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Mycobacteria causing human cervical lymphadenitis in pastoral communities in the Karamoja region of Uganda

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SUMMARY

Mycobacteria from lymph node biopsies of patients with cervical lymphadenitis reporting for tuberculosis treatment in Matany and Moroto Hospitals in the transhumant areas of Karamoja, Uganda were isolated and characterized. The AccuProbe® culture identification kits for *Mycobacterium tuberculosis* complex (MTC), *M. avium* complex (MAC) and *M. avium* were used to identify the isolates. Spoligotyping, IS*901* PCR and IS*1311* and IS*1245* restriction fragment length polymorphism (RFLP) were used to characterize the isolates. Of the 43 biopsies, ten *M. avium*, seven *M. tuberculosis*, three *M. bovis*, and two *M. intracellulare* were isolated. Two isolates could not be identified with AccuProbe® and from 19 samples no mycobacteria could be isolated. Three isolates with the Beijing spoligotype were identified from the seven *M. tuberculosis* isolates. The spoligopatterns of the *M. bovis* isolates had previously been detected in cattle in Uganda. Isolation of members of the MAC group reflects the complex interaction between the transhumant communities, water sources and their cattle. None of the *M. avium* isolates harboured IS*901*, and all showed several bands on IS*1311* and IS*1245* RFLP, in accordance with *M. avium* subsp. *hominissuis*. Composite dendrograms of IS*1311* and IS*1245* RFLP showed that the isolates were similar and identical patterns were found. The isolation of *M. bovis* confirms the human infection with zoonotic mycobacteria in areas where consumption of raw milk and meat is routine. Isolation of environmental mycobacteria also confirms their increasing role in human disease and the occupational risk of infection in the transhumant ecosystem in the absence of safe drinking water and environmental contamination.

INTRODUCTION

Tuberculosis (TB), one of the most widespread infectious diseases, is a leading cause of death from a single infectious agent among adults in the world [1]. After decades of decline, incidence rates of TB are increasing worldwide. In 2004, 2·6 million (29%) of the 8·9 million cases of worldwide TB reported to the Global Tuberculosis Programme of WHO were from Africa. This figure is considered low because many African countries, especially those with few resources, are unable to report all TB cases because of difficulties in identifying suspected cases, establishing proper diagnostic procedures, and poor recording and reporting systems [2].

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Surveillance reports on Uganda [3] suggest an increase in TB incidence and decline in case detection rate. In 2004, Uganda ranked 15th in Africa on estimated number of incident cases (all forms), with an incidence of 402/100 000 persons per year and a prevalence of 646/100 000 people respectively. New notification rates of 13 extra-pulmonary TB cases/100 000 persons per year were also reported. While the information on causative agents of human TB in developed countries may be available, the relative contribution of *Mycobacterium tuberculosis* complex (MTC) and mycobacteria outside the MTC (MOT) to the human mycobacterial syndromes in developing countries, including Uganda, is still scarce. This is attributed to weak laboratory infrastructure and inadequate financial and human resources [3]. As reported, these results were based on smears, and no information was available on the identity of the species of mycobacteria isolated in the patients.

A review of zoonotic TB [2] estimates the proportion of human cases due to *M. bovis* to account for 3·1% of all forms of TB; 2·1% of pulmonary and 9·4% of extra-pulmonary forms. The incidence of mycobacterial cervical lymphadenitis has increased in parallel with the increase in the incidence of mycobacterial infection worldwide and may be a manifestation of a systemic tuberculous disease or a unique clinical entity localized to the neck [4]. Bovine tuberculosis (BTB) has been reported in cattle in Karamoja [5] and *M. bovis* has been confirmed present in other pastoral areas as well [6]. The impact of high herd level prevalence of BTB in cattle herds in Karamoja region [5] on the health of transhumant communities continuously in close contact and highly dependent on cattle as source of food has not been investigated, but studies done in similar pastoral communities in the neighbouring Tanzania isolated *M. bovis* from 21% [7] and 10·8% [8] of culture-positive cases of tuberculous cervical lymphadenitis.

Consumption of raw milk, fresh blood and uninspected meat, documented in various studies as routes of *M. bovis* transmission to humans [9–11] are traditional practices rampant in pastoral communities. Milk-borne infection is the principal cause of cervical lymphadenopathy (scrofula) and abdominal and other forms of non-pulmonary TB [12]. In the more profoundly immunosuppressed individuals, *M. bovis* infections tend to be disseminated [13].

Like zoonotic *M. bovis*, the emergence of MOT as a classical opportunistic or true pathogen in human and animal infections is also becoming increasingly important [14–17]. In the absence of *M. tuberculosis*, MOT are increasingly being isolated in HIV-positive patients. *M. avium-intracellulare* (MAC) have been isolated in 82·8% of non-tuberculous mycobacterial infections [18]. Members of MOT are saprophytic and are normal residents in natural waters rich in organic matter [19–22]. Moreover, water has been documented as a source of infection for mycobacteria causing cervical lymphadenitis and disseminated infections in humans [14, 22–24]. In children, one study has shown a predominance of *M. scrofulaceum* (60%) followed by *M. avium* (40%) as causes of cervical lymphadenitis [25] while another report [26] documented a predominance of MAC to *M. scrofulaceum*.

The occurrence of BTB in cattle and the existence of suitable biotopes for MOT in the water sources in transhumant or pastoral areas could increase the risk of infection with MTC and MOT to livestock and cervical lymphadenitis syndromes in communities. Against this backdrop, there was a need to investigate the possible impact of zoonotic and environmental mycobacteria in human disease in high-risk transhumant communities of Karamoja, Uganda. The main objective of this study was, therefore, to isolate and characterize the mycobacteria causing cervical lymphadenitis in patients in the transhumant areas of Karamoja, Uganda.

MATERIALS AND METHODS

Collection of samples

Routine lymph node biopsies from 43 human patients with cervical lymphadenitis reporting to the TB units of Moroto and Matany hospitals were aseptically collected and frozen at −20 °C. Data on age, sex, occupation and origin were collected for each patient. Samples were transported in cooling boxes containing icepacks to the National Tuberculosis referral laboratory at Wandegeya, Kampala, where they were processed.

Tissue preparation and bacteriological examination

Fat and connective tissue were removed from the samples and about 3–10 g were placed in sterile Stomacher bags containing 30 ml physiological buffered saline and homogenized in a Stomacher machine for 7–10 min. The homogenates were transferred to sterile screw-cap tubes and decontaminated

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by the NaOH–NALC method [27]. After vortexing, the samples were left at room temperature for 15 min. Subsequently 5 ml of sterile 0·067 m phosphate buffer (pH 6·8) was added and the mixture was centrifuged at 3660 ***g*** for 15 min. The supernatant was discarded and the sediment was inoculated on Lowenstein–Jensen (Difco Laboratories, Detroit, MI, USA) media with and without pyruvate (0·6%) and incubated at 37 °C for 12 weeks. Tubes were read weekly, and typical or suspect colonies were harvested into cryotubes containing 1·5 ml Middlebrook 7H9 media (Difco) and stored at −70 °C at the National TB referral laboratory, Uganda. Samples were then transported to the National Veterinary Institute, Oslo and subsequently subcultured on Middlebrook 7H10 and Stonebrink media (Difco).

Identification of mycobacterial isolates

All acid-fast bacteria, determined by the Ziehl–Nielsen (ZN) staining technique, were examined by AccuProbe® *Mycobacterium tuberculosis* complex (MTC) identification kit (Gen-Probe Inc., San Diego, CA, USA) according to the manufacturer's protocol. Results were considered positive when relative light units (RLU) were >30 000, repeat range 20 000–29 000, and negative <20 000. Samples negative on the MTC kit were examined further on the AccuProbe® *M. avium* complex (MAC) and AccuProbe® *M. avium* culture identification kits. Final results were interpreted as follows; cultures positive on the *M. avium* and MAC culture identification kits were considered as *M. avium* and cultures negative on the *M. avium* kit and positive on the MAC kit were identified as *M. intracellulare*. Samples negative on all AccuProbe® culture identification kits were grouped as unidentified mycobacteria.

Spoligotyping

Cultures belonging to the MTC were submitted for further differentiation by the spoligotyping kit according to the manufacturer's instructions (Isogen, Life Science, The Netherlands). DNA isolation was done according to the protocol for DNA extraction for cell cultures (Qiagen™, Oslo, Norway), and PCR and hybridization were performed as previously described [28]. Amplified DNA was hybridized to a membrane containing 43 oligonucleotides in a miniblotter (MN45, Immunetics, Cambridge, MA, USA). Bound fragments were revealed by chemiluminescence after incubation with horseradish peroxidase-streptavidin (Boehringer, Mannheim, Germany) for 45 min at 45 °C and exposure of the membrane to X-ray film (Hyperfilm, Amersham, Bucks., UK) for 10–12 min. The *M. tuberculosis* H37Rv and *M. bovis* BCG were included as controls.

The results were analysed using the BioNumerics program version 3.5 (Applied Maths, Kortrijk, Belgium). The BioNumerics software was used to calculate Dice coefficients of similarity and to cluster the isolates and generate dendrograms by the unweighted-pair group method using average linkage. The optimization and tolerance settings were both set at 2·00%.

Examination by IS*1311* and IS*1245* restriction fragment length polymorphism (RFLP)

Cultures identified as *M. avium* were further examined for the insertion sequence IS*901* [29] and characterized by IS*1245* and IS*1311* RFLP. The DNA extraction, RFLP analysis and interpretation of results were performed as previously described [29], using the recommended probes for IS*1245* and IS*1311* RFLP.

RESULTS

The 43 biopsies from patients with lymphadenitis were cultured and different mycobacteria were isolated and characterized *M. avium* were found in 23·3% ($n=10$), *M. tuberculosis* in 16·3% ($n=7$), *M. bovis* in 7·0% ($n=3$) and *M. intracellulare* in 4·7% ($n=2$) of the samples. Two isolates (4·8%) were negative on all three AccuProbe® kits, and from 19 samples (45·2%) no mycobacteria could be detected. Patient data and data on mycobacterial isolates are presented in the Table.

Spoligotyping was performed on 10 isolates positive on the AccuProbe® MTC culture identification test; three isolates were identified as *M. bovis* (lacking spacers 39–43) and seven as *M. tuberculosis* (Fig. 1). Four spoligotypes of *M. tuberculosis* were identified; the Beijing genotype, lacking spacers 1–34, from three patients, one type that lacked spacers 1–24 and 33–36 and two types closely related to *M. tuberculosis* H37Rv. Two spoligotypes of *M. bovis* were identified from the three isolates

Nine of the ten *M. avium* isolates were characterized, the last isolate could not be typed due to culturing problems. None of the isolates harboured the IS*901* element, and all but one isolate showed four

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Table. *Patient occupation, sex, district of origin, age quartile and mycobacteria isolated from human cervical lymphadenitis in Uganda*

	Culture results						
	No. (%)	M.a	M.b.	M.i.	M.t.	Un.	No growth
Occupation							
Peasant	5 (11·6)	0	0	0	2	0	3
Baby/toddler	5 (11·6)	4	0	0	0	0	1
Businessman	1 (2·3)	0	0	0	0	0	1
Herding	4 (9·3)	1	0	0	0	0	3
Housewife/help	8 (18·6)	0	1	1	3	1	3
Nomad	20 (46·5)	5	2	1	2	1	8
Sex							
Female	11 (25·6)	1	1	0	4	1	4
Male	32 (74·4)	9	2	2	3	1	15
District							
Moroto	34 (79·1)	8	3	2	4	2	15
Nakapiripirit	2 (4·7)	1	0	0	0	0	1
Katakwi	6 (14·0)	1	0	0	2	0	3
Kumi	1 (2·3)	0	0	0	1	0	0
Age quartile							
<7 years	11 (25·6)	6	0	0	0	1	4
7–25 years	10 (23·3)	2	0	0	1	1	6
26–32 years	12 (27·9)	1	2	1	4	0	4
>32 years	10 (23·3)	1	1	1	2	0	5
Total (isolation)	24 (100)	10 (41·7)	3 (12·5)	2 (8·3)	7 (29·2)	2 (8·3)	—
Total (growth)	43 (100)	10 (23·3)	3 (7·0)	2 (4·7)	7 (16·3)	2 (4·7)	19 (44·2)

M.a., *M. avium*; M.b., *M. bovis*; M.i., *M. intracellulare*; M.t., *M. tuberculosis*; Un., unidentified.

identical bands on IS*1311* RFLP. The last isolate showed five bands. More bands were observed on IS*1245* RFLP. The composite dendrograms of IS*1311* and IS*1245* RFLPs yielded five different profiles (Fig. 2). The lack of IS*901* and the multibanded profile observed on IS*1311* and IS*1245* RLFP were compatible with *M. avium* subsp. *hominissuis* [30].

DISCUSSION

The study documented that *M. avium*, *M. tuberculosis* and *M. bovis* caused cervical lymphadenitis in humans in pastoral communities in the Karamoja region of Uganda. To our knowledge, this is the first report of *M. avium* as an important causative agent of these lesions in regions of Uganda where BTB is present and uncontrolled, a fact that is contrary to the previously held opinion that *M. bovis* was the most important cause of these infections [2]. However, no mycobacteria could be isolated or identified on ZN stain in 45·2% of patients' samples in this study.

M. bovis was the causative agent of cervical lymphadenitis in 12·5% of the cases where mycobacteria could be isolated, in accordance with what has been found in Tanzania [8, 31]. As documented elsewhere in Africa [2], cattle are an integral part of pastoralists' social life. Raw milk, fresh blood and meat form the main diet during transhumance. Ingestion of raw milk is the most probable route of extra-pulmonary *M. bovis* infections [2, 32, 33], although inhalation of the agent might also lead to infections [31, 34, 35]. The fact that *M. bovis* were detected from nomads aged >25 years might point to their leading roles in herding and privileged access to milk that is usually consumed raw and commonly mixed with cows' urine or fresh blood. *M. bovis* was not detected in children as reported previously [36]. However, the limited sample size might have affected this observation. The occurrence of *M. bovis* mainly in one district seems to point to the host factor-occupation type risk of transmission; although the limited patient catchment areas of the hospitals might

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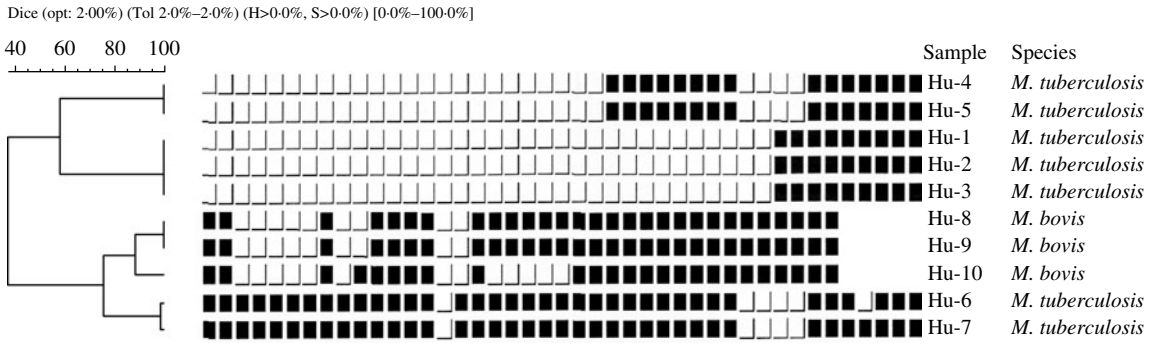

Fig. 1. A dendrogram showing spoligotypes detected in three *Mycobacterium bovis* and seven *Mycobacterium tuberculosis* isolates from human patients with cervical lymphadenitis in Uganda. For *M. bovis*, only spacers 1–38 are shown.

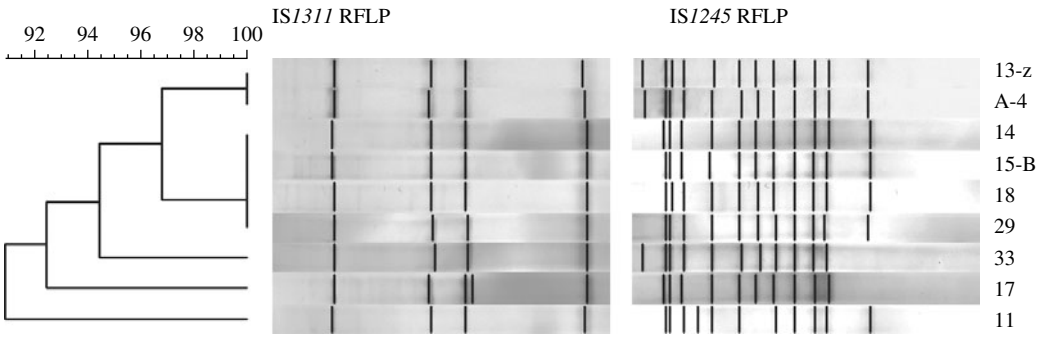

Fig. 2. A dendrogram showing the IS*1311* and IS*1245* RFLP of nine *Mycobacterium avium* isolates from human cervical lymphadenitis in Uganda.

have distorted our results. The two different spoligotypes detected in humans have previously been detected in cattle in Uganda (J. Oloya *et al.*, unpublished observations). The isolation of the same spoligotypes both in cattle (although from another location) and humans suggests cross-species transmission and might reflect the occupational risks that pastoralists face. These spoligotypes have not been detected in other African countries, and comparison of these spoligotypes with those in the database (http://www.mbovis.org) did not show any similarities, indicating that these strains might be unique.

M. tuberculosis was detected in 29·2% of the cases where mycobacteria could be isolated. In Tanzania, *M. tuberculosis* was found in 41·5% [8] to 70·5% [31] of human lymphadenitis cases. The isolation of *M. tuberculosis* did not seem to be localized to a particular occupation, sex, age or district, indicating its communicability and infectiousness. Four spoligotypes of *M. tuberculosis* were identified in the seven isolates. One of the spoligotypes (Hu-4 and Hu-5 in Fig. 1) is labelled LAM 3/S in the spoligotyping database SpolDB4, and has been isolated in 118 countries including Kenya [37], Tanzania [38] and Uganda [39]. This spoligotype is very similar to *M. africanum* II, the only difference being the presence of spacer 40 which is lacking in *M. africanum* II. Isolation of the *M. tuberculosis* Beijing spoligotype from Uganda confirms its widespread nature (a total 3758 isolates identified in 61 countries with highest isolation frequency in the Asian countries) and probably its selective advantage over other TB genotypes [40]. The two last spoligotypes detected, Hu-6 and Hu-7, have been isolated in six and 26 countries respectively. However, they have not been isolated in the East African region until now.

Isolation of MOT in 58·3% of the cases of cervical lymphadenitis where mycobacteria could be isolated corroborates other studies [25, 31, 41]. Although the data are limited, *M. avium* seems to be a problem in children in pastoral areas as previously reported [22, 42, 43]. In some countries, there may be of a shift in the frequency of isolation of previously predominant *M. scrofulaceum* to bacteria in MAC from children with cervical lymphadenitis [44, 45]. This shift could be attributed to their low immune status or other

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immunocompromising infections [46, 47], a not surprising situation in a region where 18·7% of the children are reportedly malnourished [48]. MOT was more often isolated from patients in Moroto district, probably as a result of many patients from within that district seeking medical assistance. Many MOT are found in the environment, where they are able to grow, persist and survive [45]. In absence of documented evidence of person-to-person spread of these mycobacteria [14, 49], biotopes become very important in maintaining human and animal infection. Our earlier studies in pastoral cattle in the same areas detected many avian and doubtful reactors on the tuberculin test [5], suggesting sensitization to avian tuberculin due to mycobacteria in the environment. The patients were not screened for HIV due to lack of facilities at the health centres, therefore its role can not be ruled out.

The predominance of males in this study may point to the poor social conditions of women in this highly patriarchal community. Women have less access to medical facilities than men due to customary restrictions, poverty, distance to health facilities and insecurity. Transhumant societies rarely assign women major responsibilities for large stock, while males move with the animals from place to place in the dry season in search of water and pastures. An alternative explanation is that men drink water directly from the same stagnant water sources as their animals and wild birds. As documented, water high in organic matter or animal dejections enhances growth of environmental mycobacteria [21, 47], and studies have found high MAC numbers correlated with high temperature, low pH, low dissolved oxygen and high soluble zinc [21, 41]. These findings show that water in heavily soiled swamps, as seen in the grazing areas of Karamoja, may represent major sources of mycobacterial infections, possibly connected with the higher incidence of human and animal infection in this region. The absence of safe water sources for domestic use is also a health issue in homesteads. On average, the distance to clean water sources is 5 km, often leading to families using rain surface run-off water trapped in open ponds for domestic use. Ponds in communities where 84–90% of homesteads lack toilets [50], could easily get contaminated with organic plant, animal and human matter. MAC has been recovered from water samples from ponds in several countries [21, 45, 51, 52], and soil in these ponds could be a natural habitat for MAC where water acts as vehicle for transmission [45, 47, 51, 52].

Results from IS*1311* RFLP showed only two genotypes, while IS*1245* RFLP gave a better separation of strains by generating 9–12 bands in their patterns. This low discriminatory power of IS*1311* RFLP correlates well with earlier study [29]. The different isolates showed similar patterns, and none of the isolates had the RFLP pattern earlier described as the 'bird pattern' [8] or as *M. avium* subsp. *avium* [30]. However, the lack of IS*901* and the observed multibanded RFLP patterns were compatible with *M. avium* subsp. *hominissuis* [30], an environmental mycobacteria that has been implicated as a cause of infection in humans [53]. However, more data on *M. avium* in humans, animals and the environment in Uganda are necessary in order to further elucidate the importance of this opportunistic pathogen.

In conclusion, this study has given an insight into the mycobacteria causing cervical lymphadenitis in pastoral communities in Uganda. The isolation of *M. bovis* with the same spoligopatterns from cattle and humans has once more highlighted a need for coordination of veterinary and medical policies geared towards the control of TB in developing countries [33]. The isolation of MOT from a high proportion of human cases of lymphadenitis shows the importance of bacterial culture in TB diagnostics. The isolation of the Beijing strain of *M. tuberculosis* indicates the widespread nature of this strain and potential risk associated with its resistance to drugs. The complexity of human–animal–environment interaction in pastoral production systems poses enormous challenges in control and prevention of mycobacterial pathogens.

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DECLARATION OF INTEREST

None.

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