ISOLATION AND IDENTIFICATION OF MYCOPLASMA FROM MILK OF MASTITIC GOATS IN THE SUDAN

Part I

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Introduction

The goat is an important source of milk supply in many urban and rural areas in the Sudan. Drop in milk yield due to mastitis in this species causes considerable economic loss. This fact stimulated some researchers to study and conduct treatment trials of mastitis in the Sudan (Dafaalla. 1961; Dafaalla and Gharib, 1958; Harbi et al. 1981; Ibraheim. 1962, 1968, 1969).

In fact, Bridé and Donatien (1923) were the first to report on pleuropneumonia-Like organisms as a cause of agalatia and had the privilege of isolating the causative agent in inanimate media. Three decades later, Van der Hoeden and Shamir (1954 a & b) described contagious agalatia in Sheep and goats.

In the Sudan, no substantial evidence has been given as to mycoplasmas causing mastitis in goats. Actually, Ibraheim (1969) described an organism as being **M.agalatiae** on cultural and biochemical findings only. This of course does not satisfy the criteria accepted for identification of mycoplasmas (International Committee of Systematic Bacteriology, 1979). Hence the question of agalactia in the Sudan still remains unsolved.

In the present work we give the first report of a proper identification of mycoplasma from goat milk in the Sudan. Two strains of **Mycoplasma arginini** were isolated in pure culture. To our knowledge this is the first report in the literature of these isolates associated with goat mastitis.

Materials & Methods

Specimen :

Thirteen milk samples were collected from mastitic goats in Kuku area and from Omdurman Veterinary Clinic. Some goats showed signs of involvement of the supramammary lymph glands. The samples were taken under aseptic conditions after discharging the first jet of the teat. The infected milk was often whey-like and sometimes tinted with blood.

Media:

Both liquid and solid media were used. These were Brucella broth and agar (Albimi); Mycoplasma broth and agar (Oxoid); and Bacto Heart infusion broth and agar (Difco). Media were supplemented with horse serum, yeast extract, DNA, and antibiotics as described earlier (Harbi et al 1981).

Cultural methods:

These comprised the addition of 1 ml of each milk sample into a tube containing 9 ml of broth. Serial dilutions were then made into 10 tubes to minimise the chance of contamination. By the 4th sub-passage when there was clear opalescence indicating growth of mycoplasma, we commenced streaking the culture from liquid onto the corresponding solid media. Both solid and liquid media were incubated aerobically at 37 C in a humid chamber. Plates were examined for the appearance of umbonate colonies at intervals of up to two weeks and broth cultures checked for a period of 10 days. After three passages containing bacterial inhibitors, isolates were tested for reversion to bacterial form by subjecting them to five successive subcultures in media without antibiotics (I.C.S.B., 1979).

After cloning, the isolates were tested for sensitivity to digitonin, a property characteristic of sterol-dependent mycoplasmas (Freundt et al ., 1973). Digitonin-sensitive organisms were further identified by the methods of Erno and Stipkovits (1973). Tests applied were the activity of phosphatase, hydrolysis of arginine and urea, fermentation of glucose and reduction of 2,3,5, triphenyl tetrazolium chloride under aerobic conditions. Serum digestion was tested by streaking 3-day cultures on inspissated horse serum slants. Production of film and spots was tested as described by Fabricant (1967). The only serological test used was the growth inhibition test. This was carried out by the paper disc technique (Clyde, 1964).

Reference strains and sera:

These were generously provided by Professor, Freundt, Director, FAO/WHO collaborating centre for Animal Mycoplasma, Aarhus-Denmark; and Dr. P.Perreau Head Department of Microbiology, I.E.M.V.T. France.

Bacteriological examination:

Samples were simultaneously sent to the Department of Bacteriology to test for pathogens other than mycoplasma.

Results

Of the thirteen milk samples, two strains were characterised as mycoplasmas by the digitonin test. They were designated G_2UL and G_3UL and identified as **M. arginini**. The isolates had all the properties of the type strain G 230 (Barile et al. 1968). Both of them hydrolysed arginine but failed to hydrolyse urea or ferment glucose. Triphenyl tetrazolium chloride was not reduced. They were phosphatase negative, failed to digest inspissated serum and film and spots were not produced. A clear zone of inhibition was produced by hyperimmune serum against the type strain G 230 in the growth inhibition test.

Bacteriological examination:

Staphylococcus albus & Staph. epidermidis and Klebsiella ozaenae were generously identified by the Bacteriology Department.

Discussion

The isolation of mycoplasmas from milk is rather tedious due to the possible existence of other more rapid growing organisms. The secretion of antibodies in milk may be another factor impairing their isolation.

M.arginini has been reported on several occasions as a secondary invador in pneumonic sheep and goats (Harbi et al 1981; Leach 1970; Perreau 1973). The organism has also been isolated from cattle with mastitis (Leach 1970). From goat mastitis, however, our isolation of M.arginini appears to be the first in the literature. Staphylococcus albus, & epidermidis and Klebsiella ozaenae has been isolated from the two cases in association with M.arginini. The isolation of Klebsiella ozaenae from milk is uncommon; but Staphylococcus is known as an important pathogen in goat mastitis (Ibraheim 1968). It is possible then that M.arginini has acted as a secondary invador to already damaged tissue, or by depleting arginine in the tissue rendered it succeptible to other pathogens. The last hypothesis has recently found support (Freundt, 1978). Ruffo et al, 1971, have reported that M.arginini induced experimental mastitis, but evidence presented up to date indicated that this mycoplasma species is not highly pathoegnic. Actually the results obtained by Ruffo and his collaborators were not definitely confirmed. If proved true, however, this may indicate the possible existence of virulent strains among the species. This fact stimulates the present authors to conduct experimental infection with their isolates, beside continuing the search for the true agents causing agalactia in goats probably existing in the Sudan.

Summary

Two strains of mycoplasmas were isolated in a pure form from milk of mastitic goats. By their mode of growth, biochemical and serological behaviour they were identified as M. arginini. This is the first report of isolation of M. arginini from goats with mastitis. The significance of the isolates as true pathogens, secondary invadors or mere commensals merits further studies.

Acknowledgement

The authors are grateful to the director Vet. Res. Administration for encouragement. They also express their gratitude to Professor E.A. Freundt. Director FAO WHO collaborating centre for Animal Mycoplasmas- Aarhus - Denmark; and to Dr. P. Perreau. Head Department of Microbiology, I.E.M.V.T., Maisons - Alfort France. Thanks are also due to the technical staff of the Department of Mycoplasma and to our colleagues in Kuku and Omdurman Veterinary Clinic. This paper is published by the kind permission of the P.U.S. Animal Resources-Khartoum.

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Table II.

The viable counts of **Ps. aeruginosa before and after deep-freezing.**

Count number	Counts / ML		
	B_1	B_2	B_3
Initial	2.3×10^8	2.3×10^7	2.3×10^7
1	3.7×10^8	2.9×10^8	Deep-Frozen
2	8.5×10^7	8×10^7	"
3	9×10^7	1.5×10^8	"
4	6×10^7	8×10^7	"
5	7×10^7	1.2×10^8	"
6	2.4×10^8	1.9×10^8	"
7	2×10^7	2.8×10^7	"
8	1×10^7	5.6×10^7	1×10^4
9	3×10^7	5×10^9	1.5×10^9
10	2.3×10^8	4.2×10^8	5×10^8
11	1.4×10^8	4.1×10^7	1.1×10^8
12	1.7×10^6	5.8×10^7	1×10^8
13	1.3×10^6	1.6×10^8	2.8×10^8
14	1.1×10^6	1.4×10^8	7.7×10^7
15	3.6×10^6	1.3×10^8	3.7×10^8
16	5.7×10^7	2×10^8	1.6×10^8
17	3.6×10^7	4.4×10^7	5.1×10^7
18	6.5×10^7	2×10^7	6.4×10^7
19	2.3×10^7	5×10^7	6×10^7
20	2×10^7	3×10^7	9×10^7
21	1×10^7	1×10^7	1×10^8
22	1.7×10^8	Deep-Frozen	1.2×10^8
23	2×10^6	"	2.3×10^7

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