

## Communication

### Mycoplasmas from donkeys and horses in the Sudan

H.E.E. Shams El Din <sup>1</sup>

M. El Nasri <sup>2</sup>

SHAMS EL DIN (H.E.E.), EL NASRI (M.), Mycoplasmes de l'âne et du cheval au Soudan. *Revue Élev. Méd. vét. Pays trop.*, 1990, 43 (3) : 323-324

Dix-sept isolats, soit 4,27 p. 100, ont été séparés de 398 prélèvements. Douze ont été obtenus à partir de 300 écouvillons des fosses nasales chez l'âne (4 p. 100), trois et deux (4,3 et 6,89 p 100) ont été isolés respectivement à partir de 69 prélèvements nasaux chez le cheval et 29 par lavage utérin chez la jument. Neuf isolats furent perdus au cours d'un stockage à -20 °C et les 8 restant ont été identifiés comme des mycoplasmes dont les réactions biologiques, chimiques et sérologiques ont été testées. Ils ont pu être séparés en deux groupes sur la base de la fermentation glucosique et de l'hydrolyse de l'arginine. Dans le premier groupe, aucune fermentation ni hydrolyse n'est notée. Les organismes du second groupe n'ont hydrolysé que l'arginine. *Mots clés* : Ane - Cheval - Mycoplasme - Soudan.

#### Introduction

Mycoplasmas and acholeplasmas have been isolated from genital and respiratory tracts of horses by several workers (9), but until now there were no reports on the isolation of these organisms from donkeys. They are useful animals in the Sudan since they are used for transport purposes in many parts of the country.

Isolation of mycoplasmas from donkeys and horses has not been previously attempted in the Sudan. The present work was, therefore, carried out to investigate the prevalence of mycoplasmas in these two animal species and to find out whether or not donkeys and horses harbour *Mycoplasma mycoides* subsp. *mycoides* (*M. mycoides*) and *Mycoplasma mycoides* subsp. *capri* (*M. capri*), the causal agents of contagious bovine and caprine pleuropneumonia. These two diseases are among the most important for cattle and goats in the country.

#### Materials and methods

##### Samples

Three hundred and ninety eight samples consisting of nasal swabs were collected from 300 donkeys and 69 horses in Khartoum and Wad Medani areas and 29 uterine washings from mares in Khartoum.

1. Department of Microbiology, Faculty of Veterinary Science, POB 32, Khartoum North, Sudan.

2. Department of Preventive Medicine and Public Health, Faculty of Veterinary Science, POB 32, Khartoum North, Sudan.

Reçu le 23.2.1990, accepté le 3.4.1990.

Swabs were collected with sterile absorbent cotton wool swabs and uterine washings were sent to the laboratory for bacteriological examination. Swabs collected from animals in Wad Medani area were sent to Khartoum in a transport medium. All samples were kept on ice during transport.

##### Culture media

PPLO and Brucella media (Difco) were used as solid and liquid media for isolation and propagation of cultures. The media were supplemented with 20% horse serum, 0.02% DNA, 500 IU/ml penicillin and 10% v/v of a 25% aqueous solution of yeast extract (Oxoid). PPLO broth was also used as transport and basal medium for biochemical tests.

##### Culture methods

Swabs without transport medium were streaked directly on the solid medium, then immersed in the liquid medium and stirred by an electrical stirrer for half a minute. Serial tenfold dilutions of the suspension were made up to 10<sup>-3</sup> in the liquid medium and after two days of incubation, 0.2 ml of each dilution was used to inoculate liquid and solid media (H. KIRCHHOFF, personal communication, 1988). Swabs in the transport medium were stirred and similarly treated.

Swabs from the Khartoum area were examined within two hours after collection and those from Wad Medani after 36 hours. Uterine washings were centrifuged at 3000 rpm for 10 minutes and the deposit was used to inoculate liquid and solid media. Inoculated media were incubated aerobically in a humid container at 37 °C.

##### Identification of isolates

Cloned cultures were identified by the following tests: reversion and filtrability (5), sterol requirement for growth (4) sensitivity to digitonin (6).

##### Biological and biochemical tests

The tests used were: liquefaction of coagulated serum, glucose fermentation, arginine hydrolysis, phosphatase activity (7), haemolysis of red blood cells (1), tetrazolium reduction (10) and production of films and spots (3).

##### Serological tests

Growth inhibition (2) and growth precipitation (8) tests were used. The isolates were tested against antisera prepared to *M. equirhinis*, *M. equigenitalium*, *M. subdolum*, *M. arginini*, *M. mycoides* and *M. capri*.

#### Results

##### Isolation of mycoplasmas

Seventeen isolates (4.27%) were recovered from 398 samples. Twelve isolates (4%) were obtained from 300

## Communication

donkey nasal swabs, three isolates (4.3 %) were obtained from 69 horse nasal swabs and two isolates (6.89 %) were recovered from 29 uterine washings. All isolates showed typical fried-egg colony type which appeared on microscopic examination after 24 to 48 hours of incubation. Both PPLO and *Brucella* media were suitable for isolation, but growth was better and the colonies were larger on PPLO agar.

Nine isolates, *i.e.* six from donkey nasal swabs, one from horse nasal swab and two from uterine washings, were lost during storage at  $-20^{\circ}\text{C}$ . These isolates were subjected to the reversion test only and showed no change in colony appearance. They were, therefore, assumed to be either mycoplasma or acholeplasma.

### *Biological and biochemical properties of isolates*

The remaining eight isolates showed no change in colonial morphology when grown in inhibitor-free media; they required serum for growth, were sensitive to digitonin and could be filtered through a 450 nm millipore filter. Accordingly, the organisms were identified as mycoplasmas. Only four isolates hydrolysed arginine and the other four neither fermented glucose nor hydrolysed arginine. All isolates gave negative results in the other tests.

On the basis of glucose fermentation and arginine hydrolysis, the isolates were divided into two groups. The first group included the four isolates hydrolysing arginine of which three were from donkeys nasal swabs and the fourth from horse nasal swab. The second group included the remaining four strains which neither fermented glucose nor hydrolysed arginine. Three of these strains were from donkey nasal swabs and the fourth was from horse nasal swab.

### *Serological tests*

All isolates did not react with any of the antisera used in the two tests.

## Discussion

The present work is the first study on equine mycoplasmas in the Sudan. The isolation rate from donkey and horse nasal swabs and mare uterine washings were 4, 4.3 and 6.89 %, respectively. The isolates possessed cultural and biochemical properties which justified their identification as mycoplasmas. None of the isolates reacted with any of the antisera used in the tests. It should be important, therefore, to test the isolates with antisera prepared against other mycoplasma species. It should also be noticed that mycoplasmas other than those common to horses were isolated from the respiratory tract of these animals such as *M. felis* (9). Surprisingly no acholeplasma was isolated during this study although they were reported elsewhere (9). However, nine isolates were lost before

complete identification. Some of these might have been acholeplasmas.

An important fact revealed by the serological tests was that none of the isolates reacted with antisera to *M. mycoides* and *M. capri* which indicates that horses and donkeys do not carry these cattle and goats important pathogens.

### Acknowledgements

The authors are indebted to Pr. H. KIRCHHOFF of the School of Veterinary Medicine, Hannover, Germany, for allowing the first author to train in her laboratory and for supplying some antisera. We are grateful to Pr. E.A. FREUNDT of the Institute of Medical Microbiology, University of Aarhus for supplying some of the antisera. The technical staff of Department of Microbiology offered technical help. German Academic Exchange Service (DAAD) sponsored the study and we are grateful for their financial support.

SHAMS EL DIN (H.E.E.), EL NASRI (M.). Mycoplasmas from donkeys and horses in the Sudan. *Revue Elev. Méd. vét. Pays trop.*, 1990, **43** (3) : 323-324  
Seventeen isolates (4.27 %) were recovered from 398 samples. Twelve isolates (4 %) were obtained from 300 donkey nasal swabs, three (4.3 %) and two (6.89 %) isolates were recovered from 69 horse nasal swabs and 29 mare uterine washings, respectively. Nine isolates were lost during storage at  $-20^{\circ}\text{C}$  and the remaining eight were identified as mycoplasmas and their biological, biochemical and serological reactions were investigated. The isolates could be divided into two groups on the basis of glucose fermentation and arginine hydrolysis. The first group neither fermented glucose nor hydrolysed arginine. Organisms in the second group hydrolysed arginine only.  
*Key words* : Donkey - Horse - Mycoplasma - Sudan.

### References

1. CLYDE (W.A.). Haemolysis in identifying Eaton's pleuropneumonia-like organism. *Science*, 1963, **139** : 55.
2. CLYDE (W.A.). Mycoplasma species identification based upon growth inhibition by specific antisera. *J. Immun.*, 1964, **92** : 958-965.
3. EDWARD (D.G.). An investigation of pleuropneumonia-like organisms isolated from the bovine genital tract. *J. gen. Microbiol.*, 1950, **4** : 4-15.
4. EDWARD (D.G.), FITZGERALD (W.A.). Cholesterol in the growth of organisms of the pleuropneumonia group. *J. gen. Microbiol.*, 1951, **5** : 576-586.
5. ERNO (H.), STIPKOVITS (L.). Bovine mycoplasma : cultural and biochemical studies. *Acta vet. scand.*, 1973, **14** : 436-449.
6. FREUNDT (E.A.), ANDREWS (B.E.), ERNO (H.), KUNZE (M.), BLACK (F.T.). The sensitivity of mycoplasma to sodium-polyethanol-sulfonate and digitonin. *Zentbl. Bakt. ParasitKde, Abt. I Orig.*, 1973, **225** : 104-112.
7. FREUNDT (E.A.), ERNO (H.), LEMCKE (R.M.). Identification of mycoplasma. In : BERGAN (T.), NORRIS (J.R.), ed. *Methods in microbiology*. London, Academic Press, 1979. Vol. 13.
8. HEITMANN (J.), KIRCHHOFF (H.). Acholeplasma species differentiation with growth precipitation test. *Int. J. syst. Bacteriol.*, 1978, **28** : 96-98.
9. LEMCKE (R.M.). Equine mycoplasmas. In : TULLY (J.G.), WHITCOMB (R.F.), ed. *The mycoplasmas*. New York, Academic Press, 1979. Vol. II.
10. SOMERSON (N.L.), MORTON (H.E.). Reduction of tetrazolium salts by pleuropneumonia-like organisms. *J. Bacteriol.*, 1953, **65** : 245-251.