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OCCURRENCE OF MYCOPLASMA SPECIES IN CAMELS IN EGYPT

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SUMMARY

The present study was conducted on a total of 200 one-humped camels (Camelus dromedarius) . Two hundred and ninty samples were collected from the respiratory tract. Mycoplasmas could be isolated from pneumonic lungs but not from apparently normal lungs. A total of 122 Mycoplasma and Acholeplasma strains were recovered and the highest was nasal swabs (87.50%) and the lowest was from mediastinal L.N. (16.66%). *M.arginina* and *A.laidlawii* could be identified, while *A. oculi* could be isolated from nasal and pneum onic lungs only.

Four hundred thirty samples from urogenital tract revealed the presence of 88 isolates from both female and male genital tract. The rate isolation was higher from the vagina of non pregnant she camels (22.85%) in contrast to (14.28%) among pregnant she camels. *M.arginini, A. laidlawii* could be identified , in addition to Ureaplasma species from the genital tract of camels. Its distribution in the genital tract of both male and female camels were described in details.

Examination of two hundred sixty samples obtained from the digestive system of camels revealed t he presence of 105 Mycoplasma and Acholeplasma isolates, M.ariginin and A.laidawii were the predominant isolates.

INTRODUCTION

Camels are important source of meat in Egypt beside its use in international camel races.. However, Paling et al. (1988) in Kenya recorded the prevalence of antibodies to Brucella spp., Mycobacterium partatuberculosis, Mycoplasma mycoides mycoides and other Mycoplasma species, which causes contagious caprine pleuropneumonia.

Investigations on camel mycoplasmosis in EGypt was previously studies by some authors such as, Ahmed (1974), Sabry et al. (1976), Fayad anhd Sabry (1979) and Sabry and Ahmed (1986). They could islate *M.arginini, A. Laidlawi* and untypable Mycoplasma species from examined camels.

The aim of the present investigation was to reveal the incidenc of mycoplasma organisms with speciea reference to ureaplasma from both apparently normal and diseased camels.

MATERIAL AND METHODS

Samples were collected from 180 slaughtered camels at Cairo Abattoir and 20 samples were taken from living one-humped camels (Camelus dromedarius) at Faculty of Agriculture, Cairo, University. The age of these camels ranges from 6-17 year s. The general health status was also recorded.

Nasal swabs (80), tracheal (50), brochial lymph nodes (20) apparently and pneumonic lungs (80) and Mediastinal lymph nodes (60) were obtained from the respiratory tracts.

Uterine swabs 100, ovaries 80, vaginal swabs 140, preputial 60 and urethral swabs 50 were collected from the genital tract.

Omasum 60, abomasum 50, rumen 50, liver 30 and rectal swabs 70 were taken from the digestive tract of camels.

The medium used for primary isolation of Mycoplasma and Acholeplasma (heart infusion broth and agar) and the culture procedure was done as described by Sabry and Ahmed (1975).

Primary isolation of Mycoplasma and Acholeplasma:

0.5gm of tissue was aseptically put into a sterile mortar, cut by sterile scissors and ground using sterile sand. 5ml of broth was added, then from the mixture plating was made onto agar medium and 0.2ml was transferred into broth. After 3 days incubation at 37°C this step was repeated 3 times . The plates were examined after 48 hours under a stereo microscope for the presence of mycoplasma colonies having typical Fried egg appearance.

Purification and maintenance of the isolates was made according to Freundt et al. (1973):

Genus determination: was performed using digitonin sensitivity test as described by Freundt et al., (1973).

The agar plate was inoculated with 0.02 ml of the test culture, using running drop technique, a disc impregnated in 1.5% digitonin in ethanol was pressed in the middle.

The plate was incubated at 37°C in a candle jar, examined daily under stereo microscope. The presence of inhibition zone indicates a positive test.

Primary isolation of Ureaplasma:

The LSB (modified Livingston's medium) was used for the isolation of Ureaplasma according to Livingston (1972).

The ground tissue was put in 2 ml broth medium from which 2 fold dilution was made then incubated at 37°C in a CO_2 incubator. When the colour of the broth culture was beginning to change into pink colour, 0.2 ml of this broth was tranferred onto agar plate, incubated at 37°C in jars containing CO_2 bags. The plates are examined daily under stereo microscope for the presence of the small Ureaplasma colonies.

Biochemical characterization of the Mycoplasma isolates: was carried out using glucose fermentation test, arginine deamination test, tetrazolium reduction test and film and spot test according to Erno and Stipkovis (1973).

Glucose fermentation test:

2.9 of glucose medium was inoculated into 0.1ml of the suspected Mycoplasma culture, incubated aerobically at 37°C and examined daily for seven days. A yellow colour indicates a positive test.

Arginine deamination test:

2.9ml of orginine medium was inoculated with 0.1 ml of the suspected mycoplasma culture, incubated at 37°C for 7 days A red colour indicates a positive test.

Tetrazolium reduction test:

2.9ml of tetrazolium medium was inoculated with 0.1 ml of the suspected mycoplasma culture, incubated aerobically at 37°C for 7 days A pink colour indicates a positive test.

Film and spot formation test:

0.2 ml of mycolasma broth culture was cultivated on heart infusion agar plate which was incubated at 37°C , after 3 days a glistening layer appeared owing to the presence of lipase indicating a positive test.

Arbutin and aesculin test:

Are used for Acholeplasma, 0.2 ml of broth culture was inoculated into 2 ml arbutin or aesculin test media. A black colour indicates a positive test.

Biochemical characterisation of Ureaplasma: Shepard et al. (1974).

Ureas colour test:

0.2 ml of the suspected culture was put into 2 ml urease colour test medium and incubated in CO_2 incubator . The broth culture was examined daily, a pink colour indicates a positive test.

Serotyping of Mycoplasma: was performed using growth inhibition test (GI), according to Clude (1964) and growth precipitation test, as described by Erno and Peterslund (1983).

Serotyping of Ureaplasma: was done using metabolic inhibition test (MIT), according Purcell et al. (1966).

Reference Mycoplasma and Ureaplasma antisera were kindly obtained from Diagnostic Lab Ithaca, N.Y., USA.

RESULTS

From the results recorded in Table (1), it is clear that the highest recovery rate of Mycoplasma was from the nasal swabs of camels (87.5%) while the mediastinal lymph nodes represented the site of the lowest recovery rate (16.66%).

Table 1 Incidence and Serotyping of Mycoplasma and Acholeplasma species isolated from the Respiratory tract of Camels

Type of examined samples	No. of examined samples	No. of positive samples		Total No. of Mycoplasma isolates		Typing of Mycoplasma isolates: M. Arginini		Typing of Mycoplasma isolates: Unidentified		Total No. of Acholeplasma isolates		Typing of Acholeplasma isolates: A. laidlawii		Typing of Acholeplasma isolates: A.oculi	
Nasal swabs	80	70	87.50%	40	32.78%	25	20.49%	15	12.29%	30	24.59%	25	20.49%	5	4.09%
Tracheal swabs	50	22	44%	20	16.39%	12	9.83%	8	6.55%	2	1.63%	2	1.63%	0	0.00%
Bronchial lymph nodes	20	8	40%	6	4.91%	2	1.63%	4	3.27%	2	1.63%	2	1.63%	0	0.00%
Apparently normal lungs	20	0	0%	0	0.00%	0	0.00%	0	0.00%	2	1.63%	2	10.00%	0	0.00%
Pneumonic lungs	60	12	20%	8	6.55%	5	4.09%	3	2.45%	0	0.00%	0	0.00%	0	0.00%
Mediastinal lymph nodes	60	10	16.66%	6	4.91%	2	1.63%	4	3.27%	4	3.27%	2	1.63%	2	1.63%
Total	290	122	42.06%	80	65.57%	46	37.70%	34	27.86%	4	3.27%	4	3.27%	0	0.00%
										42	34.42%	35	28.68%	7	5.73%

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Mycoplasmas could be isolated from pneumonic lungs (20%)but not from apparently normal lungs. Table (1) showed that *M.arginini* was recovered from examined respiratory system samples. The nasal samples followed by tracheal swabs revealed the highest incidence of mycoplasma isolates (32.78% and 16.39%) respectively.

Serotyping of the mycoplasma isolates revealed that 25 *M.arginini* (20.49%) from nasal swabs 12 M.arginini (9.83%) from tracheal swabs. 36 isolates gave no reaction with the antisera used for serotypin and could be classified as unidentified mycoplasma. 25 as A.laidlawii were identified (20.49%) from the nasal swabs. A.oculi was isolated from respiratory tract samples in incidence of 5.73%. The highest isolation rate was from the nasal swabs (4.09%).

As shown in Table (2), 88 isolates were recovered out of 430 samples from male and female camels (20.46%), 39 of them (44.31%) were belonging to genus Mycoplasma, 31 were Ureaplasma (35.22%) and 18 were Acholeplasma (20.45%). The highest mycoplasma recovery rate was 16 out of 70 examined urogenital tract samples from the vaginal swabs of non pregnant she camels (22.85%). 20 mycoplasma isolates were recovered out of 60 examined prepuce samples of camels (33.33%).

Table (2) also showed that *M.arginini* could be isolated from examined samples (15.90%) except the uterus and ovary of pregnant she camels. *A.laidlawii* were identified out of isolates belonging to Acholeplasmas in an incidence of 17.04%, 3 only could not be identified (3.40%). 31 Ureaplasmas were isolated from the genital system of camels (35.33%). However, they gave no reaction with known Ureaplasma antisera and could not be identified.

Table (3) showed that the highest recovery rate from the rectal swabs (42.85%) while the liver represented the site of the lowest recovery rate (33.33%). From the data recorded in Tabel (3), it is clear that *M.arginini* could be isolated from digestive tract samples . The abomasum followed by the rumen and omasum were the sites of higher isolation rate in an incidence of 3.80%, and 2.85% respectively. Also, 54 strains of A.laidlawii (51.42%) were isolated from the digestive tract. While, 10 isolates of Acholeplasma (9.52%) could not be identified.

DISCUSSION

The presence of antibody titers to *M.mycoides mycoides* and to mycoplasma species (strain 38) in the of camels in South Eastern Kenya as mentioned by Paling et al. (1988) drew attention to the possible role of camels as the source of Mycoplasmosis infection.

The present study was conducted to determine systematic recovery of the mycoplasma flora from one humped camels (Camelus dromedarius) with special reference to Ureaplasmas.

In Egypt, previous studies were carried out to recover mycoplasma from camels such as, those of Ahmed (1974), Sabry et al. (1976), Sabry and Ahmed (1986) who could isolate mycoplasna from the respiratory, digestive and genital tract of

Table 2 Incidence and serotyping of Mycoplasma, Ureaplasma and Acholeplasma isolated from the genital tract of camels

	Reproductive status	Type of examined samples	No.of examined samples	No. of positive samples		No. of Mycoplasma isolates		Mycoplasma strains				No. of Ureaplasma isolates		No. of Acholeplasma isolates		Acholeplasma strains			
								M. arginini		Unidentified						A. laidlawii		Unidentified	
Fe ma le	Non pragnant	vagina	70	16	22.85%	9	10.22%	5	5.68%	4	4.54%	4	4.54%	3	3.40%	2	2.27%	1	1.13%
		uterus	50	8	16.00%	4	4.54%	3	3.40%	1	1.13%	2	2.27%	2	2.27%	2	2.27%	0	0.00%
		ovary	40	10	25.00%	7	7.95%	2	2.27%	5	5.68%	3	3.40%	0	0.00%	0	0.00%	0	0.00%
	pregnant	vagina	70	10	14.28%	4	4.54%	1	1.14%	3	3.40%	5	5.68%	1	1.13%	1	1.13%	0	0.00%
		uterus	50	10	20.00%	4	4.45%	0	0.00%	4	4.54%	5	5.68%	1	1.13%	1	1.13%	0	0.00%
		ovary	40	3	7.50%	1	1.13%	0	0.00%	1	1.13%	2	2.27%	0	0.00%	0	0.00%	0	0.00%
Ma le		prepuce	60	20	33.33%	6	6.81%	2	3.27%	4	4.50%	5	5.68%	9	10.22%	7	7.95%	2	0.00%
		urethra	50	11	22.00%	4	4.45%	1	1.13%	3	3.40%	5	5.68%	2	2.27%	2	2.27%	0	0.00%
	Total		430	88	20.46%	39	44.31%	14	15.90%	25	28.40%	31	35.22%	18	20.45%	15	17.04%	3	0.00%

* percentages were calculated according to the total number of positive samples (isolates)

Table 3 **Incidence and serotyping of Mycoplasma and Acholeplasma isolates from the digestive tract of camels**

Type of examined sample	No. of examined samples	No.of positive samples	No. of Mycoplasma isolates	Typing of Mycoplasma species		No. of Acholeplasma isolates	Typing of Acholeplasma species	
				M.arginini	Unidentified		A.laidlawii	Unidentified
Omasum	60	20 33.33%	8 7.61%	3 2.85%	5 4.76%	12 11.42%	9 8.57%	3 2.85%
Abomasum	50	25 50%	10 9.52%	4 3.80%	6 5.71%	15 14.28%	12 11.42%	3 2.85%
Rumen	50	20 40%	12 11.42%	4 3.80%	8 7.60%	8 7.61%	8 7.61%	0 0.00%
Liver	30	10 33.33%	6 5.71%	2 1.90%	4 3.80%	4 3.80%	1 95.00%	3 2.85%
Rectalswab	70	30 42.85%	5 4.76%	3 2.85%	2 1.90%	25 23.80%	24 28.85%	1 0.95%
Total	260	105 40.38%	41 39.04%	16 15.23%	25 23.80%	64 60.96%	54 51.42%	10 9.52%

* percentages were calculated according to the total number of positive samples (strains isolated)

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camels. On the other hand, Gad et al. (1989) isolated *M.arginini* and *A.laidlawii* from the genital tract of male and female camels.

In Iraq, Al-Aubaidi et al. (1978) isolated A. oculi from camels while Palling et al. (1988) in Kenya detected antibodies to *M.Mycoides mycoides* in camels.

In the present investigation, the highest incidence of mycoplasma isolation from the respiratory tract of camels was from the nasal swabs (87.50%). *M.arginini, A.laidlawii* and *A.oculi* could be isolated from pneumonic lungs and lymph nodes but not from apparently normal ones. Fayed and Sabry (1979) also could isolate Acholeplaqsmas from the respiratory tract of camels. Machado et al. (1995) isolated *M. ariginini* from pneumonic lung of cattle suffering pleuropneumonia . Sabry et al. (1976) referred to the possible role of mycoplasma in pneumonia in camels . In the present study. *A laidlawii* was also isolated from nasal swabs and this coincides with Liberal (1989) who isolated *A.laidawii* from 144 nasal swabs of calves.

Regarding mycoplasma of the urogenital tract, it was found that mycoplasma was recovered from the uterus of pregnant she camels comparable to non pregnant ones. On the contrary, the isolation rate was lower in the vagina and ovary of pregnant she camels. These findings coincide with those of Gad et al. (1989).

M.arginini could be isolated mainly from she camels and preputeal swabs of male camels. *M.arginini* was previously isolated from cows and ewes suffering from granular vulvovaginitis. Moreover, Ahmed et al., (1981) recovered *M.arginini* from ewes with endometritis and also from she goats suffering from vaginitis. In addition, it was reported from rams and cattle affected with balanopusithitis and azospermia by Leach (1970) and Rashwan et al., (1981) Sabry et al (1976) and Sabry and Ahmed (1986) could isolate *M.arginini* from the genital tract of camels.

Ureplasmas could be isolated feom female and male genital tracts and from the urethra with higher incidence in the vagina and uterus of non pregnant females and from the prepuce and urethra of male camels. Livingston (1972) isolated Ureaplasma from Texas feedlots cattle. Meanwhile, Howard et al. (1975) isolated bovine Ureaplasmas and compared them serologically by immunofluorescence. Moreover, El-Ebeedy et al. (1982) could isolate Mycoplasmas and Ureplasmas from the vaginal discharges, prepureal washings and semen collected from female and male cows and buffaloes suffering from various forms of reproductive disorders such as vaginitis, endometritis , low fertility and abortion in some cases with absence of other pathogens which suggested that Mycoplasmas and Ureaplasms were the possible causative agents.

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