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With 2 figures in the text.

Short communication:

First Report of *Nocardia farcinica*-Induced Granulomatous Mastitis in a Saanen Goat in the Sudan

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ملخص البحث

تم عزل بكتيريا من نوع (*Nocardia farcinica*) من عنزة سعانين تعانى من إلتهاب ضرع بمستشفى كلية الطب البيطرى الجامعي التعليمى بشمبات. الضرع المصاب كان متضخماً وبه عجيرات صلبة عند التحسس ويفرز كميات قليلة من سائل مائي يحتوي على جلطات ثخينة ورمادية اللون من الحليب. لم تستجب العنزة للعلاج بعقار "النيوماستبرا"(Neomastipra). أظهر الفحص النسيجي المرضي تفاعلاً حبيبيا نموزجياً. تم التعرف على الميكروب مبدئياً بواسطة اختبارات مظهرية مختارة وتم التأكيد على هوية الميكروب **(***Nocardia farcinica***)** بواسطة تحليل المورثه 16S من الحامض النووي الرايبوسومي(rDNA).

Summary

***Nocardia farcinica* was isolated from a Saanen nany goat suffering from a chronic unilateral mastitis at the University of Khartoum Veterinary Teaching Hospital, Shambat. The infected udder was swollen with hard nodules and scanty watery secretion containing grey thick milk clots. The goat did not respond to treatment with Neomastipra. Histopathological examination revealed a typical granulomatous reaction. The isolate was initially identified on the basis of selected phenotypic properties and confirmed as *Nocardia farcinica* using 16S rDNA gene sequence analysis.**

Many eco-types of goats are raised in the Sudan; but the Nubian and the desert types are dominant. Encouraging genetic upgrading and increasing the production of goats via importing foreign breeds such as Saanen, has attracted the attention in the Sudan during the last two decades. Saanens are high producing dairy goats with an average milk yield of 960 kg per annum (Haenlein, 1996).

Mastitis in goats has drawn little attention in many countries including the Sudan, although the disease is known to cause significant economic loss (Radostitis *et al*, 2000).

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species were isolated from clinically normal milk samples of mixed dairy goat flocks, including Saanen (Ndegwa *et al*, 2001). However, in many tropical countries, the occurrence of such infections in man and animals remains unknown because these organisms can be easily overlooked during routine bacteriological examination (Goodfellow, 1998). The aim of this communication was to report untreatable chronic granulomatous *Nocardia* mastitis in a Saanen goat in the Sudan.

The goat examined belonged to a Saanen breed, which was brought to the University of Khartoum Veterinary Teaching Hospital at Shambat, with signs of mastitis. Thorough clinical examination with especial attention to udder was conducted. Milk samples were collected in sterile containers and sent immediately to the laboratory for microbiological investigations**.**

Neomastipra JR5 (Hipra, S.A., Spain) was used for treatment; half of the vial was administered daily following the evacuation of the udder. The dose was repeated for five consecutive days.

Two milk samples were collected two days apart and examined according to standard bacteriological methods as described by Maldonado *et al* (2004). Smears from milk were prepared and stained by Gram and Ziehl-Neelsen stains. The isolate was examined for selected phenotypic properties as described by Isik *et al* (1999).

Biopsy for histopathological sections was fixed in 10% formalin, prepared accoring to conventional histopathological methods and stained by Haematoxylin and Eosin (H & E).

Extraction and thin layer chromatography analysis of mycolic acids were done as described previously (Hamid *et al*, 1993). Isolation of chromosomal DNA and PCR amplification of the 16S rDNA gene was carried out according to Chun and Goodfellow (1995). The 16S rDNA nucleotide sequences were first handled manually to correct the machine errors; then edited and aligned using PHYDIT for Windows (Version 3.1., J. Chun). Edited seqences were then tested on BLAST electronic system (www.ncbi.nlm.nih.gov\blast) to establish a quick phylogenetic position. Following an assignment of the isolate with *Nocardia* spp. in the BLAST system, the sequences were compared to all known *Nocardia* spp. sequences found in GenBank database (www.ncbi.nlm.nih.gov\nucleotides). Distance estimation and tree topology were done using the neighbour-joining algorithm with the aid of TREECON for windows software (Version 3.1b, University of Antwerp, Belgium). To test the significance of the resulting tree topology, bootstrap analysis in 100 re-sampling using the TREECON programme was performed.

On clinical examination, one half of the udder was found swollen and contained five hard nodules which were 1 to 3 cm in diameter. The infected milk was scanty, watery and contained grey flakes and clots. The supra-mammary lymph nodes were swollen. Histopathologically, the nodules showed chronic inflammatory reaction with infiltration of mononuclear cells, epithelioid cells and masses of fibrous connective tissue (Fig.1).

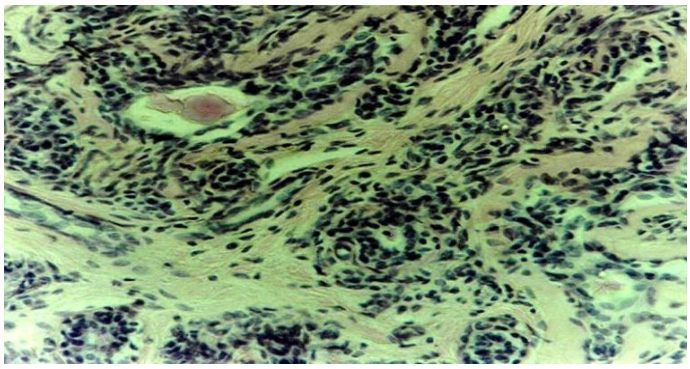

Fig. 1 : Section of mastitic udder of a saanen goat caused by *Nocardia farcinica* showing interstitial mononuclear and epithelial cells infiltration and proliferation of fibrous connective tissues (H & E x 100).

Smears prepared from infected milk and from culture revealed gram positive and acid fast branching filamentous organisms characteristic for nocardiae. This is a good property to diagnose nocardiae (Goodfellow, 1998). The two milk samples showed a similar growth onto Tryptic Soya Agar (TSA) after three days under aerobic incubation at 37^{o} C that was labelled SD1800. The colonies of the grown culture were orange, rough, concave and dry and were suggestive for a member of the genus Nocardia. The isolated strain SD1800 and the reference *N. farcinica* strain ATCC 3888 both were aerobic, non-motile, non-spore forming, catalase positive, utilized rhamnose as a sole carbon source and hydrolysed esculin and arbutin.

The organism was confirmed to be a member of the genus *N.farcinica* following the detection of the nocardo-mycolates on whole cell acid methanolysis in both the reference, *N. farcinica* ATCC 3318 and the test SD 1800 strain. The strain was confirmed as *N. farcinica* by 16S rDNA gene sequencing and subsequent aligning with relevant actinomycetes found in the electronic databases. The strain showed a 100% similarity in the 16S rDNA gene with that of the reference *N. farcinica* ATCC 3318, with a bootstrap value of 100% in the neighbour-joining tree methodology (Fig. 2).

Fig. 2: A phylogenetic tree based on 16S rDNA sequences (1456 nucleotides) showing relationships and position of the mastitis isolate (SD1800) to members of the genus *Nocardia* and related mycolic acid containing actinomycetes. The scale bar indicates 0.02 substitutions per nucleotide position.

The case did not respond to treatment with Neomastipra, which represents a range of antibiotic and antibacterial agents as well as an anti-inflammatory agent. It has been noted that nocardiae represent difficulty in treatment (Wallace *et al*, 1990).

The results of the present report conclude that a serious cause of mastitis in saanen goats is unveiled. It constitutes the first reported case among the imported saanen breed. saanen goats with their high milk production potential are disposed to mastitis notably with opportunistic organisms such as *nocardiae* and other soil-borne pathogens. It has been found that foreign breeds manifest mild tropical diseases with severe clinical signs (Ageeb and Hayes, 2000). Further studies are warranted to determine the prevalence rate and the clinical manifestations of such infections at the country level in order to estimate such serious hazard to dairy goats industry. Application of chemical as well as molecular methods, though are demanding techniques has proven to be quite reliable to establish species status in identifying an unknown organism.

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