Vet. Med. J. Giza, 37, No. 1, 111-121 (1989).

MICROBILOGICAL INVESTIGATIONS FOR MYCOPLASMAS AND BACTERIA IN EGYPTIAN FOALS

BY

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(Received: 19.9.1988)

INTRODUCTION

Microbiological investigations of the conjunctiva, respiratory systems, genital systems and rectum of foals, which included the bacteria and mycoplasmas are much restricted if not absent in the literature. Nevertheless, most extensive work carried out by Kirchhoff et al. (1973) succeeded in the identification of eight acheloplasmas isolated from the lunge of equine foeti as: *A: laidlawii* (4 strains), *A. equifetale* (4 strains).

Kirchhoff (1978) identified *A. hippikon* from lungs of equines foeti while in 1974 she recovered, for the first time, *Mycoplasma equigenitalium* from equine foeti. Lemche and Kirchhoff (1979) isolated *M. subdolum* from identical samples.

The same organism *M. subdolum* was isolated from a foetal stomach by Moorthy et al. (1976) and identified by Lemcko and Kirchhoff (1979).

Concerning the isolation and identification of acheloplasma and mycoplasma species colonizing the genitalia of equines, Langford (1974) and Moorthy et al.(1977) recovered *A. laidlawii* and *A. oculi* as well as *M. bovigenitalium* from the genital tract of mares and aborted foeti.

The current study was initiated for further investigations of mycoplasma inhabiting both the respiratory and genital tract of equines as well as the determination of the bacterial population accompanying these organisms in the same organs.

MATERIAL AND METHODS

MATERIALS:

Sampling:

Samples obtained for the study were collected by sterile cotton swabs from 40 foals procured from the Veterinary Milltary Hospital at Almasa, Egypt. These swabs were collected from the conjunctiva, nose, nasopharyngeal area, vainal orifice and rectum for primary isolation of mycoplasma and bacteria.

Media useed for isolation and identification of mycoplasma and bacteria:

1- PPLO broth medium with DNA - PN (sabry, 1973)

2- PPLO agar medium-PNP. (Sabry, 1973)

PPLO agar base (Difco) substitutod the PPLO broth and other ingredients were the came.

3- Digotonin medium for defferentiation (Ern Ø and Stipkovits, 1973).

Media for biochemical behavious of isolates:

1. Dextrose fermentation medium: (Sabry, 1968)

2. Galaetose fermentation medium : (modified from Sabry, 1968 and ErnØ and Stipkovits, 1973)

3. Arginin deamination medium:Sabry, 1968)

Spocific antisera. for mycoplasma and acholeplasma were received from the Institute of Microbiology, Faculty of Veterinary Medicine, Honover, West Cermany.

Media used for primary isolation of bacteria:

Nutriont broth, Selinite F broth medium, Nutriont agar, Blood agar, MacConkey agar medium, and 5% sheep blood agar.

Media for biochemical test:

Gelatin, nitrate reduction medium, oxidative fermentation medium, sugar media " 1 % glucose, lactose, maltose, dulcitol". (Criickshank et al., 1975).

METHODS:

Primary caltivation of swabs collected from examined animals was performed using the methods described by Sabry and Ahmed, (1975).

Morphological studies of mycoplasma colonies were done using stereo micrescope (Leitz) for the determination of the fried egg appearance exhibiting the typical morphology of mycoplasma with a marked central spot on solid media.

Sugar formentation was performed on the previously mentioned media incubated aerobically and anaerobically for 2-10 days at 37°C. Positive cases were indicated by sugar utilization and acid production which Varies according to the carbohydrate used (Alutto et al., 1970).

Differontiation of acholeplasma and mycoplasma genera using digitonin test was done for determinating the inhibition zone in case of mycoplsama species using the disc method (Freundt et al. 1973).

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Serological identification was porformed by the growth inhibition test(Sabry et al., 1971) and Metabolic inhibition test (Taylor-Robinson and Purcell, 1966).

Tests for isolation and identification of bacteria were porformed according to (Cruickshank et al., 1975).

RESULTS

A- Mycoplasmas:

All isolated colonies exhibited the fried egg morphology which is typical of mycoplasmas where a marked central spot appeared on solid medium. Mycoplasmas isolated grow well both aerobically and under 5% Co_2 at 22°C and were inhibited by 1.5% digitonin. Inhibition zones reached from 4 - 12 mm. in diameter.

All isolated straing possesed the same biochomical activities. They fermented dextrose and galactose with the production of acid only. Besides, hydrolysis of arginine was also observed in all cases.

Serological identification: Identification of the sixteen isolated strains of mycoplasma species serologically revealed the presence of two different strains: *Mycoplasma equigenitalium* (11 isolates), *Mycoplasma* pulmonis (5 isolates), according to growth inhibition and metabolic inhibition tests performed.

Data presented in the **Table 1** showed that most of the isolated Mycoplasma strainc (11 isolates) were related to *M. equigenitalium* forming 27.50% of the examined cases. These isolates were obtained from all examin d natural openings and were concentrated mainly in the conjunctive and vagina (10% and 7.50% respectively) and to a lesser extent from the nose and nasopharyngeal area (5% in both). ON the other hand, *M. pulmonis* formed only 12.5% of the examined cases and were concentrated in the nasopharyngeal area (7.5%) and to lesser extent in the nose (5%).

Table (1): Hycoplasmas isolated from 40 Egyptian foals.

Sit of isolation	No.of Positive Cases			
	M. equigenitalium		*M. pulmonis*	
	No.	%	No.	%
Conjunctiva	4	10.00 %	-	-
Nose	2	5.00 %	2	5.00 %
Nasepharyngeal area	2	5.00 %	3	7.50 %
Vagina	3	7.50 %	-	-
Total	11	27.50 %	5	12.50 %

Ⓓ Rectal swabs were negative for mycoplasmas.

Table (2): Bacteria isolated from the nasopharyngeal area of 40 Egyptian feals,

No.	species of bacteria	Isolated from the nesopharyngeal area	
		No.	%
1	Staph. epidermidis	7	17.50 %
2	Corynobacterium equi	5	12.50 %
3	M. albus	5	12.50 %
4	anthracoides	3	7.50 %
5	E. coli	1	2.50 %
Total		21	52.50 %

It was noticed that the rectum in this work appeared to be free from mycoplasma.

B- Bacteria:

Result of primary bacterial culture revealed the presence of bacterial populations in 21 cases obtained from the nasepharyngeal area out of 40 feals.

The identification of isolated purs bacteria cultures showed that 21 cultures were related to five bacterial species, viz., *Staph. opidermidis, Corynebacterium equi, Micrococcus albus, B. anthracoides* and *E.coli* were in different percentages as shown in **Table (2).**

From the table it is clear that *Staph. epidermidis* was the most predominant species among the isolated becteria (17.50%) while *Ecoli* was found only in 2.5% of the examined cases. The other tiree species were obtained in moderate percentage varying between 7.5 - 12.5 % of cases.

DISCUSSION

The work entailed in this study included the microbiological examination of conjunctiva, nose, nasopharyngeal area, vaginal orifice and rectum of 40 Egyptian foals for detection of mycoplasmas as well as bacterial species for the first time in Egypt.

The obtained results revealed the presence of 2 different strains of mycoplasmas: *Mycoplasma equigenitalium* (11 isolates) and *Mycoplasma pulmonis* (5 isolates) which were identified according to morphology of the colonies, biochemical and serological characterisitcs.

M. equigenitalium was isolated from 27.50% of examined cases and found in different percentages in all the examined orifices with the exception of the rectum which was free from mycoplasmas.

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These results go hand in hand with these obtained by kirchoof (1974) who recovered *M. equigenitalium* from equine foeti. Regarding the isolation of other mycoplasma species from the genetalia of equines only, *M. bovigenitalium* was detected by Langford (1974) and Moorthy et al. (1977), while Loncke and Kirchhoff (1979) recovered *M. subdolum* from equine foeti

Uptil the start of the presont study, no investigations were carried out locally to determine the presence of mycoplasmas in Egyptian foals.

Accordingly, the gained results are considered to be first record of mycoplasmas in equines here in Egypt. This will require further investigation to demonstrate the role of this group of micro-orgenisms in equine diseases especially when it is obviously recovered from apparebtly healthy cases as described in this work.

Concerning the bacteriological examination it is quite clear that all the isolated bactoria are of the commensal types and no obligatory pathogenic bacteric were isolated.

These bacteria were found inhabiting the nasopharyngeal area of 21 cases forming 52.50% which indicates that of *Staph. epidermidis* was the most common among isolated bacteria, (17.5%) while *E. coli* was met with only accidently in one case (2.5%). The presence of different bacterial species could be considered as an indicator of infection possibillities under stress factors which may result in complications with mycoplasmal infections.

The question about the role of such bacteria as well as their occurrence with mycoplasmas in the development of pathological processes among equines is still opened and requires more investigations.

ACKNOWLEDGEMENTS

The authors are much thankfull to Dr. Y. Zoin Elabidin, Director of the Veterinary Military Hospital at Almasa, Egypt. for the facilities of sampling and to Mr. Y. Abdel Gelil, Departmont of Microbiology for his aid in the laboratory work.

SUMMARY

A total of 40 Egyptian foals were examined for isolation of mycoplasma species and bacteria from conjunctiva, nose, nasopharyngeal area, vaginal and rectum.

Two mycoplasma strains *M. equigenitalium* were recovered from all examined sites except the rectum in different percentages, while *M. pulmonis* was found only in the nose and nasopharyngeal area. Bacteria obtained from the nasopharyngeal area were related to 5 genera: *Staphylococcus, Corynebacterium, Micrococcus, Bacillus* and *Eschirechia*. The most predominant species was *Staph. epidermidis*.

The presence of mycoplasmas and bacteria in examined cases indicates the possibility of development of infection among the tested animals.

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