On the Aetiology of Bovine Farcy in the Sudan

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Fifteen strains of the agent of bovine farcy were isolated from lymph nodes of affected cattle. Quantitative analyses of mycolic acids revealed values that allowed the assignment of these strains to the genus *Mycobacterium*. The organisms bore a greater resemblance to *Mycobacterium farcinogenes* than to *Mycobacterium senegalense*.

INTRODUCTION

Since 1970 it has been known both in Chad and in Senegal that farcy, the disease of African cattle, is really a mycobacteriosis and that in most cases it is not due to *Nocardia farcinica* as had been believed previously (Chamoiseau, 1979). Chamoiseau (1973) introduced the species *Mycobacterium farcinogenes* for the agent of bovine farcy and recognized two subspecies, *tchadense* and *senegalense*. The former contained the slow-growers with less activity when grown on amides and lower pathogenicity for guinea pigs than the latter. Later, these were raised to species status as *M. farcinogenes* and *M. senegalense* (Chamoiseau, 1979). There is evidence, however, that strains of these mycobacterial species have frequently been mis-identified as *N. farcinica* (Ridell & Goodfellow, 1983; Ridell *et al.*, 1982, 1985) and reliable methods are needed to distinguish *M. farcinogenes* from *N. farcinica*.

Lipid analyses have provided good characters for the classification and identification of many actinomycete genera (Minnikin *et al.*, 1985). Mycolic acids, which are 2-alkyl, 3-hydroxy long-chain fatty acids, have particular importance in the classification of actinomycetes with a wall chemotype IV based on *meso*-diaminopimelic acid, arabinose and galactose (Lechevalier *et al.*, 1971; Ridell *et al.*, 1982; Minnikin *et al.*, 1985).

The present study describes the development of quantitative analysis of mycolic acids in field strains of bovine farcy in the Sudan as a method for deciding whether these strains belong to the genus *Mycobacterium*.

METHODS

Strains. Fifteen strains were isolated from lymph nodes of cattle affected with farcy as previously described (El Sanousi *et al.*, 1977, 1979). The strains isolated from Nayala, Southern Darfur province, Western Sudan, were designated NF3, NF6, NF10, NF11, NF12 and NF13 and those isolated from Kadogli, Southern Kordofan Province, Western Sudan, were designated KF14, KF15, KF16 and KF17. Strains KHF1, KHF2, KHF3, KHF4 and KHF5 were isolated from three abattoirs in Khartoum, Khartoum Province, Central Sudan. *Nocardia asteroides*, *Nocardia brasiliensis* and *Mycobacterium phlei* were obtained from the Central Medical Laboratory of Khartoum. All cultures were grown in modified Sauton's medium at 37 °C as described by Mordarska *et al.* (1972).

Mycolic acid extraction. Mycolic acids were extracted from 2 g wet weight of bacteria by the method of Kanetsuna & Bartoli (1972), weighed and their melting points determined. The residues collected from all the *Mycobacterium* strains used in this study were pooled and redissolved in 15.6 ml diethyl ether and 13 1.2-ml samples were taken. Each sample received one of the following amounts of ethanol: 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, 2.5, 2.75, 3.0, 3.25 or 3.5 ml. The mixtures were stored at 0 °C for 18 h, centrifuged and the residues

weighed. The relation between the amount of ethanol used and the mycolic acid extracted was examined by the quadratic equation ($Y = a + bx + cx^2$) according to the method described by Snedecor & Cochran (1967).

RESULTS AND DISCUSSION

The mean value of three trials for the amount of mycolic acid extracted from each strain is shown in Table 1. The effect of increasing the amount of ethanol on mycolic acid extraction is shown in Fig. 1.

A maximum extraction of mycolic acid was obtained when about 2 ml ethanol was added and, thereafter, a negative effect on the amount of mycolic acid was observed with increasing amounts of ethanol. All the test strains from cases of bovine farcy gave amounts of mycolic acid greater than 8 mg per g of bacteria while the *Nocardia* strains tested gave amounts of residues less than 2 mg g⁻¹. Only 5 mg (g wet wt bacteria)⁻¹ is required for assignment to the genus *Mycobacterium* (Kanetsuna & Bartoli, 1972). The melting point of the mycolic acids extracted ranged between 50 and 52 °C compared to 150 °C for the residues isolated from *Nocardia* (Table 1).

The modification of the method of Kanetsuna & Bartoli (1972) applied in this study for extraction and quantification of mycolic acid is simple and reproducible especially for diagnostic laboratories. The procedure provides a convenient way of distinguishing between mycobacteria of veterinary importance and nocardiae.

Fig. 1. Mycolic acids extracted with different amounts of ethanol. The relationship is governed by the equation $Y = 113.88X - 30.11X^2 - 52.85$.

Table 1. Amounts of mycolic acids extracted from different strains of *M. farcinogenes* and *N. asteroides*, *N. brasiliensis* and *M. phlei*

The method of Kanetsuna & Bartoli (1972) was used. Values are the mean of three determinations.

Strain	Mycolic acid [mg(g wet wt) ⁻¹]	Melting point (°C)	Strain	Mycolic acid [mg(g wet wt) ⁻¹]	Melting point (°C)
<i>M. farcinogenes</i>			<i>M. farcinogenes</i>		
NF3	14.66	50	KHF1	13.41	51
NF6	14.24	50	KHF2	12.60	50
NF10	14.16	50	KHF3	9.87	52
NF11	13.43	50	KHF4	12.36	51
NF12	14.62	50	KHF5	12.79	51
NF13	13.56	51	<i>N. asteroides</i>	0.77	150
KF14	14.16	50	<i>N. brasiliensis</i>	1.88	150
KF15	14.21	50	<i>M. phlei</i>	9.22	50
KF16	8.47	52			
KF17	12.35	52			

Most of the strains tested were also studied by Shigidi *et al.* (1980) who suggested their placement in the genus *Nocardia*. However, mycobacteria and nocardiae share many antigens in common, and the presence or absence of a single unidentified precipitinogen can not be used to distinguish between the two genera (Ridell, 1983). Moreover, Ridell *et al.* (1985), using the same strains but designated GB 375–GB 385, concluded that *M. farcinogenes* and *M. senegalense*, but not *N. farcinica*, are the principal agents of bovine farcy in the Sudan, thus substantiating the findings of El Sanousi *et al.* (1977) and El Sanousi & Salih (1979). Since all the Sudanese strains of bovine farcy tested were of the slow-growing type and since they are highly pathogenic for guinea pigs with weak activity when grown on amide, they bear a greater resemblance to *M. farcinogenes* than to *M. senegalense*. It is also apparent that *N. farcinica* is not commonly encountered as a cause of bovine farcy in Eastern Africa.

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