Nocardia farcinica – a significant cause of mastitis in goats in Sudan

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ABSTRACT

Fifteen of 100 mastitic milk samples from goats suffering from mastitis were tentatively identified as members of the genus *Nocardia* on the basis of selected phenotypic and chemotaxonomic characteristics. Six of the 15 strains were confirmed as *Nocardia farcinica* by 16S rDNA gene sequencing and subsequent aligning with relevant actinomycetes found in electronic databases and 2 by other identification criteria. *N. farcinica* is a serious cause of mastitis with a significant prevalence (15%) among the examined goats. Efforts are needed to optimise and simplify isolation and identification methods.

Key words: goats, mastitis, *Nocardia farcinica,* Sudan.

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INTRODUCTION

Many infective agents have been implicated as causes of mastitis in cattle, but the disease is most commonly caused by *Staphylococcus aureus, Streptococcus agalactiae* and *Escherichia coli*[24]. Miscellaneous causes of mastitis in bovines, such as nocardiae, have been described[22,27]. Although mastitis in goats has been reported from some parts of the world, including Africa[1,6,20–22,27], it has received little attention in Sudan.

An early study reported the isolation of *Mycoplasma* spp., *Nocardia* sp. and *Corynebacterium pseudotuberculosis* from mastitic goats in Sudan[16], and another study confirmed *Mycoplasma agalactiae* as one of the causes of goat mastitis in Sudan[5]. To our knowledge, no general survey of the causes of mastitis among goats in Sudan has been undertaken.

Various microbial agents have been isolated from African goats with clinical or subclinical symptoms or from normal milk samples. These include *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Micrococcus* spp., *Acinetobacter* spp., *Actinomyces* spp., *Pseudomonas* spp. and coliforms[6,20,21]. Various bacteria, including *Actinomyces* spp., have been isolated from clinically normal milk samples from a mixed dairy goat flock in Kenya[21]. In Sudan there were two reports of bovine mastitis caused by nocardiae[13,26] and a single report of mastitis in a goat caused by *Nocardia asteroides*[4].

Nocardiosis is known to cause a variety of suppurative infections in humans and animals[12,19]. The most commonly reported pathogenic species are *Nocardia africana, N. asteroides, N. farcinica* and *N. nova,* followed in order of importance by *N. brasiliensis, N. otididiscaviarum, N. pseudobrasiliensis* and *N. transvalensis.* The incidence of such infections in humans and animals in tropical countries is unknown although nocardiosis has been reported from most regions of the world. However, it is well established that nocardiae can be easily overlooked during routine culture and smear examinations.

The aim of this study was to identify to species level a number of actinomycetes-like strains that had been isolated from mastitic goats in Sudan, using 16S rDNA sequence analysis of representatives of major phenotypic clusters identified recently[11].

MATERIALS AND METHODS

Animals and area of investigation

The goats examined during the course of this report belonged to the Nubian type and 1 was a Saanen type. All goats were from Khartoum State, central Sudan. Thorough clinical examination with special attention to udders was conducted. Milk samples from 100 mastitic goats were collected in sterile containers and immediately transported to the laboratory for bacteriological investigations.

Bacterial isolations

Primary isolation of the causal agents was carried out using tryptic soya agar (TSA; Difco). TSA plates were incubated aerobically at 37 °C for up to 5 days. Subsequent subcultures of the primary cultures were made using glucose yeast extract agar (GYEA: 10 g glucose, 10 g yeast extract, 14 g agar, 100 mℓ distilled water; pH 6.8).

Mycolic acid analysis

The 15 nocardia-like strains and other actinomycete-like strains were examined for the presence of nocardomycolates by thin-layer chromatography (TLC). Extraction of nocardomycolic acids and TLC analysis of extracted mycolates were performed as previously described[15]. The presence of single-spot co-chromatographs, compared with *N. farcinica* (ATCC 3888), confirmed the presence of nocardomycolates.

Phenotypic identification

Cultures were subjected to an identification scheme using selected morphological and cultural characteristics[12]. One of the diagnostic features of actinomycetes, such as nocardiae, is the presence of acid-fast branching filaments[23]. To detect strains of *Nocardia farcinica,* rapid opacification of Middlebrook 7H10 agar was also included as a test in the identification scheme[2,10].

Sequencing of 16S rDNA

Isolation of chromosomal DNA and 16S rDNA sequencing were carried out according to the method of Chun and Goodfellow[3]. The resulting PCR amplicons were separated by gel electrophoresis and purified using Nucleospin Extraction Kits (Macherey-Nagel, Dueren, Germany) according to the manufacturer's instructions. Sequencing of the almost complete 16S rDNA gene was performed as previously described[3].

Phylogenetic analyses

The resulting sequences were manually aligned against other nocardiae and representative sequences from members of the genera *Corynebacterium, Dietzia, Gordonia, Mycobacterium, Rhodococcus, Skermania, Tsukamurella* and *Williamsia*

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retrieved from the DDBJ/EMBL/GenBank databases using PHYDIT (http://plaza.snu.ac.kr/~jchun/phydit/). Evolutionary trees were inferred by using 4 treeing algorithms, namely, the least-squares[9], maximum-likelihood[8], maximum-parsimony[18] and neighbour-joining[25] using the PHYLIP suite of programs[7]. Bootstrap analyses were used to evaluate the treeing topologies of the neighbour-joining data according to Jukes and Cantor[17] based on 1000 resamplings.

RESULTS

The presence of branching filamentous organisms was taken as the cause of mastitis as they are not considered to be part of the normal udder flora; other organisms were considered contaminants. Based on phenotypic properties, 15 of the 100 isolated strains were recognised members of the genus *Nocardia.* Most of the strains were isolated in pure form from the milk samples. The 1st isolate (SD1800) was isolated in pure form from a nodular granulomatous mastitic case. The procedure was repeated 3 times, and this case was then used a benchmark for the isolation procedure. TSA is not a selective medium and therefore our procedure did not target particular organisms but rather revealed actinomycetes as they were encountered.

The 15 strains showed: a) rapid growth with rough, wrinkled colonies that varied in colour from orange (Fig. 1), to grey to cream-yellow; (b) colonies were firmly attached to the medium, were difficult to emulsify, and had sparse or non-aerial hyphae. Microscopically, the organisms were Gram-positive, weakly to non-acid fast and contained branched filaments that fragmented into short chains and, occasionally, rods.

In TLC analysis, the 15 strains were found to contain mycolic acids. These mycolates were considered nocardomycolates as they co-migrated with those typical of *Nocardia* sp.

Eight of the fifteen strains were identified phenotypically as *Nocardia farcinica* as all opacified the Middlebrook agar 7H10 whereas none of the other recognized species of *Nocardia* do. It should be noted, however, that neither *Nocardia asiatica* nor *N. puris* were included for comparison because type strains are still unavailable.

Six of the 8 strains showed a 16S rDNA gene similarity of 100 % with that of *Nocardia farcinica* ATCC 3318^T (Fig. 2). A bootstrap value of 100 % was attained in the neighbor-joining tree.

Based on these results, the eight strains could be confidently assigned to *N. farcinica.*

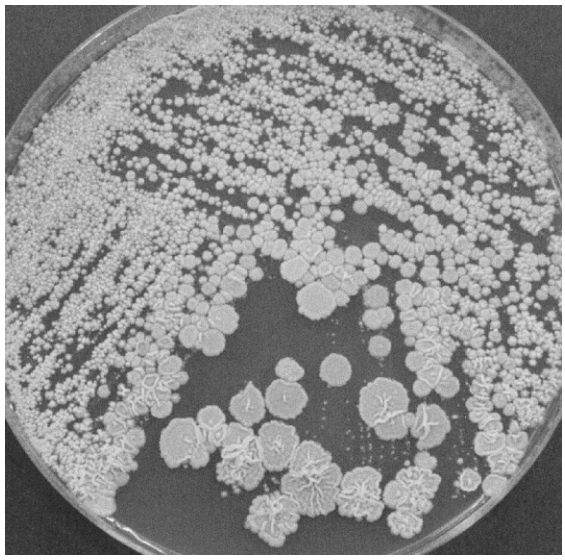

Fig. 1: **7-day-old culture of *Nocardia farcinica* isolated from milk of a mastitic goat and grown on glucose yeast extract agar.**

DISCUSSION

The number of cases of mastitis due to nocardiae reported in this study is relatively high (8/100 milk samples from mastitic goats). This is surprising in view of the lack of previous reports of mastitis in goats caused by *N. farcinica.* There are, however, reports of nocardiae causing mastitis in cattle[22] and other diseases in animals[27]and a single report of mastitis in a goat caused by *Nocardia asteroides*[4].

Little is known about the incidence of

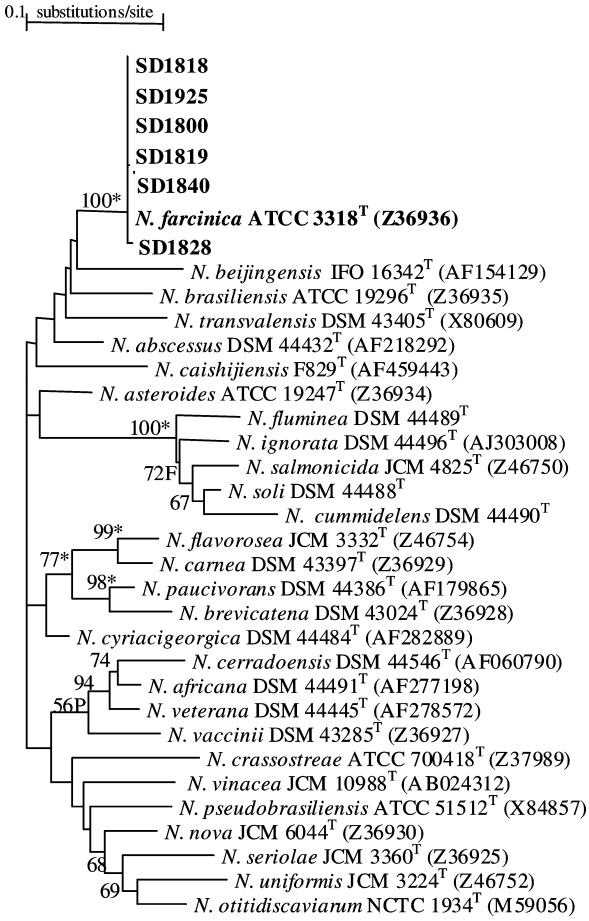

Fig: 2: **Neighbor-joining tree of almost complete 16S rDNA sequences** (1456 nucleotides) **showing relationships between the isolates and representatives of the genus *Nocardia.* The asterisks denote the branches that were also recovered using the least-squares, maximum-likelihood and maximum-parsimony methods. The numbers at the nodes indicate the level of bootstrap support** (%) **based on a neighbour-joining analysis of 1000 resampled data sets; only values above 50 % are given. The scale bar indicates 0.2 substitutions per nucleotide position.** T = type strain.

infections in goats, not only with regard to mastitis, but other diseases as well. In Sudan there are about 20 million goats, and as our pilot study suggests, a surprisingly large proprotion of the population might be suffering from infections due to *Nocardia farcinica* and possibly other actinomycetes as well.

The initial identification criteria used in the present study, namely colony morphology, opacification of Middlebrook 7H10 agar and the detection of nocardomycolates are highly diagnostic of *Nocardia farcinica,* as confirmed by 16S rDNA gene sequencing of 6 of the 15 isolated strains. Our results on opacification of agar media is in agreement with those of previous studies[2,10] in which this test was recommended as a useful adjunct to routine methods when identifying strains of *Nocardia farcinica.*

To our knowledge, this is the 1st report of mastitis in goats due to *Nocardia farcinica* and it is evident from the present study that better and more accurate methods for the quick identification of pathogenic nocardiae should be urgently sought and evaluated.

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REFERENCES

1. Bergonier D, Berthelot X, Poumarat F 1997 Contagious agalactia of small ruminants: current knowledge concerning epidemiology, diagnosis and control. *Review of Science and Technology* 16: 848–73
2. Carson M, Hellyar A 1994 Opacification of Middlebrook agar as an aid in distinguishing *Nocardia farcinica* within the *Nocardia asteroides* complex. *Journal of Clinical Microbiology* 32: 2270–2271
3. Chun J, Goodfellow M 1995 A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *International Journal of Systematic Bacteriology* 45: 240–245
4. Daffalla E N, Gharib H M 1958 A study of mastitis in goats caused by *Nocardia asteroides. British Veterinary Journal* 114: 143–145
5. el Hassan S M, Harbi M S, Mamoun I E 1986 Isolation and identification of *Mycoplasma agalactiae* from mastitic goats in the Sudan. *British Veterinary Journal* 142: 289–290
6. El Idrissi A H, Benkirane A, Zardoune M 1994 Studies on subclinical mastitis in caprine dairy herds in Morocco. *Revue d'Elevage de Médicine et Vétérinaire de Pays Tropicaux* 47: 285–287
7. Felsenstein J 1993 *PHYLIP Phylogenetic inference package* (version 3.5c). Department of Genetics, University of Washington, Seattle, USA
8. Felsenstein J 1981 Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular and Evolution* 17: 368–376
9. Fitch W M, Margoliash E 1967 Construction of phylogenetic trees: a method based on mutation distances as estimated from cytochrome c sequences is of general applicability. *Science* 155: 279–284
10. Flores M, Desmond E 1993 Opacification of Middlebrook agar as an aid in identification of *Nocardia farcinica. Journal of Clinical Microbiology* 31: 3040–3041
11. Gamal El Din O A 2003 Studies on mastitis in goats caused by actinomycetes. MVSc thesis, University of Khartoum, Khartoum
12. Goodfellow M 1998 Nocardia and related genera. In Balows A, Duerden B I (eds) *Topley and Wilson's microbiology and microbial infections,* 9th edn, vol. 2. Edward Arnold, London: 463–489
13. Hamid M E, El Sanousi S M, Minnikin D E, Goodfellow M 1998 Isolation of *Nocardia farcinica* from Zebu Cattle suffering from mastitis. *Sudan Journal of Veterinary Science and Animal Husbandry* 37: 66–71
14. Hamid M E, Maldonado L A, Sharaf Eldin G, Fathelrahman M, Saeed N S, Goodfellow M 2001 *Nocardia africana* sp. nov. from patients with pulmonary lesions. *Journal of Clinical Microbiology* 39: 625–630
15. Hamid M E, Minnikin D E, Goodfellow M, Ridell M 1993 Thin layer chromatographic analysis of glycolipids and mycolic acids from *Mycobacterium farcinogenes, Mycobacterium senegalense* and related taxa. *International Journal of Medical Microbiology, Virology, Parasitology and Infectious Diseases* 279: 354–367
16. Ibrahim A E 1968 Caprine mastitis caused by *Mycopasma, Nocardia* and *Corynebacterium pseudotuberculosis. The Sudan Journal of Veterinary Science and Animal Husbandry* 9: 31–38
17. Jukes T H, Cantor C R 1969 Evolution of protein molecules. In Munro H N *Mammalian protein metabolism* Vol. 3. Academic Press, New York: 121–132
18. Kluge A G Farris F S 1969. Quantitative phyletics and the evolution of the anurans. *Systematic Zoology* 18, 1–32
19. McNeil M M, Brown J M 1994 The medically important aerobic actinomycetes: Epidemiology and microbiology. *Clinical Microbiology Reviews* 7: 357–417
20. Ndegwa E N, Mulei C M, Munyua S J 2000 The prevalence of subclinical mastitis in dairy goats in Kenya. *Journal of South African Veterinary Association* 71: 25–27
21. Ndegwa E N, Mulei C M, Munyua S J 2001 Prevalence of microorganisms associated with udder infections in dairy goats on small-scale farms in Kenya. *Journal of South African Veterinary Association* 72: 97–98
22. Pier A C, Willers E H, Mejia M J C 1961 *Nocardia asteroides* as a mammary pathogen of cattle. *American Journal of Veterinary Research* 19: 319–320
23. Quinn P J, Carter M E, Markey B, Carter G R (eds) 1999. *Clinical veterinary microbiology,* Mosby, Edinburgh
24. Radostitis O M, Gay C C, Blood D C, Hinchcliff K W 1994 *Veterinary medicine,* 9th edn. W B Saunders, London
25. Saitou N, Nei M 1987 The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425
26. Shigidi M T A, Mamoun E 1981 Isolation of *Nocardia asteroides* from cattle with mastitis in Sudan. *Bulletin of Animal Health and Production in Africa* 29: 275–278
27. Walton A M, Libke K G. 1974 Nocardiosis in animals. *Veterinary Medicine and Small Animal Clinics* 69: 1105–1107