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MYCOPLASMA AND *UREAPLASMA* OF THE GENITAL TRACT OF CAMELS IN EGYPT

(With 6 Tables and 1 Figure)

By

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ميكوبلازما ويوريابلازما الجهاز التناسلي للجمال في مصر

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هذا البحث قد أعد لدراسة وعزل وتصنيف ميكروبات الميكوبلازما واليوريابلازما التي تصيب القناة التناسلية لذكور وإناث الجمال في مصر. وقد تم إجراء البحث علي عدد ٢٥٠ عينة، من مسحات الجراب (١٣٣) ومسحات مهبلية (١١٧) من ذكور وإناث الجمال في مصر. بالإضافة إلي أنه تم فحص عدد ٣٥ جهاز تناسلي لإناث الجمال. وقد تم جمع العينات من جمال مذبوحة بمجزر القاهرة والجيزة. وقد أوضحت نتائج الفحص أن ميكروب اليوريابلازما والذي تم عزله في هذا البحث لأول مرة في مصر قد عزل من جراب ذكور الجمال بنسبة ٣٨, ٥٠٪ ومهبل الإناث بنسبة ٧٤, ٤٢٪ قد كان أعلى من نسبة العزل لميكروب الميكوبلازما (٣٣, ٨٣٪ و ٤٨, ٣٢٪ على التوالي). كذلك أوضحت الدراسة أن أعلى نسبة عزل لميكروب اليوريابلازما و الميكوبلازما قد عزلت من المهبل بنسبة ٤٠٪ و٥٧, ٢٨٪ على التوالي- تليها نسبة العزل من عنق الرحم ٥٧, ٢٨٪ و ٤٣, ١١٪ على التوالي. وقد سجلت أقل نسبة عزل (١٤, ٢٩٪ و صفر٪ على التوالي) من الرحم وكذلك من المبايض. وقد أوضحت الاختبارات البيوكيمياتية والاختبارات السيرولوجية التأكيدية أن ميكروب الميكوبلازما أرجينيني واليوريابلازما دايفرسيم هما اللذين تم عزلهما من القناة التناسلية للجمال في مصر.

SUMMARY

With the growing awareness of the unique role the camel plays in the culture, heritage and agriculture, increasing attention has been focused on improving its health and productivity. The present study was carried out establish and identify the *Mycoplasma* and *Ureaplasma* species which colonize the genital tract of male and female one-humped camels in Egypt. A total of 250 swabs (133 preputial and 117 vaginal), in addition to 35 genital tract of she-camels were examined. All samples were collected from Cairo and Giza abattoirs and were cultured on specific media for *Mycoplasma* or *Ureaplasma*

isolation. Digitonin test was applied for genus determination. Biochemical tests were carried out to narrow the choice of specific antisera used. Serological identification as growth inhibition (GI) and growth precipitation (GP) tests were carried out with specific antisera. Indirect haemagglutination (IHA) test was applied as a serodiagnostic test. To our knowledge, this was the first record of *Ureaplasma* isolation from the genital tract of camels, the results showed that, the *Ureaplasma* isolation rate from the prepuce and vagina of camels was (50.38% and 42.74%, respectively) relatively higher than the *Mycoplasma* recovery rate (33.83% and 32.48%, respectively). On the other hand, the *Acholeplasma* species isolation rate was the lowest, it was (15.04% and 6.84%, respectively) from the prepuce and vagina of the examined camels. The recovery pattern of *Ureaplasma* and *Mycoplasma* from various organs of the genital tract of she-camel showed that, the highest isolation rate was recovered from the vagina (40.00% and 28.57%, respectively) followed by cervix (28.57% and 11.43%, respectively) and the lowest rates were from uterus and the same from ovaries (14.29% and 0.00%, respectively). The biochemical and the confirmatory serological tests showed that *M. arginini* and *U. diversum* were the only identified *Mycoplasma* and *Ureaplasma* species from the genital tract of camels.

Key words: *Camels - Genital tract - Mycoplasma - Ureaplasma*

INTRODUCTION

Of all domestic animals, the camel is relatively neglected as far as animal pathology and medicine are concerned. This is inspite of the fact that the camel plays vital socio-economic role, it supports millions of people in the dry and arid zones of Asia and Africa, also produces annually thousands of tons of consumable meat and milk (Wernery and Kumar, 1994).

In Egypt, the domesticated camel is the one-humped camel (Camelus dromedarius) which is a hardy animal and well adapted to live in a harsh desert environment. The fertility of camels is good but poor nutrition in seasons of low rainfall and resultant poor grazing is a cause of reduced sexual activity of both sexes. Also some bacterial, viral and parasitic disease directly affect health and camel productivity which indirectly influence their reproductive performance (Higgins, 1983).

Although *Mycoplasma* constitute a part of the normal flora colonizing the genital tract of most farm animals including camels, some pathogenic

strains of *Mycoplasmas* and *Ureaplasmas* act as an infective agents or preparing the soil for other bacteria or viruses to invade and produce their specific pathogenic action. A series of investigations on *Mycoplasma* of various systems of the one-humped Camelus dromedarius have been conducted in Egypt (Sabry et al., 1976; Fayad and Sabry, 1979; Sabry and Ahmed, 1986 (a,b) and Gad et al., 1989). Unfortunately, no literature concerning *Ureaplasma* isolation from genital tract of camel were available. So, the present work aimed to: 1- Establish and identify the most prevalent *Mycoplasma* and *Ureaplasma* species of the genital tract of one-humped camels in Egypt. 2- Establish the colonizing pattern of *Mycoplasma* and *Ureaplasma* at various organs in the genital tract of she-camels. 3- Detection of the antibodies against *Mycoplasma* in the blood sera of examined camels by the use of IHA test.

MATERIAL and METHODS

SAMPLES

A total of 250 swabs (133 preputial and 117 vaginal) were collected from slaughtered male and female camels. The samples were collected from Cairo and Giza abattoirs from animals ranging in age from 5 to 14 years old. The samples were collected aseptically by rubbing the mucosa of the prepuce and vagina by two sterile cotton tipped swabs, one swab was directly transported to the *Mycoplasma* isolation (heart infusion) broth media, the second dipped on U_9 broth (Shepard and Lunceford, 1970) for *Ureaplasma* isolation. Blood samples were collected from all examined animals in sterile vials for serum separation. Moreover, the genitalia of 35 slaughtered she-camels were collected, packed individually in plastic bags and transferred to the laboratory in thermos tank with ice packs. The naked eye appearance of the organs was noted and recorded. In the laboratory, swabs from each organ were taken aseptically and cultured for *Mycoplasma* and *Ureaplasma* isolation.

METHODS

For *Mycoplasma* isolation, the collected swabs were cultured on heart infusion (Difco) broth and agar media supplied by 20% horse serum, 10% fresh yeast extract (25% w/v), 1.2% DNA (0.2% w/v), 1% thallium acetate (1% w/v) and 100,000 IU (0.5%) penicillin G-sodium, the pH was adjusted at 7.8. The procedure of isolation and identification was previously described by Sabry and Ahmed (1986a) Fig.(1). The plates were examined microscopically for the characteristic fried egg appearance of *Mycoplasma*

colonies after 48-72 hrs of incubation at 37 °C in humidified jars under CO_2 tension.

Identification of *Mycoplasma* isolated from genital tract of camels

I- Digitonin sensitivity test (Freundt et al., 1973)

The *Mycoplasma* isolates were purified and subjected for genus determination on the basis of their sensitivity to 1.5% (w/v) digitonin in ethanol 95% where genus *Mycoplasma* (sterol required) are digitonin sensitive and genus *Acholeplasma* (non-sterol required) are digitonin resistant.

II- Biochemical tests

1. Fermentation of glucose:

A 24 hrs broth culture of the tested isolates was prepared and inoculated into broth media containing glucose and phenol red as indicator, the broth was incubated at 37 °C and the changes in pH was recorded, where the acid side (yellow) is positive.

2. Hydrolysis of arginine:

The isolate was prepared and inoculated into broth media contain L. arginine Hcl and phenol red as indicator, after incubation at 37 °C, the pH changes was recorded, where alkaline side (dark red) is positive.

III- Serological identification

The purified isolates of *Mycoplasma* were confirmed by employing some serological tests against *M. arginini* reference antisera as:

* growth inhibition (GI) test according to (Gourley and Howard, 1983).
* and growth precipitation (GP) test according to (Erno and Peterslund, 1983).

The used reference antisera of *M. arginini* was obtained from *Mycoplasma* Reference Laboratory in Hannover, Germany.

IV- Serodiagnostic test

Indirect haemagglutination (IHA) test (Cho et al., 1976) was applied as a quantitative test for the detection of specific antibodies in the sera of examined camels against *M. arginini*.

For *Ureaplasma* isolation and identification, as shown in Fig. (1), serial ten fold dilution of the original specimens were done in *Ureaplasma* broth medium U_9 of Shepard and Lunceford, 1970. The original and diluted

samples were incubated at 37 °C for 18 to 24 hrs, The procedure adapted for *Ureaplasma* isolation was described in detail by Hassan, 1994.

Identification of *Ureaplasma* isolated from genital tract of camels

1- Hydrolysis of urea:

The *Ureaplasma* positive samples that cultured on U_9 broth devoloped by Shepard and Lunceford (1970) were showed change in colour from yellow to pink due to hydrolysis of urea with the production of ammonia which resulted in change in pH to alkaline side as a characteristic biochemical reaction of genus *Ureaplasma*.

2- Identification of *Ureaplasma* colonies:

Positive broth samples were subcultured on A_7 agar developed by Shepard and Lunceford (1976) and incubated at 37 °C under CO_2 tension in humidified jars. By applying urease stain developed by Shepard and Howard (1970) on the surface of 48 hrs old colonies on agar media, the *Ureaplasma* colonies were stained yellow to dark brown.

RESULTS

The results in tables (1, 2 and 3) showed that the *Mycoplasma* isolation rate from the prepuce of camels was (33.83%) and (32.48%) from the vagina of examined she-camels. On the other hand, the *Ureaplasma* isolation rates were relatively higher, they were 50.38% and 42.74% from the prepuce and vagina of camels, respectively. Digitonin sensitivity test declared that, the *Acholeplasma* was isolated in a rate of (15.04%) from the prepuce of camels and it was only (6.84%) from the vagina of she-camels. Although the highest recovery rate of *Ureaplasma* as single infection was detected in the prepuce (35.34%) of camels, the isolation rate from the vagina was relatively low (30.77%), followed by the isolation of *Mycoplasma* as single infection from vagina of she-camels (18.80%) and then from the prepuce (13.53%). Although the *Acholeplasma* could not isolated singly from vagina of examined she-camels, the isolation rate from the prepuce was (9.77%).

The results showed that, the recovery rate of *Mycoplasma* and *Ureaplasma* as mixed infection from the prepuce of camels (15.04%) was higher than that from the vagina (9.40%) of she-camels. While the recovery rate of mixed infection (*Mycoplasma /Acholeplasma)* from the prepuce of camel was relatively high (5.26%), it reached 4.27% from the vagina of she-camels. On the other hand, the isolation rate of *Acholeplasma/Ureaplasma*

mixed infection from vagina of she-camels was 2.56%, but mixed infection could not be detected from the prepuce of camels.

In a trial to map the recovery pattern of *Mycoplasma*, *Ureaplasma* and *Acholeplasma* from various organs of the genital tract of she-camels, a total of 35 genital organs of slaughtered she-camels were examined. The results in table (4) showed that, although the *Mycoplasma* recovery rate from the examined vagina was (28.57%) relatively higher than the isolation rate from the cervix (11.43%), the organism could not be isolated from the uterus and ovaries.

The *Ureaplasma* isolation rate from the vagina (40.00%) was higher than that from the cervix (28.57%) and the lowest recovery rate was from the uterus and ovaries (14.29%).

The examined cervix, uterus and ovaries were free from *Acholeplasma*, but the colonization rate in the vagina was (5.71%).

Biochemical screening tests were useful for differentiation of the isolates into different species and to provide reliable basis for subsequent serological tests and narrowing the choice of specific antisera used. The results of the biochemical tests in table (5) showed that, the digitonin non-sensitive isolates (*Acholeplasma*) which were identified from the vagina of she-camels (8.20%) were relatively lower than that from the prepuce of camels (15.15%).

All *Mycoplasma* isolates recovered from the genital tract of camels (93 isolates) were (arginine +ve) and (glucose -ve) so classified as *M. arginini*. The recovery rate of *M. arginini* from the genital tract of she-camels (39.34%) was slightly higher than that from the prepuce (34.09%) of camels.

A total of 131 isolates could hydrolyse urea and identified as *Ureaplasma* and as the *Ureaplasma* species which colonized animals was *U. diversum*, so, the recovery rate of *U. diversum* from the genital tract of she-camels (52.46%) was slightly higher than that from the prepuce (50.76%) of camels.

The results of the serological activities of blood sera of examined camels against *M. arginini* was examined by IHA test and tabulated in table (6) and showed that, while the *M. arginini* recovery rate from the genital tract of she-camels was (31.58%), the indirect haemagglutinating antibodies in their blood sera were relatively low (10.53%). On the other hand, while the isolation rate of *M. arginini* from the prepuce of camels was (33.83%), the IHA antibodies reached only 3.76%.

DISCUSSION

The camel is an important animal for meat production in Egypt. There are a great demand to throw more light on this animal specially its reproductive capacity and related problems. The bacterial flora of the genital tract and its role on fertility of the she-camels are among the most important subjects to be studied (Awad et al., 1978).

Since members of the family *Mycoplasmatceae*, including the genera *Mycoplasma* and *Ureaplasma* are frequently isolated from apparently healthy animals, some species of them cause disease in susceptible animals resulting in considerable losses in production (Doig et al., 1981 and Doig and Ruhnke, 1986).

Since the data concerning *Mycoplasma* and *Ureaplasma* of the genital tract of camels is very scantily, it was preferable to refer to some cattle literatures that might throw some light on this subject.

The results showed that, the isolation rate of *M. arginini* from the prepuce of camels (33.83%) was slightly higher than that from the vagina of she-camels (32.48%). In this regard, the results are in agreement with that of Sabry and Ahmed (1986b) and Gad et al. (1989) who reported a high incidence of *M. arginini* in the prepuce of male camels than from the vagina of she-camels.

Blom and Friis (1983)and Pal et al.(1984), stated that, the incidence of *Mycoplasmas* in the external genitalia of bulls and buffalo bulls reached 9-63%. Ball (1990) and Eaglesome et al. (1992) explained this phenomena, as the bulls carried a latent *Mycoplasma* infection in their prepuce and act as a permanent source of infection which might become pathogenic under certain condition when transferred to cow's genital tract during coitus.

The recovery rate of *U. diversum* from the prepuce of camels (50.38%) was relatively higher than the isolation rate from the vagina of she-camels (42.74%). The high incidence of *U. diversum* in the prepuce and vagina of cattle was reported by many authors, who concluded that, *U. diversum* is a common contaminant of the prepuce and distal urethra of bulls. The organism was isolated from 29-100% from preputial samples cultured and generally not associated with clinical signs (Doig et al., 1981 and Ball, 1990). On the other hand, Ball and Mc Caughey (1979) and Amano et al. (1990) stated that, *U. diversum* is prevalent in the vagina of cows and the isolation rate ranged from 11 up to 100%.

Concerning the *Acholeplasma*, table (3) showed that, the *Acholeplasma* recovery rate from the prepuce of camels (15.04%) was

relatively higher than from the vagina of she-camels (6.84%). On the other hand, Gad et al. (1989) identified *Acholeplasma laidlawii* from the prepuce of camels in a rate of (60.00%) and (66.62%) from the vagina of non-pregnant she-camels.

The results showed that, the recovery rate of *Mycoplasma* and *Ureaplasma* as mixed infection from the prepuce of camels (15.04%) was relatively higher than from the vagina (9.40%) of she-camels. On the other hand, the colonization rate of mixed *Acholeplasma/Mycoplasma* from the vagina of she-camels was (4.27%) relatively higher than the isolation rate of *Acholeplasma* and *Ureaplasma* (2.56%). Awad et al. (1978) supported these results, they stated that more than one organism were isolated from each genital organ of she-camels and also more than one strain of the same organism could be isolated from the same organ. Moreover, mixed infection was previously reported by Hassan (1994) who reported that, the prepuce of bulls as well as the vagina of cows and buffalo-cows colonized more than one species of *Mycoplasma* and/or *Ureaplasma*.

The results of the present study showed that, the recovery rate of *M. arginini* from the vagina (28.57%) was relatively higher than the isolation rate from the cervix (11.43%), but no organism could be isolated from both uterus and ovaries.

Concerning the *U. diversum* isolation rate, the highest recovery rate (40.00%) was detected in the vagina followed by (28.57%) from the cervix and the lowest recovery rate was (14.29%) from the uterus and ovaries. These results were supported by reports of Ruhnke et al. (1978), Ball and Mc Caughey (1979) and Harings (1987) who stated that, the *Mycoplasma* and *Ureaplasma* are most prevalent in the vagina, followed by the vestibule, then the cervix and less common in the uterus and oviduct. Ball et al. (1981) and Doig et al. (1981) explained that the *Mycoplasma* and *Ureaplasma* uterine and oviductal infections usually lasted and cleared from the organ in a period ranges from 7 days up to 3 weeks that explained why these organisms are rarely isolated from the upper reproductive tract.

The results of the present study are in agreement with that of Sabry and Ahmed (1986a) , who reported that, the *Mycoplasma* colonization rate from the vagina was higher than from the cervix followed by uterus and the least was from the ovaries. Ruhnke (1994) reported that uterine swabs are less often culture-positive since the organism may remain in the uterus for only a short time.

The biochemical tests were useful to establish the biochemical pattern of the isolate and to simplify their serological identification. The results of

the study showed that, *M. arginini* was the only identified *Mycoplasma* species from the genital tract of examined one-humped (dromedary) camel in a rate of 36.61%. These results are in agreement with that reported by Sabry and Ahmed (1986b) and Gad et al. (1989) who identified *M. arginini* as the only *Mycoplasma* species isolated from the genital tract of camels.

M. arginini is ofently isolated from a wide range of hosts including bulls that had semino- vesiculitis (Leach , 1970), from cattle and sheep with granular vulvovaginitis (Barile et al., 1968 and Al-Zeftawi et al., 1981) and from buffaloes with endometritis problems (Allam and Sabry, 1978).

The present results showed that, a total of 131 isolates were hydrolyzed urea and identified as *U. diversum* in a recovery rate of 52.46% from the genital tract of she-camels and 50.76% from the prepuce of camels as the first report of identification of *U. diversum* from the genital tract of camels in Egypt.

Ureaplasma was recognized on the basis of rapid growth, anaerobic atmosphere and urease activity. So, it is not necessary to go beyond biochemical identification because no widely accepted serotyping scheme is as yet established and no standardized serologic procedure for *Ureaplasma* diagnosis in cattle is currently available. The immune response to *Ureaplasma* is relatively poor in field cases, making serodiagnosis very difficult (Rosendal, 1994).

A total of 30 isolates were classified as *Acholeplasma* species, with a recovery rate of 8.20% and 15.15% from the genital tract of female and prepuce of male camels respectively.

Unfortunately, the *Acholeplasma* isolates obtained in the present study could not be identified to its species, but further work is in progress for identification. *Acholeplasma* has been isolated from almost all animals survived including cattle (Hoare, 1969) and was previously isolated from camels by many authors (Al-Aubaidi et al., 1978; Fayad and Sabry, 1979; Sabry and Ahmed, 1986 and Gad et al., 1989).

In the present study, the indirect haemagglutination (IHA) test was used as a quantitative serodiagnostic test for the detection of specific antibodies against the isolated *M. arginini* species in the sera of examined camels. A total of 285 samples (152 from she-camels and 133 from camels) were examined. The results showed that, the IHA antibodies in their blood sera were (10.53% and 3.76%) respectively.

The use of IHA as a serodiagnostic test was previously advised by Cho et al., 1976 who cited that, the IHA test is highly specific and the most sensitive and reliable serologic test.

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Figure (1) Protocol for isolation and identification of *Mycoplasma* and *Ureaplasma* from genital tract of camels.

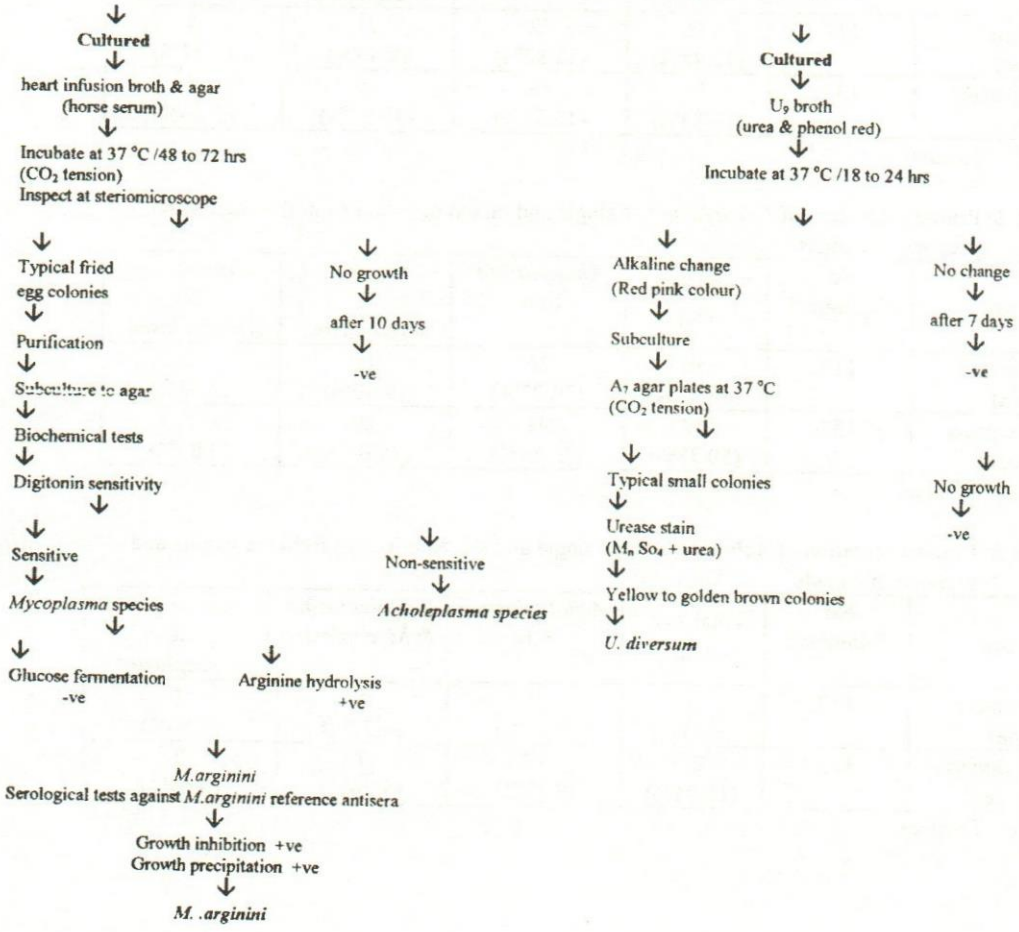

Table 1: Primary isolation of *Mycoplasma* as single and mixed infection from the vagina and prepuce of camels.

Sex	No. Examined	Total +ve* (%)	*Mycoplasma* only	*Mycoplasma* & *Ureaplasma*	*Mycoplasma* & *Acholeplasma*
She-camels (vagina)	117	38 (32.48%)	22 (18.80%)	11 (9.40%)	5 (4.27%)
Male camels (prepuce)	133	45 (33.83%)	18 (13.53%)	20 (15.04%)	7 (5.26%)

* +ve = Positive.

Table 2: Primary isolation of *Ureaplasma* as single and mixed infection from the vagina and prepuce of camels.

Sex	No. Examined	Total +ve* (%)	*Ureaplasma* only	*Ureaplasma* & *Mycoplasma*	*Ureaplasma* & *Acholeplasma*
She-camels (vagina)	117	50 (42.74%)	36 (30.77%)	11 (9.40%)	3 (2.56%)
Male camels (prepuce)	133	67 (50.38%)	47 (35.34%)	20 (15.04%)	0 (0.00%)

* +ve = Positive.

Table 3: Primary isolation of *Acholeplasma* as single and mixed infection from the vagina and prepuce of camels.

Sex	No. Examined	Total +ve* (%)	*Acholeplasma* only	*Acholeplasma* & *Mycoplasma*	& *Ureaplasma*
She-camels (vagina)	117	8 (6.84%)	0 (0.00%)	5 (4.27%)	3 (2.56%)
Male camels (prepuce)	133	20 (15.04%)	13 (9.77%)	7 (5.26%)	0 (0.00%)

* +ve = Positive

Table 5: Biochemical tests for differentiation of the isolates from the genital tract of camels.

Organ examined	No. of total isolates	Digitonin -ve** No. (%)	Glucose -ve Arginine +ve* No. (%)	Urea hydrolysis No. (%)
		Species *Acholeplasma*	Species *M. arginini*	Species *U. diversum*
Vagina and genital tract of she-camels	122	10 (8.20%)	48 (39.34%)	64 (52.46%)
Prepuce of camel	132	20 (15.15%)	45 (34.09%)	67 (50.76%)
Total	254	30 (11.81%)	93 (36.61%)	131 (51.57%)

* +ve = Positive. ** -ve = Negative

Table 6: Indirect haemagglutination test for the detection of antibodies against *Mycoplasma* in blood sera of camels.

Sex	No. of examined serum samples	Isolation rate No. +ve* (%)	IHA** test (1/160) (%)
She-camels	152	48 (31.58%)	16 (10.53%)
Male camels	133	45 (33.83%)	5 (3.76%)

* +ve = Positive. ** IHA = Indirect haemagglutination.

Table 4: *Mycoplasma*, *Ureaplasma* and *Acholeplasma* isolated from the genital tract of 35 she-camels.

No. of examined genital tract	Species identified	Vagina No. +ve* (%)	Cervix No. +ve (%)	Uterus No. +ve (%)	Ovaries No. +ve (%)
35	*Mycoplasma*	10 (28.57%)	4 (11.43%)	0 (0.00%)	0 (0.00%)
	Ureaplasma	14 (40.00%)	10 (28.57%)	5 (14.29%)	5 (14.29%)
	Acholeplasma	2 (5.71%)	0 (0.00%)	0 (0.00%)	0 (0.00%)

*+ve = Positive