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SOME EPIDEMIOLOGICAL STUDIES ON CAMEL MYCOPLASMOSIS IN EGYPT

By

Mona A. Mahmoud [1]; Wassif, I. M.[1]; El-Sayed, A.A. [2]; Awad, W.S.A. [2];

Eman A. Noaman [1]and El-kattan, A. M. [1]

[1]Department of Animal Health, Desert Research Center, Cairo, Egypt.

[2]Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University.

ABSTRACT

The prevalence of Mycoplasmosis in diseased and apparently healthy camels (in contact) was studied in consideration of the effect of season, age, sex and locality in relation with mycoplasmas, *Mycoplasma bovis* and *Mycoplasma bovirhinis* isolation rates.

It was significantly higher during summer than winter and the most susceptible age group was from five to ten years except diseased group with mycoplasmas and *Mycoplasma bovis* infections. Male camels were found to be more susceptible to Mycoplasmosis than she-camels and higher prevalence in samples obtained from El-Basateen abattoir than those obtained from the Northwestern coastal zone of Egypt except only diseased group with *Mycoplasma bovis* infection in which the prevalence was higher in she-camels and at Northwestern coastal zone.

Keywords:

Mycoplasmosis, Camel, Epidemiology.

INTRODUCTION

Camel is a multi-purpose animal with a huge productive potential and the most suitable domestic animal for use in climatic extremes **(Wernery, 2007**). The world's camel population is about 28 million heads,80% of them lives in Africa, with 60% in the Horn of Africa, Arabian camels (Dromedaries) constitute 94% of the world's camel population **(FAO STAT, 2014 and Yam and Khomeiri, 2015)**. During the last two decades, camels breeding industry has become in continuous development to achieve maximum benefit from camels' milk and meat productive potential, as well as hide and hair manufacturing **(Ahmad *et al.,* 2010)**. *Mycoplasmas* are distinguished phenotypically from other bacteria by their minute size (125-150 mill micron) and total lack of a cell wall that explains many of the unique properties

of the *mycoplasma*, such as sensitivity to osmotic shock and detergents, resistance to penicillin, and formation of peculiar fried-eggs shape colonies. Clinical mycoplasmosis often lacks pathognomonic characteristics sharing the symptoms of some other clinical infections **(DaMassa,1996).** Under certain conditions *Mycoplasma*, either solely or following bacterial and/or viral infection cause health problems such as pneumonia, abortion and arthritis **(Freundt,1985)**.In spring 2011,a severe respiratory disease occurred in Iran, Pakistan and Afghanistan affecting several thousand dromedaries with high mortality. Several promed reports were released. From Iran, CVRL (Central Veterinary Research Laboratory, Dubai, and UAE) received blood and nasal swabs from diseased animals. *Mycoplasma* spp. were isolated from several swabs **(Wernery and Kinne 2012)**. **Awol *et al.* (2011)** mentioned that, *Mycoplasma* is one of the causative agents of camel's respiratory disease outbreak since 1995 in Ethiopia.This work was designed to throw spot light on some epidemiological studies of Mycoplasmosis in diseased and apparently healthy camels including the effect of season, age, sex and locality. The isolates of this work; *Mycoplasma bovis* and *Mycoplasma bovirhinis* were identified in previous study **(Mona *et al*., 2017)**.

MATERIAL AND METHODS

Animals and samples:

A total of 369 randomly selected dromedary camels from August 2014 to June 2016 (Table 1) were examined clinically for respiratory manifestations as cough and nasal discharges, in live camels whereas slaughtered camels were examined for the pathological condition of their lungs to be divided into apparently healthy (in contact) (179) and diseased groups (190) of both sexes (175 males and 194 females for nasal swabs samples, (Table 2) and lung tissue samples,(Table3) with various ages (five to over than ten years) were involved. Investigated live camels were belonging to Northwestern coastal zone (Matrouh Governorates and Maryout station of the Desert Research Center that lies 35 Km southwest of Alexandria), whereas, investigated camels belonged to El-Basateen Abattoir were imported from Sudan for slaughtering.

Table (1): Number and type of collected samples from camel.

	Health condition of the tested camels					
	Diseased			Apparently healthy		
Locality / Samples	Northwestern coastal zone	El-Basateen abattoir	Total	Northwestern coastal zone	El-Basateen abattoir	Total
Nasal swabs	75	51	126	95	50	145
Lung tissues	0	53	53	0	45	45
Total	75	104	179	95	95	190

Table (2): Number of nasal swabs collected according to Season, Age, Sex and Locality.

Health condition / According to		Diseased	Apparently healthy	Total
Season	Summer	56	69	125
	Winter	70	76	146
Age	5-10 Y	103	116	219
	> 10 Y	23	29	52
Sex	Male	37	48	85
	Female	89	97	186
Locality	Northwestern coastal zone	75	95	170
	El-Basateen abattoir	51	50	101

Table (3): Number of Lung tissues collected according to Season, Age, Sex and Locality.

Health condition / According to		Diseased	Apparently healthy	Total
Season	Summer	25	25	50
	Winter	28	20	48
Age	5-10 Y	53	45	98
Sex	Male	50	40	90
	Female	3	5	8
Locality	El-Basateen abattoir	53	45	98

2- Bacteriological, biochemical and molecular identification of isolated mycoplasmas:

The studying mycoplasmas isolates in this work were previously identified in **Mona *et al.*, (2017).** A sterile cotton swabs were inserted into nostrils of examined camels, immediately inoculated into Modified Hayflick's broth medium **(Rosendal, 1994)**.

Lung tissues collected from slaughtered camels were packed in sterile plastic bags separately. Samples were transported in an icebox to the laboratory for immediate bacteriological examination or stored in a refrigerator (4°C) or in deep freeze (-20°C) until be examined.

The collected lung tissues were seared in the laboratory using a red-hot spatula, cut open and swab was taken from the cut and inoculated into Modified Hayflick's broth medium. Swabs from nostrils and lung tissues were cultivated on Modified Hayflick's solid medium **(Rosendal, 1994)** by direct method (samples were spread directly on agar plates) and indirect method (samples were inoculated into broth, which incubated for 3 and 6 days then plated, or re-inoculated in broth which plated after 3- and 6-days incubation). All plates were incubated at 37°C in humidified candle jar for two weeks and examined microscopically using stereomicroscope every 2-3 days until "fried egg" colonies appeared, followed by purification of the isolates according to **Sabry and Ahmed (1975)**. Isolated Mycoplasms colonies were maintained at-20°C either as 2-3 ml aliquots of actively growing broth culture or as agar blocks/strips in a sterile screw capped vial. Purified Mycoplasms isolates were tested for Digitonin sensitivity **(Freundt, 1983)** to be differentiated into *Mycoplasma* (Digitonin sensitive, showing 6-10 mm zone of inhibition around Digitonin discs) and *Acholeplasma* (Digitonin resistant, showing no inhibition zone). Identified isolates as *Mycoplasma* were tested for Arginine utilization, Glucose fermentation **(Sabry, 1968**) and Film and spot formation **(Cottew,1983)**. Molecular identification of all biochemically-grouped *Mycoplasma* on the genus level was done using PCR for detection of 16S rRNA gene belonging to all *Mycoplasma*, followed by identification on the species level for detection of specific 16S rRNA gene for each of *M. bovis* and *M. bovirhinis*. The identification was fully described in **Mona *et al*., (2017)**.

3-Statistical analysis:

The obtained data were statistically analyzed using M.S. Excell 2010 software to get significance ($P < 0.005$).

RESULTS AND DISCUSSION

This work was designed to study the epidemiology of Mycoplasmosis in camels as little is known about its epidemiology in this neglected animal species **Jenberie *et al.*, (2012)**. Bacteriological and molecular identification of isolated mycoplasmas were done in previous study **(Mona *et al*., 2017)** as 67 (18.2 %) mycoplasmas were recovered from 369 nasal swabs and

lung tissues samples, which were differentiated using Digitonin sensitivity test into *Mycoplasma* spp. (35, 9.5%) and *Acholeplasma* spp. (32, 8.7 %). From 35 isolated *Mycoplasma* spp., *M. bovis* (24, 82.9%) and *M. bovirhinis* (6, 17.1%) were identified biochemically and confirmed by specific PCR as five isolates could not be identified. Concerning the seasonal effect on the prevalence of mycoplasmas in camels as showed in (Table 4), Fig. (1), a higher prevalence rate was recorded during summer (11.4%) than winter (7.7%) probably due to the effects of climatic changes as suggested by **(Ahmed and Musa 2015)** and mycoplasmas are normal inhabitant in the respiratory tract under stress condition as winds in early summer they may cause pneumonia alone or together with viruses and or bacteria as reported by **Freundt (1985),** transmitted through direct contact with respiratory secretions (air born infection) as described by **Caswell and Archambault (2008)** then Colonization in respiratory epithelium as mentioned by **Baskervile (1981)** and its affinity to the junction of alveolar duct and gas exchange site as mentioned by **Caswell and Williamas (2007)** due to its minute size and self-multiplication as said by **Razin *et al.* (1998)**.

Table (4): Effect of Season, Age, Sex and locality on the prevalence of mycoplasmas isolated from diseased and apparently healthy camels.

Factor	Variable	Camel Health condition						Total		
		Diseased			Apparently healthy					
		No. 1	No. 2	%	No. 1	No. 2	%	No. 1	No. 2	%
Season	Summer	81	5	6.2	94	15	16	175	20	11.4
	Winter	98	7	7.1	96	8	8.3	194	15	7.7
Age	5: 10 Y	156	10	6.4	161	22	13.7	317	32	10.1
	> 10 Y	23	2	8.7	29	1	3.5	52	3	5.8
Sex	Male	87	6	6.9	88	20	22.7	175	26	14.9
	Female	92	6	6.5	102	3	2.9	194	9	4.6
Locality	North-western coastal zone	75	5	6.6	95	6	6.3	170	11	6.5
	El-Basateen Abattoir	104	7	6.7	95	17	17.9	199	24	12.1

No. 1: Number of examined animals.

No. 2: Number of bacteriologically positive animals for mycoplasmas.

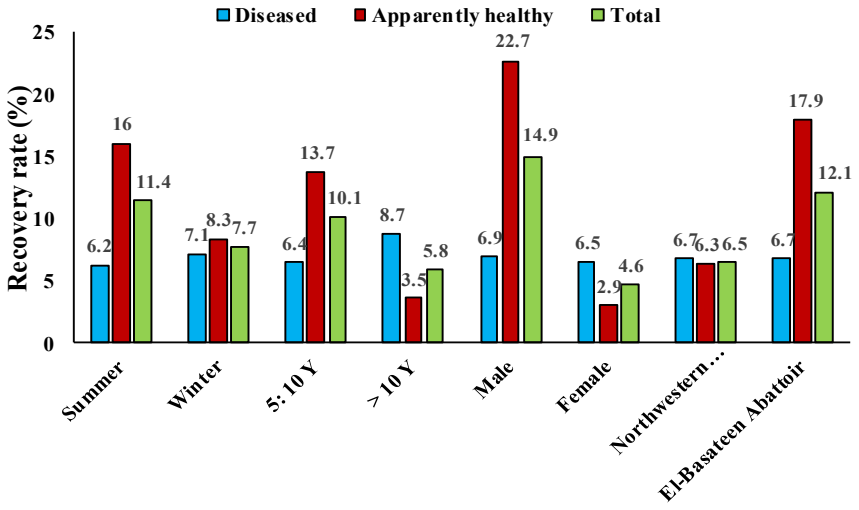

Fig. (1): Effect of Season, Age, Sex and locality on the prevalence of mycoplasmas isolated from diseased and apparently healthy camels.

Concerning the camel age, camels of (5-10 years) showed higher isolation rate of mycoplasmas than the older camels (>10 years), however the isolation rate from diseased camels was higher in the older group in agreement with **Hasaneen *et al.* (2013) and Mederos-Iriarte *et al.* (2014)** who suggested that, from five to ten years camels were more infected due to their higher susceptibility to acute and chronic infection. From the gender point of view, the isolation rate of mycoplasmas was higher in male than female (Table 4), Fig.(1). This result may be explained by most of male samples collected from El-Basateen abattoir in which many stress factors are present such as environmental change, extremes of climatic conditions, transportation and shortage of feeds and/or water as reported by **Awol *et al.* (2011)** and most of female samples collected from Northwestern coastal zone in which She-camels were stable in this area.The effect of locality on the prevalence of Mycoplasmosis from camels is investigated. The higher rate of isolation found in abattoir (12.1%) than Northwestern coastal zone (6.5%) (Table 4), Fig. (1). It may be attributed to many stress factors facing camels during transportation to abattoir as they are mostly imported from different countries that enhancing the opportunistic bacteria as mycoplasmas in agreement with **Wareth *et al.* (2014)**. On the other hand, in Northwestern coastal zone camel's accommodation to their environment (low stress condition) as reported by **El-Gmaal (2007).**

Concerning the seasonal, age, sex and locality effects on the prevalence of total *M. bovis* isolates**,** a higher prevalence rate was recorded during summer (6.9%) than winter (6.2%), mostly, the isolation was from camels five to ten years old (6.6%), infection was higher in male (9.7%) than female (3.6%) and the higher rate of isolation found in abattoir (7.5%) than Northwestern coastal zone (5.3%) as showed in (Table 5) , Fig. (2). But the prevalence of *M. bovis* in diseased camels was higher during winter than summer, over ten years age than youngers, females than males and Northwestern Coastal Zone than El- Basateen Abattoir on the opposite the prevalence of *M. bovis* isolated from apparently healthy (in contact) camels. This result may be attributed to the wide pathogenicity of *M. bovis* **(Wassif *et al.,* 2017**) as **Tully, (1979)** reported that Mastitis caused by *M. bovis* has been correlated with its presence in the nose. This described the higher prevalence in she-camel and in winter as the incidence of mastitis increase. Beside the chronicity characteristic of *M. bovis* in old age **(Wassif *et al.,* 2017**).The prevalence of *M. bovirhinis* as showed in (Table 6), Fig. (3), a higher prevalence rate was recorded during summer (3.4%) than winter (0%) and within camels five to ten years (1.9%), infection was higher in male (2.9%) than female (0.5%) and the higher rate of isolation found in abattoir (3%) than Northwestern coastal zone (0%).

Table (5): Effect of Season, Age, Sex and locality on the prevalence of *M. bovis* isolated from diseased and apparently healthy camels.

Factor	Variable	Camel Health condition						Total		
		Diseased			Apparently healthy					
		No. 1	No. 2	%	No. 1	No. 2	%	No. 1	No. 2	%
Season	Summer	81	1	1.2	94	11	11.7	175	12	6.9
	Winter	98	6	6.1	96	6	6.3	194	12	6.2
Age	5: 10 Y	156	5	3.2	161	16	10	317	21	6.6
	> 10 Y	23	2	8.7	29	1	3.5	52	3	5.8
Sex	Male	87	3	3.5	88	14	16	175	17	9.7
	Female	92	4	4.3	102	3	3	194	7	3.6
Locality	Northwestern coastal zone	75	4	5.3	95	5	5.3	170	9	5.3
	El-Basateen Abattoir	104	3	2.9	95	12	12.6	199	15	7.5

No. 1 means Number of examined animals.

No. 2 means Number of bact. positive animals for *M. bovis*.

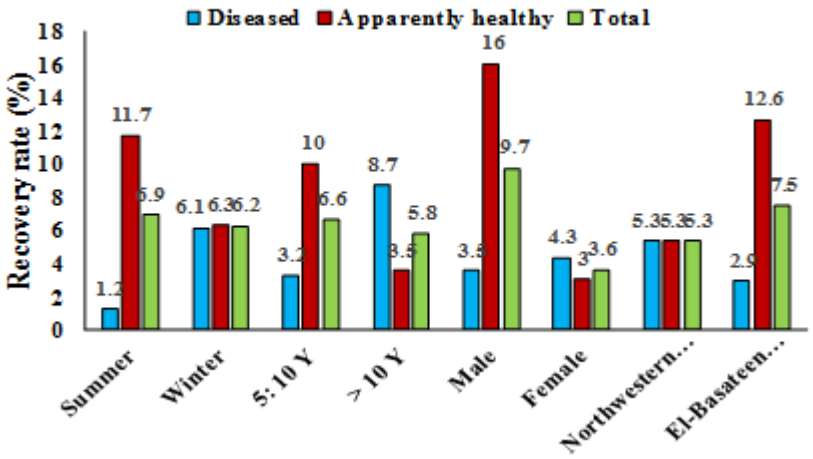

Fig. (2): Effect of Season, Age, Sex and locality on the prevalence of *M. bovis* isolated from diseased and apparently healthy camels.

Table (6): Effect of Season, Age, Sex and locality on the prevalence of *M. bovirhinis* isolated from diseased and apparently healthy camels.

Factor	Variable	Camel Health condition						Total		
		Diseased			Apparently healthy					
		No. 1	No. 2	%	No. 1	No. 2	%	No. 1	No. 2	%
Season	Summer	81	3	3.7	94	3	3.2	175	6	3.4
	Winter	98	0		96	0		194	0	
Age	5: 10 Y	156	3	1.9	161	3	1.9	317	6	1.9
	> 10 Y	23	0		29	0		52	0	
Sex	Male	87	2	2.3	88	3	3.4	175	5	2.9
	Female	92	1	1.1	102	0		194	1	0.5
Locality	Northwestern coastal zone	75	0		95	0		170	0	
	El-Basateen Abattoir	104	3	2.9	95	3	3.2	199	6	3

No. 1 means Number of examined animals.

No. 2 means Number of bacteriologically positive animals for *M. bovirhinis*.

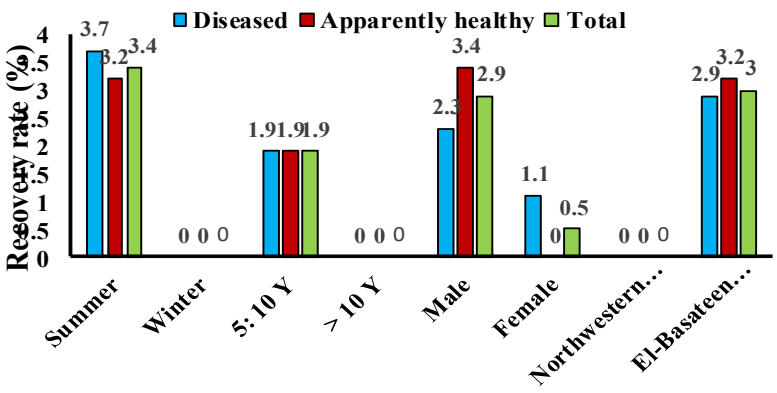

Fig. (3): Effect of Season, Age, Sex and locality on the prevalence of *M. bovirhinis* isolated from diseased and apparently healthy camels.

CONCLUSIONS

In the present epidemiological study on camel mycoplasmosis due to different mycoplasmas (*M. bovis*-*M. bovirhinis*) and *Acholeplasma* spp.which were isolated from nasal swabs and lung tissues of diseased and apparently healthy camels.High prevalence of mycoplasmas in the samples obtained from El- Basatian abattoir than those originating from north-western coast. Male camels were found to be more susceptible to mycoplasmosis than she-camels. The most susceptible age is the mean age (5:10 Y) in apparently healthy camel. However, high prevalence of mycoplasmosis in old diseased age (> 10 Y). The prevalence of mycoplasmosis in samples collected during summer was significantly higher than among samples collected in winter.

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