PREVALENCE OF FILAMENTOUS AND TUBERCULOUS MYCOBACTERIA IN CASEOUS LESIONS FROM SLAUGHTERED CATTLE IN SOUTH DARFOUR STATE, SUDAN

El Tigani-Asil[1,*], A.E.; El Sanousi[2], S.M.; Aljameel[3],M. A.; El Beir[4], H.; Maha[5], F.; Mahgoub [2],A.; Terab [1], N. M. and Abdellatif[1], M.M.

[1] Department of Pathology and Microbiology, Fac. of Vet. Sci, Uni. of Nyala, Sudan
[2] Department of microbiology, Fac. of Vet. Med, Uni. of Khartoum, Sudan.
[3] Dep. Of Patho.Veterinary Research Laboratory, Nyala, National Vet. Res. Corporation, Sudan
[4] Dep. Of tuberclosis, Institute of Tropical Diseases, Ministry of Science and Technology, Sudan
[5] Dep. Of Tuberculosis, National Health Laboratory (Stack), Ministry of Health, Sudan

المستخلص

اجريت هذه الدراسة للفحص والتعرف على الجراثيم الصامدة للاحماض في الافات القيحية للابقار المذبوحة في ولاية جنوب دارفور؛السودان. تم فحص 6680 راس من ذبائح الابقار للتقصي عن الافات العيانية والقيحية المتجبنة في بعض سلخانات الولاية المختلفة خلال الفترة من 2007-2009م . تم جمع وفحص 400 (6%) عينة متقيحة لاثبات وجود المتفطرات الصامدة للاحماض باستخدام تقنيات الفحص المجهري والتزريع. تم التعرف على العزلات باستخدام بعض الاختبارات البيوكيميائية.

نتائج الفحص اظهرت ان معدل الاصابات القيحية بلغ 400 (6%) منها 145 (36.3%) عينة حاملة لجراثيم خيطية صامده للاحماض بينما 22 (5.5%) عينة حاملة لعصيات صامد للاحماض عرفت كمتفطرات سلية بقرية. من بين المتفطرات الخيطية الصامدة للاحماض 26 عزلة عرفت كمتفطرات جقيلية بينما 6عزلات عرفت كمتفطرات سنغالية. في هذه الدراسة وجد ان معدل إنتشار الافات الجبنية مقرونة بمعدل الاصابة بالمتفطرات المسببه لجقيل البقركان منخفضا(2. 2%) مقارنة مع الدراسات السابقة (7%) في منتصف السبعينيات لنفس المنطقه.

Abstract

The aim of the present study was to detect and identify acid fast bacilli (AFB) from slaughtered cattle in South Darfur state, Sudan. A total of 6680 bovine carcasses were examined for caseous lesions at different abattoirs in South Darfur State during 2007-2009. Four hundred specimens were examined for presence of AFB using microscopic and culture techniques. Isolated mycobacteria were identified with selected biochemical tests. Out of the 6680 bovine carcasses examined, 400 (6%) had caseous lesions; 145 of them (36.3%) revealed acid fast filaments whereas 22 (5.5%) were harboring tuberculous AFB. Out of the filamentous AFB, 26 isolates were identified as *M. farcinogenes*, 6 as *M. senegalense* whereas the 22 non-filamentous AFB were identified as *M. bovis.* The prevalence of caseous lesions was lower in this study compared to previous ones. There was a marked decrease in prevalence of filamentous AFB causing bovine farcy (2. 2%) compared to 7% that had been detected during the mid 1970s from the same region.

Key words: *Mycobacterium farcinogenes, M. senegalense, M. bovis,* bovine farcy, bovine tuberculosis, Darfur, Sudan.

Introduction

The genus *Mycobacterium* comprises a wide range of organisms, including obligate parasites causing serious human and animal diseases, opportunistic pathogens, and saprophytic species found in nature Kim *et al.,* (1999). Most of mycobacterial infections are caused by *M. tuberculosis* complex (MTC) strains; however, non tuberculous mycobacteria (NTM) infections are increasing particularly in immunocompromised patients (Mokaddas and Ahmed, 2007).

Conventional definitive diagnosis of mycobacterial infections is laborious and time consuming (Kim *et al.* 1999). Runyon phenotypic criteria classified mycobacteria to slowly growing, rapidly growing, pigmented and non-pigmented species (Runyon, 1959). The rapidly growing mycobacteria (RGM) include 56 environmental species. Fifteen

species are incriminated in human and animals diseases and they belong primarily to *Mycobacterium chelonae–abscessus*, *Mycobacterium fortuitum* and *Mycobacterium smegmatis* groups. (Pfyffer *et al*., 2003 and Brown-Elliott & Wallace**,** 2002), whereas *M. senegalense, M. farcinogenes* and *M. porcinum* are pathogens of veterinary importance. *M. senegalense* and *M. farcinogenes* cause bovine farcy in tropics and they are classified as rapid growing mycobacteria (Chamoiseau, 1979; Roth *et al.,* 2000), however, El Sanousi *et al.*, (1979) considered *M. farcinogenes* slow growing mycobacterium. Mohan, (1985) isolated *M.sengelense* from bovine tuberculous lesions in Nigeria. Oh *et al*. (2005) reported *M. senegalense* in human catheter-related bloodstream infection in a cancer patient in Korea while, Wong, *et al.*, (2005) reported *M. farcinogenes* in human hip joint arthroblasty. Mycobacteria can be identified by the traditional cultural properties on solid media such as growth rate, pigments and consistency (Ridell and Goodfellow 1983; Hamid and Goodfellow 1995 and Dwight *et al.,* 2004). Different biochemical reactions described by (Ridell *et al.,* 1985; Palomino *et al.,* 2007; Chamoiseau, 1979; Goodfellow and Wayne 1982 and Viencet *et al.*, 2009) have been used in identification of *Mycobacterium* spp. El Sanousi *et al.,* (1979) and Tag Eldin *et al.,* (1988) reported that the farcy agents were strongly catalase positive, urease negative and arylsulphatase negative. While Ridell and Goodfellow (1983) and Ridell *et al*, (1985) found that *M. farcinogenes* was catalase negative in contrast to *M. sengelense*. Chamoiseau (1979) and Goodfellow and Wayne (1982) recorded that both *M. farcinogenes* and *M. sengelense* were catalase and nitratase positive and urease negative. Viencet *et al.,* (2009) recorded that *M. sengelense* was nitratase, urease and arylsulphatase positive. Murray *et al.*, (2007) stated that *M.farcinogenes* was urease positive.

The aim of the present study was to detect and identify acid fast bacilli (AFB) from slaughtered cattle in South Darfur state, Sudan.

Materials and Methods

Study area and target host

This study was conducted in South Darfur State, Western Sudan, while some samples were collected from Bagara cattle slaughtered at Al Sabalouga slaughterhouse in Omdurman after antemortem inspection.

Pathological observations

At necropsy any tuberculous or caseated lesion was recorded, fully described and $1cm^3$ portions of the target lesions were immediately fixed in 10% buffered formal saline for routine histopathology according to methods described by Hewitson and Darby (2010).

Sampling

Four hundred tuberculous or caseated bovine tissues were collected in sterile containers for mycobacteriology according to methods described by Burnett and Crocker (2005).

Microscopy

Direct smears were prepared from caseated material while, homogenized smears were made by incision and mortaring of tuberculous lymph nodes and lung tissues using 0.5-1ml sterile saline to obtain homogenized suspension according to Konemann *et al.,* 2005; Rieder *et al.,* (2007) and Goldman and Green, (2009) with some modification in the incubation period of decontaminant which hold at 10 and 5 minutes respectively instate of 15 minutes.

Culturing

Culturing was applied in duplicate Löwenstein-Jensen (LJ) medium slants either pyruvate (0.4%) or glycerol supplemented and incubated at 37C for up to 8 weeks as described by Kent and kubica, (1985). Direct

culturing (n = 100) was applied after disinfecting the outer tissues surfaces then a loopfull of inner exudate inoculated into LJ culture medium. While**,** aliquots of treated homogenate (n = 300) were inoculated into LJ medium according to Munyeme *et al.*, (2009).

Herein the samples were labeled by two letters and serial numbers. The letters indicated the genus and species name e.g. (Mb11: *Mycobacterium bovis*) followed by the sample's number. Growth was confirmed by Zeil Nelson stained films for demonstration of acid fast bacteria which were designated as either bacilli or filamentous.

Phenotypic properties

Observation of growth rate, pigment production, colony of organism morphology and determination of biochemical tests followed methods described by Kent and Kubica, (1985)

Results

Pathological observations revealed both lymphatic and pulmonary Mycobacterium infection characterized by complete caseation, tuberculous lesions and calcifications. Histopathological sections showed typical granulomatous lesions. These lesions were found microscopically harbor either acid fast filaments or bacilli (Table 1) and (Fig. 1).

Recovered Mycobacterium

Direct culturing of (100) caseated tissues and indirect cultures of homogenate (300) samples revealed 59 isolates of filamentous shape and 18 rods as in table (2). Seven filamentous isolates were missed during routine laboratory work.

Phenotypic properties of cultures

The majority of filamentous acid fast bacteria which were tentatively identified as *M. farcinogenes* produced white - grey or yellow colonies. The scotochromogenic isolates produced yellow (13 isolates), white- grey (38 isolates) and brown colonies (1 isolate). Some colonies grew deeply embedded into the medium and were strongly adhesive to slants. The shape and consistency of the colonies was granular- rough bread crumb-like (30 isolates), rough – button and rounded colonies (16 isolates) or waxy (6 isolates) (Fig.2).

Fifty farcy isolates (84.7%) were considered as slow growing Mycobacteria (SGM). While 9 isolates (15.3%) were classified as rapid growing Mycobacteria (RGM)

Biochemical tests

Results of biochemical investigation of 32 filamentous Mycobacterium isolates are shown in table (3). Accordingly 6 strains were considered *M. sengelenses* and the remaining 26 isolates were identified as *M. farcinogenes*. Twenty farcy isolates (MF: 2, 4, 14, 17, 18, 34, 35, 36, 37, 38, 39, 43, 75, 136, 140, 162, 171, 185, 225 and 252) were not biochemically investigated due to weak growth and dryness of indirect cultured colonies.

Table (1): Incidence and type of Mycobacterium infection among the examined carcasses

Type of infection	No. of Carcasses examined	No. of caseated tissues	No. of acid Fast Filaments (%)	No. of acid Fast bacilli (%)
	6680	400 (6%)	145 (36.3)	22 (5.5)
Lymphatic form			137 (34.3)	18 (4.5)
Generalized form			8 (2)	4 (1)

Table (2): Recovery rate of *Mycobacterium* spp from infected tissues

	No. of tissues examined	No. of acid Fast Filaments (%)	No. of acid Fast bacilli (%)	Total No. of acid fast bacteria
	400 (100%)			
Acid fast positive films		145 (36.3)	22 (5.5)	167 (41.8%)
Positive acid fast cultures		59 (14.8)	18 (4.5)	77 (19.7%)
Direct culture	100	27 (27)	0	27 (27)
Culture of homogenates	300	32 (10.7)	18 (6)	50 (16.7)
Recovery rate (out of smear +ve films)		59 (40.7)	18 (81.8)	77 (46%)

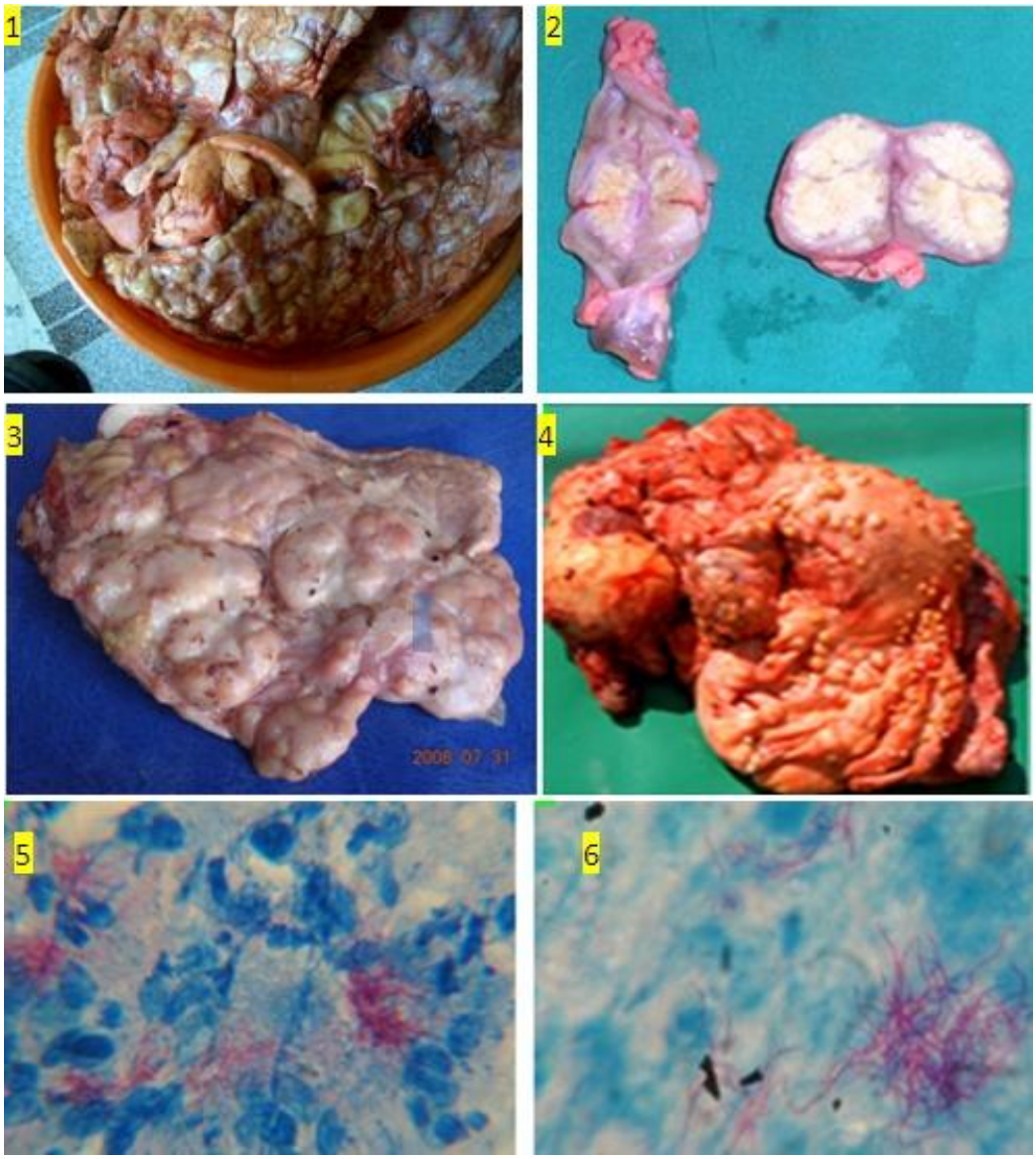

Fig.(1): bovine farcy Lesions and ZN stained films noted in Nyala abattoir

1, 3: lungs lesions showing complete granulomations, caseation and calcifications.

2. Embedded lung nodules contain yellow caseation.

4. Mediastinal lymph node showing enlargement and tuberculous lesions

5. Thin acid fast filaments prepared from picture (2).

6. Thick acid fast filaments prepared from picture (3).

Table: (3): Phenotypic and biochemical properties of filamentous Mycobacterium isolates

No	Code	Culture type	Catalase 68C	Nitrate test	Urease test	Arylsulphatase	Growth rate/ morphology	pigments
1	MF1	H	+ve	+ve	-ve	-ve	S-GR	G-W
2	MF13	H	+ ve	+ ve	+ ve	-ve	S-Waxy	Yellow
3	MF 202	H	+ ve	+ ve	- ve	-ve	S-BR	G-W
4	MF204	H	+ ve	+ ve	- ve	-ve	S-BR	G-W
5	MF208	H	+ ve	+ ve	- ve	-ve	S-BR	G-W
6	MF216	H	+ ve	+ ve	+ ve	-ve	S-Waxy	Yellow
7	MF217	H	+ ve	+ ve	-ve	-ve	S-Waxy	Yellow
8	MF219	H	+ve	+ve	-ve	-ve	S-Waxy	Yellow
9	MF244	D&H	+ ve	+ ve	- ve	-ve	S- BR	G-W
10	MF245	D&H	+ ve	+ ve	-ve	-ve	S-BR	G-W
11	MF246	D&H	+ ve	+ ve	-ve	-ve	S-BR	G-W
12	MF260	D&H	+ ve	+ ve	-ve	-ve	S-BR	G-W
13	MF264	D&H	+ ve	+ ve	-ve	-ve	S-BR	G-w
14	MF265	D&H	+ ve	+ ve	-ve	-ve	S-GR	yellow
15	MF266	D&H	+ ve	+ ve	+ve	-ve	S-GR	Brown
16	MF267	Ind	+ ve	+ ve	-ve	-ve	S-Waxy	Yellow
17	MF270	Ind	+ ve	+ ve	-ve	-ve	S-BR	G-W
18	MF271	Ind	+ ve	+ ve	-ve	-ve	S-BR	G-W
19	MF274	Ind	+ ve	+ ve	-ve	-ve	S-BR	G-W
20	MF284	D&H	+ ve	+ ve	-ve	+ve	S-BR	G-W
21	MF287	D	+ ve	+ ve	-ve	-ve	S-GR	G-W
22	MF288	D&H	+ ve	+ ve	-ve	-ve	S-GR	yellow
23	MF304	D	+ve	+ ve	+ve	-ve	S-BR	G-W
24	MF305	D	+ ve	+ ve	+ ve	-ve	S-GR	G-W
25	MF306	D	+ ve	+ ve	-ve	-ve	S-GR	G-W

26	**MF310**	**D**	**+ ve**	**+ ve**	**+ ve**	**+ve**	**S-GR**	**yellow**
27	**MF314**	**D**	**+ ve**	**+ ve**	**+ ve**	**+ve**	**S-GR**	**G-W**
28	**MF319**	**D**	**+ ve**	**+ ve**	**+ ve**	**++ve**	**S-GR**	**G-W**
29	**MF320**	**D**	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**S-GR**	**G-W**
30	**MF321**	**D**	**+ ve**	**+ ve**	**+ ve**	**+ve**	**S-BR**	**G-W**
31	**MF322**	**D**	**+ ve**	**-ve**	**+ ve**	**-ve**	**S-BR**	**G-W**
32	**MF323**	**D**	**+ ve**	**+ ve**	**-ve**	**-ve**	**S-waxy**	**G-W**

Keys:

MF= *M. farcinogenes*, **H**. = homogenate culture, D. = direct culture. D&H = both direct and homogenate culture, ND = Not done, S-GR = Slow -Granular Rough colony, S-BR= Slow-Button shaped rough colonies and G-W= Grey to White colony

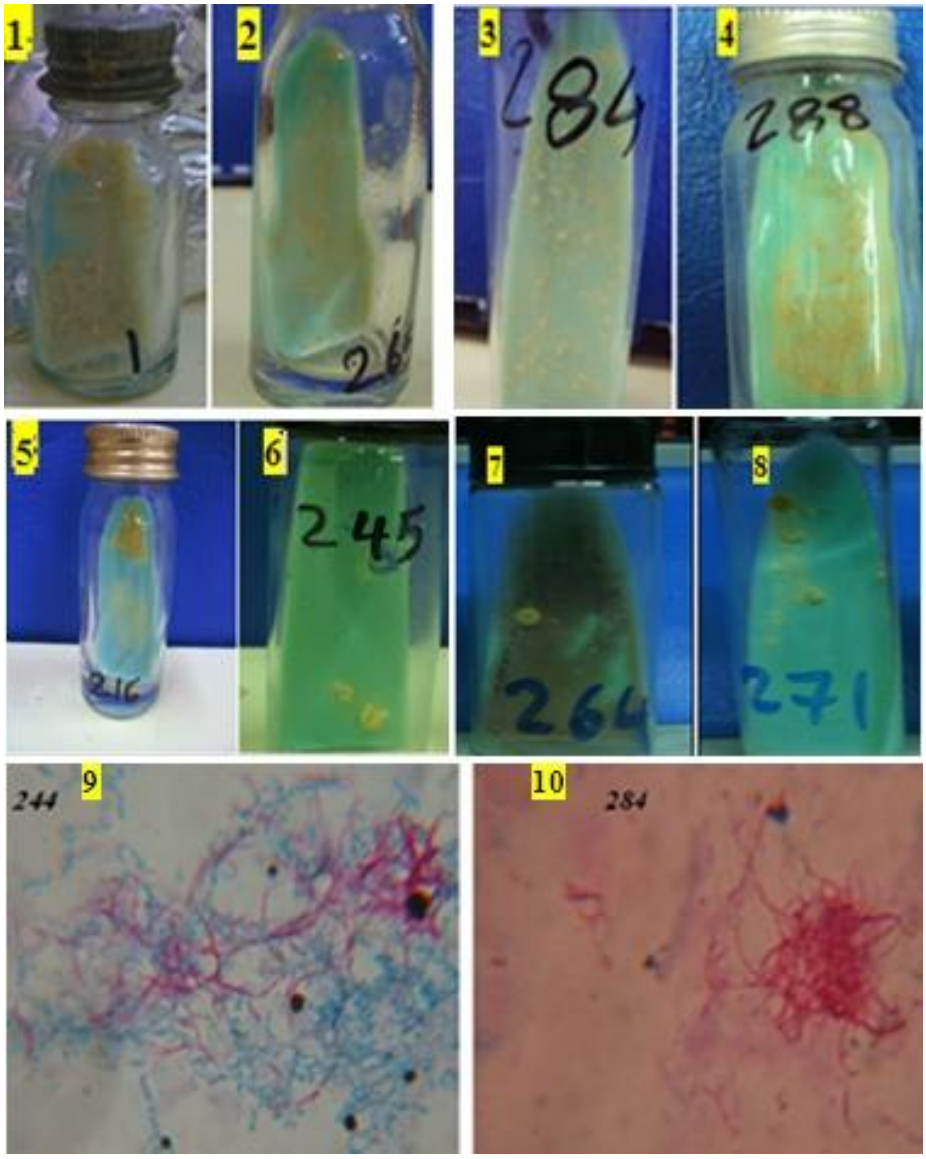

Fig.(2):

1-4: Direct cultures of *M. farcinogenes* grown on LJ medium, showing grey rough colonies (MF1), yellow rough colonies (MF265 and 288) and button gray – white colonies (MF284).

5-8: Culture of homogenate lesions on LJ medium showing yellow waxy colonies (MF216) and Grey- white rough button shaped colonies (MF, 245, 264 and 271).
9: ZN stained film from fresh farcy lesion showing contaminated acid fast filaments.
10: ZN stained film from homogenate culture showing pure acid fast filaments

Discussion

The gross pathology of bovine farcy infection showed tuberculous lesions similar to those seen in tuberculosis infection (Mohan, 1985).

Conventional microscopic and culturing methods still have a golden diagnostic value in mycobacteriosis. Microscopic examination demonstrated 167 (41.8%) films harboring acid fast bacteria morphologically classified to rods 22 (5.5%) and filamentous 145 (36.3%) mycobacteria. Cultivation of 400 caseated tissues revealed 77 (19.3%) isolates of these 18 (4.5%) were acid fast bacilli and 59 (14.8%) acid fast filaments identified farcy agents. The reduction in growth rates of farcy agents was due to toxic decontaminant effect used in decontamination of homogenates cultures of *Mycobacterium* spp (Kent and Kubica, 1985). The present study showed that farcy agents were affected by 5% oxalic acid within 10-15 minutes and cultures produced very small colonies which soon got dried when incubated for long periods. However, the organisms withstood incubation in the same decontaminants concentration with holding time of 5minutes and produced good and pure growth in LJ medium reliable for chemical and biochemical analysis.

Filamentous mycobacteria (52) were found to be slow growers (41) and produced grey- white, folded or button shaped colonies with rough consistency. These observations agree with Ridell and Goodfellow (1983) and El Sanousi *et al.* (1979) and not with Roth *et al.* (2000) who recorded that both farcy agents were rapid growing Mycobacteria. Other (5) slow

growers produced waxy yellow or gery- white colonies not easy emulisifiable in water. The rest filamentous agents (6) were rapid growers and produced yellow, granular rough colonies, not easy to emulsify in water. These were isolated from Umbararu breeds but unfortunately they dried and their chemical and biochemical profile were not determined. *M. farcinogenes* isolates grew slowly in both LJ medium with pyruvate or glycerol supplementation but produced good growth during the 4th week of incubation as described by El Sanousi *et al.* (1979).

Biochemical analysis of 32 isolates illustrated that all tested filamentous Mycobacteria were catalase 68Ċ positive and 96.9% reduced nitrate which agrees with El Sanousi *et al.* (1979), Chamoiseau (1979) and Goodfellow and Wayne (1982). However, two isolates (3.1%) MF1 and MF322 were nitrate negative. Urease production was negative in 65.6% as reported by El Sanousi *et al.,* (1979) while 34.4% only were urease positive (MF266, 304, 305,310,314,319, 321 and322) and this agrees with the results obtained by Murray *et al.,* (2007). Arylsulphatase test showed negative reaction for 26 tested strains which were considered as *M. farcinogenes* while, 6 strains (MF: 284, 305, 310, 314, 319, 320, 321) were arylsulphatase positive and were considered *M. sengelense* strains as recommended by Ridell and Goodfellow (1983), Mohan, (1985) and Viencet *et al.,* (2009) .

Chamoiseau, (1979), El Sanousi *et al.* (1979) and Hamid and Goodfellow (1995) reported that *M. farcinogenes* was the sole causative agent of bovine farcy in the Sudan. In this study both *M. farcinogenes* and *M. sengelense* were isolated and identified. However, isolation of *M. sengelense* was not recorded before in the Sudan, in spite of its incrimination as one of the bovine farcy causative agents in many other African countries (Chamoiseau, 1979 and Mohan, 1985).

Conclusions and recommendations

Phenotypic properties of *Mycobacterium* still remain as reliable diagnostic tools in developing countries. Bovine farcy in the Sudan is caused by both *M. farcinogenes* and *M. sengelense*. Farcy agents were found sensitive for 5% oxalic acid and many cells were damaged when incubated for 10 minutes.

Further screening and identification of *Mycobacterium* spp. is needed especially in developing countries to build a concrete base to control infection caused by these fastidious bacteria which have recently merged as public health hazard.

Acknowledgment

Special thanks must be paid to Mr. Sulieman Nuga, Nyala Regional Research Laboratory, Mr. Glal and Mrs. Shima in the laboratory of tuberculosis, National health laboratory, Sudan for their technical efforts to do the conventional analysis.

References

Brown-Elliott, B. A. and Wallace, R. J. (2002). Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. *Clin Microbiol Rev* **15**, 716–746.

Burnett, D. and Crocker, J. (2005). The Science of laboratory.2nd ed. Willy& sons. Pp, 566.

Chamoiseau, G. (1979). Etiology of farcy in African bovines: nomenclature of the causal organisms *Mycobacterium farcinogenes* and *Mycobacterium senegalense*. *Int. J. Syst. Bacteriol.* **29,** 407-410.

Dwight, C.H.; Maclachlan, N.J. and Richard, L.W. (2004). Mycobacterium in: Veterinary microbiology, 5th edition. Blackwell publications. P, 223-234.

El Sanousi, S. M.; Salih, M. A. M.; Musa, M. T.; Tagel-Din, M. H. and Soad, A. (1979). Further studies in the properties of the etiology of bovine farcy in the Sudan. *Rev. Elev. Med. Vet. Pays Trop*. **32,** 135-141.

Goodfellow, M. and Wayne, G. (1982). Taxonomy and nomenclature. In the Biology of Mycobacteria. **I,** 472-521.

Goldman, E. and Green,L.H.(2009). Practical handbook of Microbiology.2nd edition. P, 493-504. Taylor and Francis group, London.

Hamid, M.E and Goodfellow, M. (1995). Rapid growth and increased biomass yield of Mycobacterium farcinogenes and some related taxa in broth and agar media. *Vet. Med.* 42, 397-404.

Hewitson, T. D. and Darby, I. A. (2010). Histology Protocols. Pp, 225. Human press.

Kent, P.T. and Kubica, G.P. (1985). Public health Mycobacteriology: Guide for the Level III Laboratory. Centers for Disease Control, US Department of Health and Human Services, Atlanta, GA. 31–70.

Kim, B.J.; Lee, S.H.; Liu, M.; Kim, S.; Bai, G.; Kim, S.J.; Chae, G.; Kim, E.; Cha, C. and Kook, Y. (1999). Identification of mycobacterial species by comparative sequences analysis of the RNA gene (rpoB). *J. Clin. Microbiol*. **37**, 1714-1720.

Konemann, E.W.; Allen, S.D.; Janda, W.M (2005). Mycobacteria in: *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*, 6th ed. Philadelphia: Lippincott William & Wilkins. **p,** 1496-1514

Mohan, K. (1985). *Mycobacterium sengelense* from bovines in Eastern Nigeria. *J. appl. Bacteriol*. **59**, 277-281

Mokaddas, E. and Ahmed, S. (2007). Development and evaluation of m-PCR for rapid detection and differentiation between *Mycobacterium tuberculosis* complex members from Non- tuberculous Mycobacteria. Jpn. J. Inf. Dis. **60**, 140-144.

Munyeme, M., Rigouts, L., Shamputa, I.C., Muma, J.B., Tryland, M., Skjerve, E. and Dionne, B. (2009). Isolation and characterization of Mycobacterium bovis strains from indoginous Zambian cattle using spacer oligonucleotide typing technique. *BMC.microbiol.* **144**, 1-8.

Murray, P.R., Baron, E.J., Jorgensen, J.H., *et al, eds.* (2007). Manual of Clinical Microbiology. 9th ed. Washington, DC: ASM Press**.** 1078-1081.

Oh, W.S., Ko, K. S., Song, J.H., Lee, M. Y., Ryu, S.Y., Heo, S., Kwon, K.T., Lee, J.H., Peck, K.R. and Lee, N.Y.(2005). Catheter-associated bacteremia by *Mycobacterium senegalense* in Korea . Case report. *BMC. Infec. Dis*. **5**, 1-5.

Palomino, J.C.; Leãod, S. C. and Ritacco, V. (2007). Tuberculosis textbook from basic science to patient care. First Edition. Bourcillier Kamps.com. Pp, 687.

Pfyffer, G. E.; Brown-Elliott, B. A; Wallace, R. J. *(*2003). Mycobacterium*:* general characteristics, isolation, and staining procedures. In: Manual of Clinical Microbiology, 8th edition. P, 532-539.

Ridell, M. and Goodfellow, M. (1983). Numerical classification of *Mycobacterium farcinogenes*, *Mycobacterium senegalense* and related taxa. *J. Gen. Microbiol*. 129, 599–611.

Ridell, M., Goodfellow, M. and Abdulle, M.A. (1985). Identification of Actinomycetes isolated from cases of bovine farcy in the Sudan. *Zent. Bakteriol. Microbiol. hyg*. A259, 43-50.

Rieder, H. L.; Deun, A.V.; Kam, K.M.; Kim, S. J.; Chonde, T. M.; Trébucq, A. and Urbanczik, R. (2007). Priorities for Tuberculosis Bacteriology Services in Low-Income Countries 2nd edition. P, 12-27.

Roth, A., U. Reischl, A. Streubel, L. Naumann, R. M. Kroppenstedt, M. Habicht, M. Fischer, and H. Mauch. (2000). Novel diagnostic algorithm for identification of mycobacteria using genus-specific amplification of the 16S- 23S rRNA gene spacer and restriction endonucleases. J. Clin. Microbiol. **38:**1094–1104.

Runyon, E. H. (1959). Anonymous Mycobacteria in pulmonary disease. *Med. Clin. North Am*. **43,** 273–290.

Tag Eldin, M.H.**; El Sanousi, S.M.; Suliman, T.A. and Fawi, M.T. (1988)**. Concurrent infection with *Mycobacterium farcinogenes* and *Actinobacillus ligniersi* in slaughtered cattle. *J. Comp. pathol.* **99**, 431-437.

Vincent V, **Brown-Elliot B, Jost KC. And Wallace RJ**. **(2009).** Mycobacterium: Phenotypic and genotypic identification. Manual of Clinical Microbiology 8th edition. **p**, 560-584.

Wong, T.C.; Chan, M.W.F.; Tsang, F.W. L.; Fhkcos, F.; Yeung, S.H.; and Keung, F. I, (2005). *Mycobacterium farcinogenes* Infection after Total Hip Arthroplasty.*The J. Arthropl.* **20**, 684-687.