OCCURRENCE OF M. ARGININI IN RESPIRATORY AND EYE INFECTIONS OF SUDANESE SHEEP & GOATS

BY

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Introduction

Quite a number of Mycoplasmas has been isolated from sheep and goats with pneumonias, pleuropneumonias, agalactia, eye infections and joint involvements. These mostly included M. mycoides subsp. Mycoides, M. mycoides subsp. capri, Mycoplasma ovipneumoniae, M.agalactiae, M. conjunctivae, Mycoplasma strain F38 and M.arginini. (Al Aubaidi **et al.**,1972, Alley **et al.**, 1975, Barile **et al.**, 1968, Carmichael et al 1972, Cottew 1971, Harbi **et al.**, a & b, MacOwan and Minette 1976, Masiga and Rurangirwa 1979, Mohamed Ali 1977, Muna El Mahi 1974, Nicolet and Freundt 1975, Ojo 1976 b, Perreau 1973, Rosendal **et al.**, 1979 Surman 1973, Trotter **et al.**, 1977, Watson **et al.**, 1968). In the majority of the above reports M.arginini has often been implicated as a potential pathogen or a mere secondary invador.

In the Sudan much work has been done on diseases of Mycoplasmas of sheep and goats particularly on pleuropneumonia (Abdulla and Lindley 1967, Babiker 1968, El Nasri 1964, Harbi **et al.**,1981 a & b, Lindley and Abdulla 1969, Mohamed Ali 1977, Muna El Mahi 1974). Agalactia and eye infections in these two species also received attention though to a limited scale (Ibrahim 1969, Harbi et **al.**, 1981 b).

In the present report although our interest was mainly to isolate the mycoplasmas causing disease yet M. arginini was accidentally isolated in most of the cases alone or in association with other pathogens. Muna El Mahi (1974) isolated M. arginini from normal and suspect material from lungs of sheep. Some time later Harbi et al (1981 b) working in sheep and goats found the organism in conjunction with bacteria or other mycoplasmas. In this study the authors enumerate the strains of M. arginini which they isolated from cases of established diseases and discuss its pathogenicity.

Materials and Methods

The species and localities of the animals examined are given in table I. Specimens were collected from sheep and goats in Veterinary Clinics, Animal Production Centres and Animal Quarantines. The majority of specimens were from incidences of clinical pneumonia or diagnosed cases of pleuropneumonia. Swabs were collected from infected eyes of sheep specifically from Kadaru quarantine (Khartoum Province). Pleural fluid and lung tissues were taken by the techniques described before (Abdulla 1966), Harbi et al (1981 b).

Media: These were PPLO serum Broth and Agar (Difco) and mycoplasma Broth and Agar (Oxoid CM 403). The media were regularly supplemented with ingridients referred to elsewhere (Mohamed Ali 1977). Lung pieces were inoculated into broth without homogenisation for the reasons raised by Barile (1974). The swabs were inoculated directly into broth or streaked on Agar. Plates were put into polythene bags which furnish a humid envirnoment and incubated at 37°c in atmospheric air.

Cultural, biochemical and serological properties : The need for cholesterol was determined by the digitonin sensitivity test (Freundt et al 1973). Other biochemical properties were tested by the conventional method of Erno and Stipkovits (1973) were the phosphatase activity, catabolism of arginine and urea, fermentation of sorbitol and glucose reduction of tetrazolium and digestion of inspissated serum. Film and were detected in media prepared after Fabricant and Freundt (1967). The growth inhibition test was done against hyperimmune serum to M.arginini (type strain G230) using the disc technique described by Clyde (1964).

Animal inoculation: Inoculation trials were made to reproduce the disease in 6 Nubian goats and 3 Sudanese Desert sheep aging (1-2) years. Inoculations were done by the endobronchial intratracheal route (Abdulla 1966), two sheep were inoculated subconjunctivally (Trotter et al 1977).

Results

From the cultural and biochemical characteristics together with the growth inhibition by a definite antiserum, the strains were identified as M.arginini (Table II). All the isolates hydrolysed arginine with a distinct alkaline shift. None of the strains fermented glucose or sorbitol. Phosphatase activity and diges-

tion of coagulated serum were negative. No "film and spots" were produced. The strains also failed to reduce tetrazolium or catabolise urea.

Clear zones of inhibition ranging between 4-7 mm were given in the growth inhibition test. Apart from being very specific this latter test helped to distinguish M.arginini from other arginine metabolising organisms.

Strain (KVC 1) which appears in the test (Table II) has already been referred to (Harbi et al 1981 b). It was isolated from a goat with pleuropneumonia in association with another glucose-fermenting organism. The latter was identified as M.mycoides subsp. mycoides. It disappeared after the 4th subculture and only M.arginini(KVC 1) was finally identified.

The inoculation of Ps.aeruginosa and M.arginini (Harbi et al 1981 b), induced an acute pneumonia with extensive lesions. However Ps.aeruginosa alone gave less severe infection with milder lesions but M.arginini alone induced no infection. This reflects the synergy between M.arginini and other pathogens. The subconjunctival inoculation with M.arginini gave a negative result.

Discussion

Mycoplasma arginini was first isolated from cell culture, sheep and goats (Barile et al 1968). Subsequently this species was reported to have been isolated from the respiratory tract, eyes and joints of the above ruminants. (Al Aubaidi et al 1972, Cottew 1971, Foggie and Angus 1972, Harbi et al 1981 b, Muna El Mahi 1974, Nicolet and Freundt 1975, Perreau 1973, Surman 1973, Trotter et al 1977).

Actually several instances have been cited in which M.arginini seemed to be closely associated with disease (Leach 1970). Al Aubaidi et al (1972) reported that this organism may be pathogenic when they found it occurring in pneumonic lungs of bighorn sheep and in arthritic joints and pneumonic lungs of domesticated goats. On the other hand Watson (1968) found no consistent clinical or pathological abnormality ascribable to the injection of M.arginini. Perreau (1973) considered them as secondary invadors in bacterial pneumonias of goats. The intranasal and intratrachial inoculation of M. arginini into caesarian derived specific pathogen free lambs produced minimal lesions (Foggie and Angus 1972). These authors considered that the lesions were rather due to the broth in which the mycoplasma was suspended. It is possible to speculate that multiplication of this agent may occur in already damaged tissue. It is also liable to interpret the presence of M. arginini in the animal organ rendering the tissue to attract other pathogens. Freundt (1978) has analysed the possible effect of lung lesions as inflicted by the depletion of arginine in the lung by M. arginini. In the Sudan, Our limited studies on pathogenicity with pure cultures of M. arginini in sheep and goats consistently demonstrated lack of infection. We have encountered acute pneumonias with severe lung lesions when M. arginini was endobronchially inoculated with Ps. aeruginosa in sheep and goats. M. arginini alone failed to induce infection while with Ps. aeruginosa alone less severe form of disease was produced with minimal pulmonary changes. This would indicate an important synergistic action between mycoplasma arginini and other infective agents. In a recent report (Harbi et al 1981 b).M. arginini was isolated from the eyes of sheep with conjunctivitis. The organism grown in broth culture and used experimentally to induce conjunctivitis in sheep failed to do so. On the other hand, Surman (1973) has succeeded in inducing keratoconjunctivitis using the conjuntival scraping of an infected sheep with naturally occurring disease. The scrapings, he used, were found to contain a mixed culture of mycoplasmas, including both M. conjunctivae and M. arginini. Success of the above author to induce keratoconjunctivitis with the pure culture of M. conjunctivae alone and not with M. arginini would again suggest that the latter is a mere commensal or a secondary invador. In a single case among our isolates M. arginini (strain KVC l) was associated in early cultures with a glucose fermenting mycoplasma. The biochemical and serologic characteristics of this glucose fermenting organism, though relatively inconsistent from the start, convinced the present authors to ascribe it to the species M. mycoides subsp mycoides (Harbi et al 1981 b). Actually the inoculum, which later proved to be a mixed culture of M. arginini and M. mycoides subsp, mycoides produced typical pleuropneumonia in goats up to the 4th subculture. The pathogenic strain was lost in subsequent passages most probably because it was overgrown by the more rapidly adapted M. arginini. (Harbi et al 1981).

Although the evidence presented up to date indicates that this mycoplasma is not very pathogenic, it remains the interest of the present authors to find

whether more virulent strains of M. arginini exist or not. In order to explore the host range of this organism and to obtain authentic information on its pathogenic role more work is required using pure cloned cultures of M. arginini.

Summary

A number of mycoplasmas have been isolated from diseased sheep and goats in the Sudan and identified as M. arginini. Preliminary trials showed that this organism is not pathogenic although its synergy with other pathogens was evident. Definitive studies have not been done to establish the role of this agent in the aetiology of disease. This merits further consideration.

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Table I

Localities and designations of strains of Mycoplasma arginini isolated in the Sudan

strain designation	Host	Recovery Site	Locality
C 58	goat	Lung	Khartoum Province (Kh. Vet. Clinic)
C 59	goat	Lung	Khartoum Province Kh. Vet. Clinic
C 68	goat	Lung	Khartoum Province Kh. Vet. Clinic
K V C 1 *	goat	Lung	Khartoum Province Kh. Vet. Clinic
56	Sheep	Lung	Kordofan Province (Obeid Vet. Clinic)
538	Sheep	Lung	Blue Nile Sennar Vet Clinic
533	Sheep	Lung	Gezira (Huda Animal Production Farm)
0 16	Sheep	Luug	Khartoum Province Kh. Vet. Clinic
PS 192	sheep	Lung	Khartoum Province Kh. Vet. Clinic
PS 414	Sheep	Lung	Khartoum Province Kh. Vet. Clinic
K 9	Sheep	Eye	Khartoum Province Kadaru quarantine
K. 10	Sheep	Eye	Khartoum Province Kadaru quarantine

* Harbi et al 1981 b

Table II

Biochemical characteristics of some M. arginini strains isolated from sheep and goats in Sudan

Strain designation	Fermentation of Glucose	Fermentation of sorbitol	Catabolism of arginine	Hydrolysis of Urea	Reduction of tetra-zolium	Digestion of serum	Phosphatase activity	film & spots
C58	–	–	+	–	–	–	–	–
56	–	–	+	–	–	–	–	–
PS 414	–	–	+	–	–	–	–	–
K 10	–	–	+	–	–	–	–	–