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Further studies on the properties of the aetiology of bovine farcy isolated from Sudanese cattle

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RÉSUMÉ

Etudes sur les caractéristiques de l'agent causal *Mycobacterium farcinogenes* du farcin du bœuf au Soudan

Le farcin du bœuf a fait l'objet d'enquêtes dans trois départements du Soudan, de janvier à avril 1978, qui ont montré que l'affection atteignait 5,19 p. 100 du cheptel bovin au Nyala (Darfur), 4,19 p. 100 au Nuba Mountain (Kordofan) et 3,49 p. 100 à Omdurman (Khartoum)

Un développement très abondant de l'agent causal a été obtenu par l'incorporation dans le milieu de culture modifié de Sauton de pyruvate de sodium, le pH optimal se situant entre 7 et 9 et avec des écarts de température allant de 28° à 40 °C.

A 45 °C la croissance est légèrement freinée, elle cesse à 20 °C et à 50 °C. Le germe peut supporter l'addition de 11 p. 100 de chlorure de sodium dans le milieu de culture et le traitement avec 5 et 15 p 100 d'acide oxalique, pendant respectivement 20 et 5 minutes consécutives. Les propriétés biochimiques du germe sont semblables à celles déjà décrites pour la souche du Tchad, qui est pathogène pour le cobaye, les porcs et les veaux. Le nombre de subcultures en milieu artificiel est un facteur limitant des propriétés pathogéniques. Les souches ont montré des résistances variées à l'INH, PAS et à la streptomycine. L'acide mycolique a été chimiquement extrait des cultures ainsi que par la technique chromatographique en couches minces. Il a été identifié par son point de fusion et sa résistance à l'action dissolvante d'un milieu à base d'eau et de méthanol.

I. INTRODUCTION

Bovine farcy is an infectious disease affecting cattle in certain countries in West Africa as well as in Central and Eastern Africa. The disease is caused by *Mycobacterium farcinogenes* formerly *Nocardia farcinica*. The resemblance to atypical *Mycobacterium* was based on the presence of betahydroxy fatty acids of the mycolic type, pyrolysable with the liberation of tetra casanoic acid which characterizes cultures of atypical Mycobacteria. However the latter was excluded because of the pathogenicity of the farcy organism for guinea pigs and the activity on amides (1).

In the Sudan, the disease was tackled exclusively from the epidemiological and pathological points of view (MOSTAFA, 1966). Meager studies were conducted concerning the bacteriology of the disease (MOSTAFA, 1966, 1967). Lately, SALIH, EL SANOUSI and TAG EL DIN (13) made seasonal surveys of the disease in Western and central Sudan. They also studied the predilection sites and the distribution of lesions in the affected cattle. EL SANOUSI, TAG EL DIN and ABDEL WAHAB (3), using the method of KANETSUNA and BARTOLI (4), were able to extract mycolic acid in quantities that permit designation of *Mycobacterium* to the genus. CHAMOISEAU (1) postulated that results and observations made on chadiense strains could be applied to sudanese strains. It is felt necessary that further investigation on the properties of the aetiology of bovine farcy in the Sudan is deemed essential. Therefore, this work was undertaken to study the bacteriological properties of the bovine farcy organism isolated from sudanese cattle.

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II. MATERIAL AND METHODS

1. Surveys

Southern Darfur and Southern Kordofan Provinces were selected for the survey between the periods January-April 1978. Strains were also collected from Omdurman abattoir. The method of isolation was previously described (13) 1 705 animals were inspected ; 1 059 at Nyala slaughter house (Southern Darfur), 358 at slaughter houses of Kadogli and Dilling (Southern Kordofan) and 288 animals were inspected at Omdurman central abattoir.

2. Media trails

Modified Sauton's medium (M. S. M.) was prepared in Roux flasks of one litre capacity according to LECHEVALIER (5).

Two flasks were prepared of each of the following patterns :

1. M. S. M.
2. M. S. M. + Bovine Albumin (B. A.) 0.2 p. 100.
3. M. S. M. + Horse Serum (H. S.) 0.5 p. 100.
4. M. S. M. + Sodium Pyruvate (S. P.) 0.4 p. 100.
5. M. S. M. + Yeast Extract (Y. E.) 0.2 p. 100.
6. M. S. M. + Glucose (3 p. 100).
7. M. S. M. + B. A. + H. S.
8. M. S. M. + B. A. + H. S. + S. P.
9. M. S. M. + B. A. + H. S. + S. P. + Y. E.
10. M. S. M. + B. A. + H. S. + Y. E. + 3 p. 100 Glucose.
11. M. S. M. + B. A. + Glycerine (5 p. 100).

Bovine albumin and horse serum were added as filter sterilized after autoclaving. Glucose was prepared as 20 p. 100 sterilized separately and added aseptically. A few colonies scraped from lowenstein Jensen (L. J.) medium (about 3 colonies) were used for sowing of each flask. Flasks were incubated at 37 °C in the horizontal position. Flasks were observed daily for growth.

3. pH

Modified sauton's medium was prepared in test tubes. pHs were selected for the study 4, 5, 6, 7, 8 and 9. IN HCl and 0.5 N NaOH were used for adjusting pH prior autoclaving. Few tubes in each pH were sacrificed for reading of the final pH after autoclaving. The resultant pH was used for the study. Two colonies nearly of the same size scraped from L. J. medium were used to inoculate each tube. Cultured tubes were incubated at 37 °C, and observed for four weeks.

4. Growth temperature

Subcultures on Lowenstein Jensen medium were made and incubated at : 20 °C, 28 °C, 40 °C, 45 °C, 50 °C.

5. Oxalic acid

Growth was scraped from several L. J. media and divided into two portions. One portion was resuspended in sterile 5 p. 100 oxalic acid. Samples were removed at intervals of 5, 10, 15, 20 and 25 min. washed twice with sterile normal saline and subcultured in fresh L. J. media. Bottles were incubated at 37 °C and observed for growth for four weeks. The other part was distributed in different concentrations of oxalic acid i. e. 5, 10, 15 and 20 p. 100 for five minutes then washed twice with sterile normal saline and subcultures were made on L. J. media, Bottles were incubated at 37 °C and observed for growth for four weeks.

6. Biochemical tests

The strains were tested for ability to reduce nitrate and possession of catalase, urease, aryl-sulphatase enzyme and hydrolysis of tween 80 by fixation of neutral red according to the procedures of VESTAL (14). *Mycobacterium fortuitum* was used as a positive control.

7. Sodium chloride tolerance

The following concentrations of sodium chloride (Analar) were incorporated in L. J. medium 1-15 p. 100 W/V.

8. Loss of pathogenicity for guineapigs by subculturing

Five grams of pus scrapped from an infected lymph node were suspended in 5 ml of phosphate buffer. Five guinea pigs were inoculated intraperitoneally with one ml each of the suspension. The same lymph node was treated as previously described, and sown on L. J. medium. Growth was scraped from 5 McCartney bottles and suspended in 3 ml buffer saline. Each of three guinea pigs received one ml intraperitoneally. Guinea pigs were observed for a month, after which survivors were euthanized and autopsied.

A further subculture was made and three fresh guinea pigs were inoculated and observed. The procedure was repeated till the sixth subculture.

9. Pathogenicity for calves

Scrapings of growth in three MacCartney bottles were suspended in normal saline. Three Zebu calves, 3 years of age were used. Two calves, designated A and B, were inoculated with 10 ml each intravenously. The third calf (C) was inoculated with five ml subcutaneously in the fore limb. The animals were observed for 4 months.

10. Sensitivity to INH, PAS and Streptomycin

Laboratory techniques for determination of sensitivity of eight *Mycobacterium farcinogenes* strains to isoniazid (INH), para aminosalicylic acid (PAS) and streptomycin (ST), were carried out on L. J. medium according to the Resistance-Ratio Method as described by the Medical Research Council (7). The results were expressed as resistance ratio namely the ratio of the minimal inhibitory concentration for the farcy test strain to the minimal inhibitory concentration for the standard sensitive strain, H_{37} RV.

11. Mycolic acid detection

Growth was scraped from several Lowenstein Jensen media. The method of KANETSUNA and BARTOLI (4) was applied for extraction and characterization of mycolic acid. Further identification of mycolic acid was confirmed using thin-layer chromatography (8).

12. Serodiagnosis trials

Antigen of various strains was prepared according to NICHOLLS (11) and agglutination levels were assessed using the modified Widal test of NICHOLLS (12).

The antigen was tested against sera collected from animals infected with bovine farcy.

III. RESULTS

1. Survey

The incidence of bovine farcy at Nyala and Nuba Mountains are shown in table I. The distributions of bovine farcy lesions among lymph nodes of affected animals is shown in table II.

2. Media trials

Results are shown in table III.

Table I Incidence of bovine farcy at different abattoirs in Western Sudan and Omdurman slaughter house between January-April, 1978.

Area visited	N° of animals slaughtered	N° of animals showing external lesions	N° of positive cases smearwise after P.M.	percentage age of infection
Nyala	1 059	30	55	5.19 p.100
Nuba Mountains (Kadogli and Dilling)	358	7	15	4.19 p.100
Omdurman Slaughter House	N° of animals inspected 288	None (because of selection for commercial purpose)	10	3.47 p.100
Total	1 705	37	80	4 69 p.100

Table II Distribution of bovine farcy lesions among lymph nodes of affected animals. During January, February, March and April, 1978.

Area visited	N° of animals proved to be positive	Total N° of lymph nodes affected	Infection percentage age of :										
			1	2	3	4	5	6	7	8	9	10	11
Nyala area	55	122	6,55	8.20	11.5	22.95	7.40	9 83	7,40	8.20	4.85	9.84	3.28
Mountains Kadogli and Dilling	15	32	12,5	9.38	6.25	15.63	28,1	6 25	6.25	6 25	0	6.25	3.13
Total	70	154	7,79	8.44	10.39	21.4	10,6	8.44	7.14	7.80	4.55	9.09	3.25

1. parotid ; 2. submaxillary ; 3. retropharyngeal ; 4. prescapular ; 5. cervical ; 6. mediastinal ; 7. mesenteric ; 8. crural ; 9. popliteal ; 10. inguinal ; 11. supramammary.

Table III Results of modified Sauton's media trials.

Ingredients of media	Growth (days)			
	11	15	21	28
1. M.S. (alone) (control)	0	0	±	+
2. M.S. + 0,2 p.100 bovine albumin	±	±	0	0
3. M.S. + Bovine albumine + horse 28 serum (0,5 p.100)	±	±	0	0
4. M.S. + H.S.	+	+	+	++
5. M.S. + Bovine albumin + H.S. + sodium pyruvate	+	+	+	+
6. M.S. + sodium pyruvate	+++	++++	+++++	++++++
7. M.S. + Bovine albumin + H.S. + sodium pyruvate + Y.E.	+	±	0	0
8. M.S. + Y.E.	++	++	+++	++++
9. M.S. + Bovine albumin + sodium pyruvate + Y.E. + 3 p.100 glucose	+	+	+	+

0 = no growth ; + = very poor ; ++ = poor ; +++ = moderate ; ++++ = good ; +++++ = luxurient-confluent ; ++++++ = further improvement ; ± = doubtful.

3. pH

pH growth appeared after seven days incubation at pH 7, 8 and 9 as whitish pellice. At the eighth day growth appeared in pH 6 and in the 13 th day in pH 5 as scant growth which was improved by further incubation.

4. Temperature of incubation

Normal growth took place at temperatures 35°, 40 °C and 28, growth slightly delayed at 45 °C. At 50 °C no growth took place and colonies died. At 20 °C no growth took place but colonies remained alive for four weeks. Incubation period was found to be between 11-14 days for primary isolation.

5. Oxalic acid

Growth took place in subcultures suspended in 5 p. 100 oxalic acid for 5, 10 15, and 20 min. No growth occurred when suspended for 25 min. Growth occurred in cultures suspended in 5 p.100 and 10 p. 100 oxalic acid for 5 min. At 15 p. 100 growth was delayed. No growth occurred in 20 p. 100 for 5 min.

6. Biochemical tests

Catalase : All tested strains were strongly catalase positive.

Urease : None of the tested strains were positive.

Arylsulphatase : No enzyme detected during 3, 15 and 21 days incubation. *Mycobacterium fortuitum* was found to possess arylsulphatase enzyme.

Tween 80 *hydrolysis*

No fixation of neutral red was detected up to 12 days incubation in all strains tested.

Nitrate reduction

All strains were able to reduce nitrate to nitrite approx. 75 p. 100 development of red colour).

7. Sodium chloride tolerance

Concentration 1-15 p. 100

1 p. 100 ++++	Growth seen earlier.
2 p. 100 ++++	
3 p. 100 ++++	
4 p. 100 ++++	
5 p. 100 ++++	
6 p. 100 ++++	Delayed growth.
7 p. 100 +++	
8 p. 100 ++	
9 p. 100 + (±)	Some colonies started to disintegrate.
10 p. 100 +	
11 p. 100 +	
12 p. 100 —	
13 p. 100 —	
14 p. 100 —	
15 p. 100 —	(complete disintegration).

Normal growth occured in concentrations 1-4 p. 100 within one week. In concentrations 5-10 p. 100 the growth was delayed i.e. 10 days. Poor growth appeared in concentration 11 p. 100 in 14 days, as slimy growth. 12-15 p. 100 no growth occurred up to 24-28 days. Inoculum started to disintegrate and die.

8. Loss of pathogenicity for guinea pigs

Table IV Effect of subculturing on virulence of etiology of bovine farcy organism for guinea-pigs

Inoculum	N° of guinea-pigs	Deaths	Survivors
Pus from infected cows	5	5/5	0/5
Primary isolation	3	3/3	0/3
2nd subculture	3	2/3	1/3 ·
3rd subculture	3	1/3	2/3 ·
4th subculture	3	0/3	3/3::::
5th subculture	3	0/3	3/3::::
6th subculture	3	0/3	3/3::::

: Oedema and pus were observed at site of inoculation. No internal lesions were observed.
:::: No lesions observed when autopsied.

Results are shown in table IV. Pus was formed at the site of inoculation. Peritonitis with multiple abscessation was the most prevailing lesion in all dead guinea pigs. White yellowish focci were spreading in the liver, kidney, spleen, mesenteric lymph nodes and pancreas. Stained smears revealed acid fast branching filaments indistinguishable from *Mycobacterium farcinogenes* microscopically. The organism was recovered in L. J. medium. Survivors of the second and third subcultures showed œdema and pus at the site of inoculation, but no lesions were seen in the internal organs. Euthanized guinea pigs of fourth, fifth and sixth subcultures did not show any lesions.

9. Pathogenicity for calves

Calf (A) died after 84 days post inoculation with typical lesions of milliary form. Calf (B) was slaughtered 4 months post inoculation and no lesions were detected at post-mortem. Calf (C) no lesions were detected.

10. Sensitivity to INH, PAS and streptomycin

Out of the eight strains tested, six strains were resistant to the three drugs. Of the remaining two, one was sensitive to PAS and streptomycin and the other was sensitive to PAS only.

11. Mycolic acid detection

13.5 mg dry weight were isolated from each one g wet weight of bacteria. The melting point was found to be 50-52 °C.

12. Serodiagnosis trials

Only traces of agglutinin were recorded.

IV. DISCUSSION AND CONCLUSIONS

Slight increase observed in the incidence of infection in Nyala, Darfur, 5.19 p. 100 compared with the previous studies 3.120 p. 100. This could be attributed to cattle merchants purchasing whole herds, culling apparently affected ones for slaughtering and sending the rest for export.

However a drop in the incidences was observed in Nuba Mountains (Kordofan) 4.19 p. 100 compared with previous studies 10.2 p. 100 (13). Recently, it was noticed that cattle with apparent lesions of farcy are not attractive to merchants who only supply the slaughter houses because of condemnation of the affected carcasses without any compensation and hence the tendency towards selection. This may give an explanation to the reduction of incidences of the disease in the slaughter houses of Nuba Mountains compared with the previous records. Most of our chemical and biochemical studies on the properties or the composition of the aetiology of bovine farcy were hindered by the poor growth obtained in various media tried before (L. J. and modified Sauton's medium). The harvested material, needed for further study, was apparently very small and it took along period to collect the required amounts. Modified Sauton's medium alone did not support a good growth, hence various additives were tried to improve the growth. A convenient medium was achieved for such a purpose by addition of sodium pyruvate (0.4 p. 100) to the modified Sauton's medium. Addition of further ingredients did not improve the growth. On the contrary a retardation in growth was observed in some combinations. We were not able to explain the mode of such retardation. As regard to the pH, neutral pH with a slight shift to alkalinity was preferred to the acidity. A wide range for growth temperature was noted. No striking difference in growth was observed in cultures incubated at 28°, 35°, 37°, 40 °C. However, at 45 °C there was slight delay in some strains and complete inhibition in others. At 50 °C, colonies disintegrated and died. At 20 °C, culture failed to multiply but remained alive and grew when reincubated at 37 °C after four weeks incubation at 20 °C. This was concerning strains subcultured several times. However, for primary isolation, organism failed to grow neither at 28 °C nor at 40 °C. They remained alive for four weeks at 28 °C and

showed growth later when re-incubated at 37 °C. When incubated at 40 °C they failed to grow when temperature was lowered to 37 °C.

The Sudanese strains of *Mycobacterium farcinogenes* showed a remarkable resistance to sodium chloride. Though poor yet, growth occurred in concentrations up to 11 p. 100. Concentrations 5 p. 100 and 6 p. 100 delayed growth, on further incubation maximum growth was attained. Contrary to the findings of MOSTAFA (9), our strains were able to withstand treatment with 5 p. 100 oxalic acid for up to 20 min. MOSTAFA (9) reported a maximum time of ten minutes for survival. The strains were highly pathogenic for guinea pigs. This pathogenicity is governed by the number of subsequent subculturing in artificial medium. Partial attenuation occurred by the second subculture. Complete loss of pathogenicity was achieved by the fourth subculture. The importance of such an observation is a beneficial knowledge for vaccine production to counteract such an increasingly economical important malady. Also whenever pathogenicity of the farcy disease is to be studied, the numbers of the previous subcultures should be clearly stated. A full description to the milliary type of farcy observed in one calf is discussed elsewhere (2). Drug sensitivity tests are used not only as a guide to chemotherapy but also to support and amplify classification (6). Although the number of the strains tested were too small to draw a valid conclusion, yet, it can be stated that most of the strains tested were primary drug resistant and hence they simulate atypical mycobacteria in this respect. The studied strains showed a remarkable resistance to INH, PAS and streptomycin apart from one strain which was sensitive to PAS and streptomycin and another one which was sensitive to PAS only.

The method of KANETSUNA and BARTOLI (4) applied previously by EL SANOUSI, TAG EL DIN and ABDEL WAHAB (3) is a simple and reliable method for extraction of mycolic acid. At the present studies, 13.5 mg dry weight of mycolic acid were extracted from each one g wet weight of bacteria, in comparison with previous studies 8 mg were extracted. Though both values lie safely in the range of mycolic acid of the genus *Mycobacterium* yet such values could be stabilized by standardizing the ratio of ethanol to ether to be used for the extraction. The melting point was the same as in the previous studies i.e. 50-52 °C.

A further evidence to the identity of mycolic acid was confirmed by thin layer chromatography. When spots were washed with a mixture of methanol water (5 : 2 v/v), all spots with the exception of those corresponding to the mycolic esters, were removed. Work is in progress to use the mycolic acid extract as an intradermal injection for allergy testing in affected cattle.

The serodiagnosis of the diseases by the method of NICHOLLS (11) though gave negative results for sera of clinically infected cattle should not be abandoned as an insensitive test unless a significant number of positive sera is tested. Also, the failure of such a test to detect agglutinin in positive sera could be attributed to the type of antibodies produced. We therefore suggest for further studies that experimental infection should be made for calves and then following the titre of the agglutinin throughout the course of the disease.

These studies showed beyond doubt that etiology of bovine farcy in the Sudan is a *Mycobacterium* and not a *Nocardia*. The strains were found to possess similar characters to those of the chadians as formerly postulated by CHAMOISEAU (1) ; perhaps the intermingling of cattle between the common borders of the two countries has facilitated dissemination of common strains.

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SUMMARY

Bovine farcy was surveyed between January-April, 1978 in three provinces of the Sudan. Nyala (Darfur), 5.19 p. 100 Nuba Mountains (Kordofan) 4.19 p. 100 and Omdurman (Khartoum) 3.49 p. 100. A luxurient growth was obtained

in modified Sauton's medium when sodium pyruvate was incorporated with optimum pH of 7-9 and a wide range of growth temperature 28-40 °C with slight delay at 45 °C and no growth at 20° and 50 °C. The organism resisted 11 p. 100 sodium chloride in the medium and treatment with 5 p. 100 and 15 p. 100 oxalic acid for 20 and 5 min. respectively. Biochemical properties were similar to those of the chadian strain. The organisms are pathogenic for guinea pigs and calves. Pathogenicity is governed by number of subcultures in artificial medium. Strains varied in their resistance to INH, PAS and streptomycin. Mycolic acid was extracted chemically and by thin layer chromatography and was confirmed by its melting point and by resisting dissolving mixtures of methanol and water.

RESUMEN

Estudios sobre las características del agente causal, *Mycobacterium farcinogenes* patógeno del buey al Sudan

La enfermedad causada por *Mycobacterium farcinogenes* fue objeto de encuestas en tres departamentos del Sudan, de enero a abril de 1978, que mostraron que la afección alcanzaba 5,19 p. 100 de los bovinos en Niala (Darfur), 4,19 p. 100 en Nuba Mountain (Kordofan) y 3,49 p. 100 en Omdurman (Khartoum).

Se obtuvo un desarrollo muy abundante del agente causal por la incorporación en el medio de cultivo modificado de Sauton de piruvato de sodio, el pH optimo siendo entre 7 y 9 y con diferencias de temperatura entre 28° y 40 °C.

A 45 °C el desarrollo es ligeramente reducido, cesa a 20 °C y a 50 °C. El germen puede soportar la adición de 11 p. 100 de cloruro de sodio en el medio de cultivo y el tratamiento con 5 y 15 p. 100 de acido oxálico, durante respectivamente 20 y 5 minutos consecutivos. Las propiedades bioquímicas del germen son semejantes a las ya descritas para la cepa del Chad, que es patógena para el cobayo, los cerdos y los terneros. El número de subcultivos en medio artificial es un factor limitando las propiedades patogénicas. Las cepas mostraron resistencias variadas al INH, PAS y estreptomicina.

Se ha extraído de los cultivos el acido micólico quimicamente y por el técnico cromatográfico en capas delgadas. Ha sido identificado por su punto de fusión y su resistencia a la acción disolvente de un medio a base de agua y de metanol.

BIBLIOGRAPHIE

1. CHAMOISEAU (G.). *Mycobacterium farcinogenes*, agent causal du farcin du bœuf en Afrique. Rev. Elev. Méd. vét. Pays trop., 1974, **27** (1) : 61-65.
2. EL SANOUSI (S. M.), SALIH (M. A. M.). Miliary bovine farcy experimentally induced in a Zebu calf. *Vet. Path.* (in press), 1979.
3. EL SANOUSI (S. M.), TAG EL DIN (M. H.), ABDEL WAHAB (E.). Classification of the bovine farcy organism. *Trop. anim. Hlth. Prod.*, 1977, **9** : 124.
4. KANETSUNA (F.), BARTOLI (A.). A simple chemical method to differentiate *Mycobacterium* from *Nocardia*. *J. gen. Microbiol.*, 1972, **70** (2) : 209-212.
5. LECHEVALIER (M. P.), HORAN (A. C.), LECHEVALIRE (H.). Lipid composition in the classification of Nocardiae and Mycobacteria. *J. Bact.*, 1971, **105** : 313-318.
6. MARKS (J.). System for the examination of tubercle Bacilli and certain mycobacteria. *Tubercle*, 1976, **57** : 207-225.
7. MEDICAL RESEARCH COUNCIL. *Lancet* 1953, **11** : 213.
8. MINIKIN (D. E.), ALSHAMAONY (L.), GOODFELLOW (M.). Differentiation of *Mycobacterium*, *Nocardia* and related taxa by thin layer chromatographic analysis of whole organism methanolysates. *J. gen. Microbiol.*, 1975, **88** (1) : 200-204.
9. MOSTAFA (I. E.). Studies on cattle nocardiosis bovine farcy in the Sudan. *Sudan J. vet. Sci. anim. Husb.* 1960, **7** (1) : 1-9.
10. MOSTAFA (I. E.). Part of a thesis for ph. D. University of London. *Comp. Pathology*, 1967, **77** : 231-237.
11. NICHOLLS (A. C.). A serodiagnostic test for tuberculosis. *J. Clin. Path.*, 1975, **28** : 850-853.
12. NICHOLLS (A. C.), PEASE (P. E.), GREEN (I. D.). Agglutinin response to bacterial infection in acute exacerbation of chronic bronchitis. *J. clin. Path.*, 1975, **28** : 279-283.
13. SALIH (M. A. M.), EL SANOUSI (S. M.), TAG EL DIN (M. H.). Predilection sites of bovine farcy lesions in sudanese cattle. *Bull. anim. Hlth. Prod. Afr.*, 1978, **26** (2) : in press.
14. VESTAL (A. L.). Procedure for the isolation and identification of mycobacteria. New Publication, 1975, CDC : 75-8230.