

Association of *Fusobacterium necrophorum* with ovine and bovine hepatic abscesses and necrosis

Hind El Rayah Osman, Umalaleem Abakar Idris and Sulieman Mohammed El Sanousi.

Dept. of pathology, Faculty of Veterinary Medicine, Sudan University for Science and Technology; Dept. of pathology, Faculty of Veterinary Science, Khartoum University; Dept. of microbiology, Faculty of Veterinary Science, Khartoum University.

الخلاصة

تم تجميع 73 عينة من خراجات وتنكزات أكباد الضأن وعجول التسمين المذبوحة في مجزر أمدردمان المركزي لفحصها باكتريولوجيا و باثولوجيا. عزل ميكروب فيوزوباكتيريوم نكروفوروم من اربع عينات من جملة 54 عينة من أكباد الضأن و ثلاث عينات من جملة 19 عينة من أكباد العجول التي تم فحصها. عزل الميكروب وحيدا من عينتين ومع ميكروبات أخرى من بقية العيناتز وجدت الأوساط البكتيرية الآتية

Brain Heart Infusion Blood Agar supplemented and VL Media

هي الأنسب لاستنبات وعزل بكتيريا فيوزوباكتيريوم نكروفوروم

Abstract

For bacteriological and pathological investigations, fifty four ovine and nineteen bovine liver samples that contain abscesses and necrosis were collected from young lambs and heavy bulls slaughtered at Omdurman

Central Abattoir.

Fusobacterium necrophorum was isolated from three bovine and four ovine livers out of fifty four ovine and nineteen bovine livers with hepatic abscesses and necrosis. The organism was isolated in pure culture from two cases and in mixed culture from the remaining ones. Brain heart infusion blood agar supplemented and VL media were found to be the most suitable media for culturing *Fusobacterium necrophorum*.

Introduction

Fusobacterium necrophorum are Gram-negative, non spore-forming (Nagaraja, *et al.*, 2005), nonmotile rods that produce indole, and acid from glucose (Aulbaek, 1971). They are strict anaerobes that fail to grow under any but the most rigorous anaerobic condition (Hubbert *et al.*, 1975).

Fusobacterium necrophorum occurs in long filaments that showing vacuolation in Gram's stain (Hubbert *et al.*, 1975; Brude *et al.*, 1986). It is said to be pleomorphic and tends to fragment to be shorter and some times occurs as bacillary or coccoid form (Merchant and Paker, 1967). It was specified by Brude *et al.*, 1986 as the only *Fusobacterium* species that produces lipase, and he reviewed that a lipase negative *Fusobacterium* which is indole positive and produces propionic acid from lactate is a *Fusobacterium necrophorum* (Brude *et al.*, 1986).

Fusobacterium necrophorum is a primary or secondary etiological agent in a variety of necrotic and purulent infections (Narayanan, *et al.* 2002). It is isolated from abscesses as a pure culture or mixed with other bacteria (Nagaraja, *et al.* 1999) and determined as an abortifacient organism in sheep (Boye *et al.*, 2006). It was isolated from symptomatic foot rot infected sheep (Bennetta *et al.*, 2009).

The term necrobacillosis covers a wide variety of lesions in both animal and man that is largely due to infection with the non-spore forming anaerobic bacillus known as *Fusiformis necrophorum* - *Fusobacterium necrophorum* (Wilson and Miles, 1964). The organism is associated with necrobacillosis in white-tailed deer (Chirino, *et al.* 2003), calf diphtheria, foot rot in sheep and gangrenous dermatitis in horses and mules. It had been frequently isolated from hepatic lesions in cattle, sheep and pigs (Wilson and Miles 1964). Those non-spore forming anaerobic bacilli were first observed by Loeffler (1884) in a case of calf diphtheria and Bang (1890) established its identity.

Materials and methods

Samples from fifty four ovine and nineteen bovine livers with abscesses and necrosis were collected at Omdurman Central Abattoir and transported cooled in an isotherm-flask. Portions of those samples were fixed in 10% formal-saline for histopathology. The samples were cultured using eight culture media: Nutrient Agar, Blood Agar, Brain Heart Infusion Agar, Brain Heart Infusion Blood Agar, Brain Heart Infusion Blood Agar Supplemented,

Cooked Meat Media, Reinforced Clostredial Media and VL Media. Those cultural media were reconstituted as described by Husein (1973), Hussein and Shigidi (1974) and Shapton and Board (1971). Inoculated media were incubated anaerobically at 37°C for at least 48 hours in a McIntosh Jar (McIntosh and fildes, 1916). The anaerobic jar was set as described by Holdman and Moore (1975). Aerotolerance, morphological and biochemical tests of isolated bacteria were done according to Cowan and Steel (1966), Cruickshank *et al.* (1975), Noel and John (1986), Barrow and Feltham (1993)

Pathological methods.

Tissue samples were prepared and stained with haemotoxylin and eosin (H &E) stain (Drury and Walungtone, 1980).

Results

Bacteriological Findings

Brain heart infusion blood agar Supplemented (BHIBAS) and VL media were found to be the most suitable media for the growth of *Fusobacterium necrophorum*. Visible colonies were seen 48 to 72 hours after incubation of inoculated media. Two colonies were found to be obligate anaerobes when tested for aero-tolerance. One was small to medium size, convex, glistening, non haemolytic, entire and with translucent margins and the other was of

medium size and non translucent margins. Those identified as colonies of *Fusobacterium necrophorum* were Gram-negative filamentous organisms with beaded appearance and tend to fragment after two subcultures. They were negative for catalase and oxidase tests, and positive for glucose, haemolysis of red blood cells, lipase and indole tests. *Fusobacterium necrophorum* was isolated from three (15.7%) bovine and four (7.4%) ovine livers. It was isolated in pure culture from two samples (twenty eight per cent) and in mixed culture from the remaining ones (seventy two per cent).

Pathological findings

Liver sections from which *Fusobacterium necrophorum* was isolated revealed coalescing necrotic lesions (Fig. 1.) in which there is proliferation of bile ducts, polymorphnuclear and mononuclear cells infiltration (Fig.2.).

Discussion

The present findings indicate that *Fusobacterium necrophorum* is incriminated in the aetiology of ovine and bovine hepatic abscesses and necrosis. It was isolated from 7.4% and 15.75% ovine and bovine livers inspected respectively. This seems to be far less than the values given by Hussein and Shigidi (1974) for cattle in the Sudan, and when compared with other reports elsewhere (Simon and Stovell, 1971; Berg and Scanlan, 1982; Newsom, 1938).

Fusobacterium necrophorum seems to be the only bacteria isolated from the secondary metastatic lesions (Wilson and Miles, 1964; Scanlan and Berg, 1983). The histopathological picture currently observed in affected livers seems to be similar to that described by Lectenbergh *et al.* (1988).

References:

Aulbaek, B. S. (1971). A study of 23 strains of *Fusobacterium necrophorum*. Acta vet. Scand. **12**: 344-364.

Bang, (1890). Cited by Huttyra, F.; Marek, J.; Mohler, J.R. and Eichhorn, A. (1926) *q. v.*

Barrow, G. Feltham, R. K. A. (1993) Cowan and Steel manual for the identification of medical bacteria, 3rd Ed Cambridge University press, London.UK.

Bennetta, G.; Hickford, J.; Sedcolea, R. and Zhoua, H. (2009).

Dichelobacter nodosus, Fusobacterium necrophorum and the epidemiology of footrot. Anaerobe. 15, (4): 173-176

Berg, J. N. and Scanlan, C. M. (1982). Studies of *Fusobacterium necrophorum* from bovine hepatic abscesses, biotypes, quantitation, Virulence and antibiotic susceptibility. Am. J. vet. Res. **43**: 1580 – 1586.

Boye, M. Aalbaek, B. Agerholm, J. S. (2006). *Fusobacterium necrophorum* determined as abortifacient in sheep by laser capture microdissection and fluorescence in situ hybridization. *Mol cell probes*. 20 (6) 330-336.

Brude, I.A.; Devis, C. and Fierer, J. (1986) " Infectious diseases and medical microbiology" 2nd ed. London: Saunders company.

Chirino, T. M.; Woodbury, M. R.; Huang, F. (2003). Antibiotic and biochemical characterization of *Fusobacterium* spp and *Arcanobacterium pyogenes* isolated from farmed white-tailed deer (*Odocoileus Virginianus*) with necrobacillosis. *Zoo Wildl Med*. 34, 3: 262-268.

Cowan, S. T.; Steel, K. J. (1966). "Manual for the identification of medical bacteria" 2nd ed. London: Cambridge University press.

Cruickshank, R.; Duguid, J. P.; Marmon, B. P. and Swain, R. H. (1975).

"Medical microbiology". 12th ed. London: Long man group limited.

Drury, R. A. and Walungtone, E. A. (1980) "Carlton's Histological Technique" 5th ed. New York: Oxford Univ. Press.

Gillies, R. R. Todds, T. C. (1954). " Bacteriology illustrated". London: E and S Livingstone LTD.

Holdman, L. V. and Moore, W. E. (1975) "Anaerobic laboratory manual" 3rd ed. Virginia: Southern Printing Co.

Hubbert, W.T.; McCulloch, w. f. and Schnurrenberge, p. r. (1975). "Diseases transmitted from animal to man " 6th ed. Illinois: Charles C. Thomas.

Hussein, E. H. (1973). A bacteriological study of liver abscesses in cattle and sheep in the Sudan. M. Sc. Thesis, Khartoum University. Khartoum.

Hussein, E. H. and Shigidi M. T. (1974). Isolation of Spherophorus necrophorus from bovine liver abscesses in the Sudan. Trop. Anim. Hlth. Prod. 6: 253-254.

Lechtenberg, K. F.; Nagaraja, T. G.; Leipold, H. W. and Chengappa, M. M. (1988). Bacteriologic and histologic study of hepatic abscesses in cattle Am. J. Vet. Res. **49**: 58 – 62.

Loeffler F. (1884). Mitt Reichsgesundh Amt., **2**: 421. Cited by Wilson, G. S. and Miles. A. A. (1964) *q. v.*

McIntosh, J. and fildes, P. (1916). A new apparatus for isolation and cultivation of microorganisms. Lancet **1**: 768 Cited by Gillies, R. R. and Todds, T. C. (1954) *q.v.*

Merchant, I. A. and Paker, R. A. (1967) "Veterinary bacteriology and virology" 7th ed. Iowa; Univ. press.

Nagaraja, T. G.; Beharka, A. B.; Chengappa, M. M.; Carroll, L. H.; Raun, A. P.; Laudert, S. B.; Parrott, J. C. (1999). Bacterial flora of liver abscesses in feedlot cattle fed tylosin or no tylosin. Anim. Sci.77, 4: 973-978.

Nagaraja, T. G.; Narayanan, S.K.; Stewart, G. C.; Chengappa, M. M. (2005).

Fusobacterium necrophorum infections in animals: pathogenesis and pathogenic mechanisms. *Anaerobe*, 11 (4) 239-246.

Narayanan, S.; Stewart, G. C.; Chengappa, M. M.; Willard, L.; Shuman, W.;

Wilkerson, M.; Nagaraja, T. J. (2002). *Fusobacterium necrophorum*

Leukotoxin induced activation and apoptosis of bovine leukocytes. *Infect. Immun.* 70, 8: 4609-20.

Newsom, I.E. (1938). A bacteriologic study of liver abscesses in cattle. *J.*

Infect. Dis. **83**. 232 - 237.

Noel, R. and John, G. (1986). "Bergey's Manual of systematic

bacteriology". London: Williams and Wilkins.

Scanlan, M. C. and Berg, J. N. (1983). Bacteriologic and pathologic studies

of hepatic lesions in sheep. *Am. J. Vet. Res.* 15: 363-366.

Shapton, D. A. and Board, R. G. (1971) "Isolation of anaerobes " London:

Academic press.

Simon, P. C. and Stovell, P. L. (1971). Isolation of *Sphaerophorus*

necrophorus from bovine hepatic abscesses in British Columbia. *Can. J.*

Comp. Med. **35**: 103 – 106.

Wilson, G. S. and Miles, A. A. (1964). "Topely and Wilson's principles of

bacteriology and immunity" 5th ed. London: Edward Arnold Ltd.

Figure. 1. A chain of coalescing necrotic lesions in ovine liver
(Haematoxylin and eosin, X 10).



Figure. 2. polymorphnuclear and mononuclear cells infiltration
and bile duct proliferation in a necrotic area in ovine liver section
(Haematoxylin and eosin, X 20)

