussain K, Rabbani AH, Ali A, Ijaz M, Khan YR, Shahid M, Sohail ML, Naseer O, Ahmad AS and Prince K, 2022. Bacterial diseases affecting sheep and goats. In: Abbas RZ, Khan A, Liu P and Saleemi MK (eds), Animal Health Perspectives, Unique Scientific Publishers, Faisalabad, Pakistan 2: 97-102. https://doi.org/10.47278/book.ahp/2022.47

Konigsson MH, Bolske G and Johansson KE, 2002. Intraspecific variation in the 16S rRNA gene sequences of *Mycoplasma agalactiae* and *Mycoplasma bovis* strains. Veterinary Microbiology 85 (3): 209-220.

Kumar V, Rajneesh R, Somya M and Pramod KR, 2013. Isolation and characterization of *Mycoplasma mycoides* subspecies capri from milk of natural goat mastitis cases. ISRN Veterinary Science https://doi.org/%2010.1155/2013/593029

Mahmoud MA, Wassif IM, El-Sayed AA, Awad WSA, Noaman EA and El-kattan AM, 2019. Some epidemiological studies on camel mycoplasmosis in Egypt. Egyptian Veterinary Medical Association 79 (3): 699-709. https://www.researchgate.net/publication/337654586

Majoor CJ, Magis-Escurra C, van Ingen J, Boeree MJ and van Soolingen D, 2011. Epidemiology of *Mycobacterium bovis* disease in humans. The Netherlands, 1993–2007. Emerging infectious diseases 17: 457-463.

Mayumi W, Shigemi H, Miki G and Yuichi H, 2017. Bloodstream infection due to *Mycoplasma arginini* in an immunocompromised patient. Clinical Microbiology and Infection 50(9): 3133-3135. https://doi.org/10.1128/JCM.00736-12

Modipane L, Reva O, Magazi BT, Osei Sekyere J, Antiabong JF and Mbelle NM, 2019. Draft-genome sequences of two clinical *Mycobacterium bovis* BCG strains from South Africa. International Journal of Infectious Diseases 87: 32-38. https://doi.org/10.1016/j.ijid.2019.08.010

Nguyen V-L, Colella V, Greco G, Fang F, Nurcahyo W, Hadi UK, Venturina V, Tong KBY, Tsai Y-L, Taweethavonsawat P, Tiwananthagorn S, Tangtrongsup S, Le TQ, Bui KL, Do T, Watanabe M, Rani PAM, Dantas-Torres F, Halos L, Beugnet F and Otranto D, 2020. Molecular detection of pathogens in ticks and fleas collected from companion dogs and cats in East and Southeast Asia. Parasites & Vectors 13: 420. https://doi.org/10.1101/2020.05.27.118554

Nicholas R, Ayling R and McAuliffe L, 2008. Respiratory Diseases of Small Ruminants. In: *Mycoplasma* Diseases of Ruminants. 1st Ed. CABI International, Oxfordshire, UK, pp: 169-198.

Olusola MO, Anna K, Stuart RB, Kelly AT, Philip FM, Marc SM and Glenn FB, 2015. Genetic diversity of *Mycoplasma arginini* isolates based on multilocus sequence typing. Veterinary Microbiology 180(1–2): 123-128.

Oliveira FMD, Procopio VO, de Lima Menezes G, Silva RAD, Kipnis A and Junqueira-Kipnis AP, 2022. *Mycobacterium bovis* PknG R242P mutation results in structural changes with enhanced virulence in the mouse model of infection. Microorganisms 10: 673. https://doi.org/10.3390/%20microorganisms10040673

Pettersson B, Tully JG, Bolske G and Johansson KE, 2000. Updated phylogenetic description of the *Mycoplasma hominis* cluster based on 16S rDNA sequences. International Journal of Systematic and Evolutionary Microbiology 50: 291-301. https://doi.org/10.1099/00207713-50-1-291

Pettersson B., Uhlen M and Johansson KE, 1996. Phylogeny of some mycoplasmas from ruminants based on 16S rRNA sequences and definition of a new cluster within the hominis group. International Journal of Systematic and Bacteriology 46 (4): 1093-1098.

Pilo P, Fleury B, Marenda M, Frey J and Vilei EM, 2003. Prevalence and distribution of the insertion element ISMag1 in *Mycoplasma agalactiae*. Veterinary Microbiology 92 (1-2): 37-48.

Sandip C, Amit K, Ruchi T, Anu R, Yash M, Kuldeep D, Amar P and Minakshi P, 2014. Advances in the diagnosis of respiratory diseases of small ruminants. Veterinary Medicine International 2014: 508304. https://doi.org/10.1155/2014/508304

Shaw SE, Kenny MJ, Tasker S and Birtles RJ, 2004. Pathogen carriage by the cat flea *Ctenocephalides felis* (Bouché) in the United Kingdom. Veterinary Microbiology 102(3-4): 183-188.

Skuratowicz W, 1967. Pchly-Siphonaptera (Aphaniptera). *Klucze do Oznaczania Owadów Polski* [Keys for the identification of Polish Insects] 29: 1-141.

Smith RM, Drobniewski F, Gibson A, Montague JD, Logan MN, Hunt D, Hewinson G, Salmon RL and O'Neill B. 2004. *Mycobacterium bovis* infection, United Kingdom. Emerging Infectious Diseases 10(3): 539-541.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30 (12): 2725-2729.

Tevere VJ, Hewitt PL, Dare A, Hocknell P, Keen A, Spadoro JP and Young KK 1996. Detection of *Mycobacterium tuberculosis* by PCR amplification with pan-*Mycobacterium* primers and hybridization to an *M. tuberculosis* specific probe. Journal of Clinical Microbiology 34: 918-923.

Thompson JD, Higgins DG and Gibson TJ, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22 (22): 4673-4680.

Torres-Gonzalez P, Cervera-Hernandez ME, Martinez-Gamboa A., Garcia-Garcia L, Cruz-Hervert LP, Valle M-B, Ponce-de Leon AP and Sifuentes-Osornio J, 2016. Human tuberculosis caused by *Mycobacterium bovis*: a retrospective comparison with *Mycobacterium tuberculosis* in a Mexican Tertiary Care Centre, 2000–2015. BMC Infectious Diseases 16: 657. https://doi.org/10.1186/s12879-016-2001-5

Uphoff CC, Denkmann S-A and Drexler HG, 2012: Treatment of *Mycoplasma* contamination in cell cultures with plasmocin. Journal of Biomedicine and Biotechnology 2012: 267678. https://doi.org/10.1155/2012/267678

Valsala R, Rana R, Remesh AT and Singh VP, 2017. *Mycoplasma arginini*: High frequency involvement in goat pneumonia. Turkish Journal of Veterinary & Animal Sciences 41: 393-399. https://doi.org/10.3906/vet-1604-75

Volokhov DV, Simonyan V, Davidson MK and Chizhikov VE, 2012. RNA polymerase beta subunit (rpoB) gene and the 16S-23S rRNA intergenic transcribed spacer region (ITS) as complementary molecular markers in addition to the 16S rRNA gene for phylogenetic analysis and identification of the species of the family *Mycoplasmataceae*. Molecular Phylogenetics and Evolution 62 (1): 515-528.

Wassif IM and Balata MAE, 2020. Comparative study of pathogenic factors of *Mycoplasma agalactiae* isolates. International Journal of Clinical Skills 14(1): 301-305.

Woods JE, Brewer MM, Hawley JR, Wisnewski N and Lappin MR, 2005. Evaluation of experimental transmission of *Candidatus Mycoplasma haemominutum* and *Mycoplasma haemofelis* by *Ctenocephalides felis* to cats. American Journal of Animal and Veterinary Sciences 66(6): 1008-1012.

Wu C, Guo XQ, Cheng S, Li G and Liu P, 2021. Viral and bacterial zoonotic diseases. In: Abbas RZ and Khan A (eds), Veterinary Pathobiology and Public Health, Unique Scientific Publishers, Faisalabad, Pakistan, pp: 418-430. https://doi.org/10.47278/book.vpph/2021.035