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A BACTERIOLOGICAL STUDY OF SHEEP LIVER ABSCESSES

BY

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INTRODUCTION

It has long been recognized that the condemnation of large numbers of sheep livers, due to the presence of hepatic abscesses, constitute a considerable economic problem (Butozan et al., 1961 and Berg and Scanlan, 1982). The Literature contains little references to the bacteriologic findings in sheep liver abscesses.

Rumenitis and hepatic abscesses in sheep constitute a disease complex in which the ruminal lesions are the primary focci of infection and hepatic abscesses are the secondary focci of infection (Thomson et al 1968; Kanoe et al., 1978 and Gacia et al., 1986).

The disease is usually found in healthy appearent sheep at slaughter. The liver is usually enlarged and contains a greater or lesser number of walnut to small egg sized abscesses deeply in the liver tissues and prominences on the surface. The abscesses are surrounded by thick capsules and mostly contain thick greyish white pus (Kujumgiev, 1955; Katitch et al., 1969 and Benno et al., 1983).

The primary etiology agent of sheep hepatic abscesses is considered to be Fusobacterium necrophorum in pure

culture or in combination with other aerobic or anaerobic organisms in an incidence varied from 67% up to 90% (Simon and Stovell, 1971; Kanoe et al., 1978 and Garcia et al., 1986).

The significance of some clostridial organisms in the livers of sheep at post-mortem is undertaken to investigate by some authors and many of the sheep had liver fluke infection (Butozan et al., 1961; Thomson et al., 1968, and Scanlan and Berg, 1983). Isolation of anaerobic bacteria other than F. necrophorum from sheep hepatic abscesses have occurred rarley (Moore et al., 1969 and Kanoe et al., 1978).

Some investigators also have reported that other aerobic and facultative anaerobic organisms often are present in sheep hepatic abscesses including staphylococcus spp, Streptococcus spp., Pseudomonas, Corynebacterium spp, and members of family Enterobacteriaceae (Kanoe et al., 1976; Szazados and Tokaco, 1978 and Berg and Scanlan, 1982).

The objectives of this study were to determine the aerobic and anaerobic bacteria causing sheep liver abscesses and to characterize selected predominant isolates by antibiotic susceptibility tests and pathogenicity for laboratory animals.

MATERIALS AND METHODS

The material used in this work had been obtained from 92 carcasses of sheep. These consectively slaughtered animals were presented from different abattoirs within a period from July 1989 up to November, 1990. Seventy two sheep liver abscesses were collected, out of these, 32 revealed liver flukes. The abscessed portions removed for study were surrounded by two or three inches of apparently tissues. Twenty livers not showing abscess formation were included as control.

All specimens were processed the same day they were collected or frozen at -20°C until processes. The abscesses were reared with a not spatula prior to incision. Scraping were made with a sterile surgical blade.

Material thus obtained was inoculated for primary isolation in Robertson's meat broth (for the culture of anaerobic) and directly into 1% serum broth medium (for aerobes). Both sets were incubated at 37°C either anaerobically in Brewer's anaerobic jar in 90% H_2 and 10% Co_2 atmosphere or aerobivally at 37°C for a period of 24 hrs. An ionculum for both sets were seeded onto two plates of enriched brain heart infusion blood agar which were prepared as previously described by Berg and Loan (1975) as well as onto two plates of sheep blood agar media. One set represented each media were incubated anaerobically at 37°C for 5 days and examined periodically. The other set from different media were incubated at 37°C for 3 days and examined daily. All colony types were identified morphologically, culturally and then subcultured for further identification.

An organism was considered to be F. necrophorum if it was a Gram-negative, non sporulated, non motile, pleomorphic anaerobic, and showing the following characteristics: (a) Production if offensive odour.
(b) Filamentous forms that would break down, usually after one to five days into short rods and coccoid containing metachromatic granules.
(c) Agglutination 2% ORH negative human erythrocytes and, (d) Produce fluffy growth in Brewer's thioglycolate broth (Oxiod) which did not extend to the surface when incubated aerobically.

All aerobic and anaerobic isolates were identified biochemically according to Smith and Holdeman (1968) and Colline and Cummins (1986).

Sensitivity of the most prevalent representing anaerobic isolates to various chemotherapeutic agents in vitro was evaluated using the disc assay method according to Kneman et al., (1983). A 48 hrs. old culture in Brewer's thioglycollatc broth medium was swabbed onto the surfaec of sheep blood agar media and different discs were placed and incubated anaerobically in Brewer's anaerobic jars in 90% H_2 and 10% Co_2 atmosphere at 37°C for 48 hrs, followed by measuring and recording the diameter of growth inhibited zones in mms.

F. necrophorum was tested for pathogenicity in mice by S/C injection. Six pure cultures of F. necrophorum were tested by inoculating three mice from each culture in a dose of 2 mg (net weight) per mouse (25 to 30 g.). The viable numbers in the liver and spleen were recorded as an index of pathogenicity. Quantitative counts were done with plate dilution procedures on anaerobic brain heart infusion blood agar plates according to Scanlan and Berg (1983). The mice were observed for 3 days post-inoculation for clinical signs and re-isolation. They were killed for counting the viable bacteria in organs after 8, 12, 18, 24 and 36 hours post-infection.

RESULTS AND DISCUSSION

Recently, some investigators have found an increased incidence of telangienctatic lesions predispose to hepatic abscesses and were proferential sites for the colonization of many bacterial pathogens.

F. necrophorum strains were isolated from 33 (45.8%) in pure form as well as from 12 abscessed liver (16.7%) present in combination with other aerobic and anaerobic micro-organisms out of the 72 abscessed liver examined in the present work. The 62.5% overall isolation of F. necrophorum among sheep liver abscesses corresponds closely with the 67% of Simon

Table (1) : Results of bacterial examination of 72 abscessed sheep liver.

Bacterial isolates	Abscessed Liver (72)					
	With Liver flukes (32)		without Liver flukes (40)		Total	
	No.	%	No.	%	No.	%
I-As Single infection						
F.necrophorum	14	43.8	19	47.5	33	45.8
Cl. pyogenes	6	18.7	4	10.0	10	13.8
Staph.aureus	5	15.6	4	10.0	9	12.5
Ps.aeruginosa	1	3.1	2	5.0	3	4.2
Bacteroides fragilis	3	9.4	0	-	3	4.2
Peptostreptococcus anaerobius	0	0	2	5.0	2	2.8
II As Mixed infection:						
F.necrophorum + C.perfringens	2	6.3	5	12.5	7	9.7
F.necrophorum + E.coli	0	0	3	7.5	3	4.2
F. necrophorum + Str.pyogenes.	1	3.1	1	2.5	2	2.8
	32	100.0	40	100.0	72	100.0

Table (2): Results of culture of 20 normal livers showing types and distribution of organisms.

Type of organisms .	Distribution of organisms on the basis of single livers.	
	No.	%
Sterile	7	35.0
Staph. saprophyticus	4	20.0
Str. faecium	3	15.0
Pr. vulgaris	2	10.0
Serratia marcesoens	2	10.0
Citrobacter diversus	1	5.0
E. coli	1	5.0
Total	20	100.0

and Stovell (1971) and 74.3% of Kanoe et al., (1978). F. necrophorum is the predominant organism to be found in sheep liver abscesses. Similar results were also obtained by Thomson et al., (1968) and Garcia et al., (1986). these workers suggest that the protal circulation may serve as a pathway for hematogenous spread of the F. necrophorum from the intestinal canal.

During this study, two types of liver abscesses were noted; one with liver flukes and the other without. It was noticed that there were no significant differences in the incidence of F. necrophorum as a single infection in both types of liver abscesses. It was also observed that F. necrophorum mixed with aerobic and anaerobic bacteria were more in sheep without liver flukes than from those with liver flukes. This study utilized additional criteria that no great variations were observed in bacterial pathogens of hepatic sheep abscesses suffering from liver flukes or not. The significance of F. necrophorum and other organisms in the liver of sheep that came to slaughter can not be determined on the basis of parasitic liver fluke changes in the liver.

From Table (1), it was found that other organisms as single infection associated with liver abscesses were: C. pyogenes (13.8%), Staph. aureus (12.5%), Ps. aeruginosa and Bacteroides fragilis (4.2% each), and Peptostreptococcus anaerobius (2.8%). The comparison of the present data with those described by Thomson et al., (1968); Moore et al., (1969); Kanoe et al., (1978) and Scanlan and Berg (1983) shows little considerable differences in the incidence of isolates. This may be due to variety of media used for the isolation of causal agaent of infection.

It was of interest to note that mixed microorganisms recovered from individual abscessed liver were : F. necrophorum and C. perfringens as they isolated from seven cases. Besides, F. necrophorum and E. toli

Table (3) : Results of sensitivity of the predominant anaerobic organisms isolated from sheep liver abscesses.

Therapeutic agent used ⊗	Concentration mg	F.necrophorum (10 strains)	C.perfringens (7 isolates)	Bacteroides fragilis(3)	Peptostreptococcus anaerobius (2)
Penicillin -G	1.5 unit	S	S	R	R
Chloramphenicol	30	R	R	R	R
Colistine	10	R	R	R	R
Flumequine	30	R	R	S	R
Erythromycin	30	S	S	S	S
Gentamicin	10	S	S	S	S
Oxytetracycline	10	S	R	S	S
Streptomycin	10	R	R	R	R
Furazolidone	50	R	R	R	R
Sulfa methoxazole trimethoprim	12.5 + 23.75 mcg.	R	R	R	R

S = Sensitive
R = Resistant

being recovered in mixed infection in 3 cases (4.2%) and F. necrophorum and Str.pyogenes in 2 cases(2.8%) in mixed infection.

Table (2) shows the organisms obtained from 20 apparently normal livers, F. necrophorum was not isolated from these specimens. In additon, Staph. Saprophyticus, Str. Faecium, Pr. vulgaris, Serratic marcescens, C. diversus and E. coli were recovered in order of their frequency from 13 out of 20 normal livers. All these organisms were principally contaminants picked up between the time of slaughter and their subsequent culturing.

As shown in Table (3), the sensitivity of representative anaerobic organisms to various chemo-therapeutic agents was studied. It was found that all strains of F. necrophorum were completely sensitive to penicillin-G, erythromycin, gentamicin and oxytetracycline. These findings agreed with those obtained by Berg and Scanlan (1982) who proved that only four antibiotics included erythromycin; penicillin-G; tylosin and oxytetracyclin were mainly used in treatment or prevention of infections caused by F. necrophorum such as "foot rot" or hepatic abcesses. From the present study, one can conclude that erythromycin, oxytetracycline, penicillin-G and gentamicin may find an important place in the control and treatment of F. necrophorum infection.

In the present work, most of the examined strains of C. perfringens, B. fragilis and Peptostr. anaerobius proved to be sensitive to erythromycin, gentamicin, oxtetracycline and penicillin-G. Similar findings were reported by love et al., (1980) who tested 237 anaerobic bacteria isolated from abscesses and found that all isolates were sensitive to some antibiotics namely: penicillin, gentamicin, chloramphenicol and erythromycin.

Table (4) : Virulence to mice inoculated S/C with selected strains of F.necrophorum.

Strain No.	Examined organ	Mean Number of viable bacteria in organ per gram				
		8 H ⊘	12 H.	18 H.	24 H.	36 H.
Pre-infection.	Liver	0	0	0	0	0
	Spleen	0	0	0	0	0
1	Liver	420	490	1290	D	-
	Spleen	133	155	340	D	-
2	Liver	600	650	1020	1540	D
	Spleen	215	280	440	490	D
3	Liver	670	710	1040	1620	1840
	Spleen	300	350	460	550	580
4	Liver	280	390	510	1060	1260
	Spleen	55	80	120	240	250
5	Liver	540	820	1220	1280	D
	Spleen	160	190	220	250	D
6	Liver	660	810	1060	1350	D
	Spleen	190	190	320	540	D

D = Death occurred during the observation time.

⊘ = Three mice were killed and examined at each time.

As shown in Table (4), the number of viable F. necrophorum in the livers of inoculated mice were markedly increased than those present in spleen. Numous viable F. necrophorum were highly present in both liver and spleen adjacent to lesions during 18-24 hours post-infection. F. necrophorum was isolated in pure culture from hepatic necrobacillosis lesions and spleen specimens of all the 18 mice killed at 8, 12, 18, 24 and 36 hrs. after inoculation. The mean number of viable bacteria in the liver and spleen per gram was determined in Table (4).

SUMMARY

* 62.5% overal isolation of F. necrophorum among sheep liver abscesses as compared with 37.5% of other aerobic and anaerobic micro-organisms recovered from the same lesions. F. necrophorum was the predominant organism to be found in sheep liver abscesses.

* The significance of F. necrophorum and other organisms present in examined sheep liver abscesses can not be determined on the basis of parasitic liver fluke infestations.

* C. pyogenes (13.8%), Staph. aureus (12.5%), Ps. aeruginosa and Bacteroides fragilis (4.2%) and Peptostreptococcus anaerobius (2.8%) were found as single infection in sheep hetatic abscesses. Mixed infection were also detected.

* All the organisms recovered from 20 apparently normal livers were principally contaminants and picked up between the time of slaughter and their subsequent culturing.

* F. necrophorum strains were completely sensitive to erythromycin, gentamicin, penicillin-G and oxytracycline. C. perfringens, Peptostreptococcus anaerobius and Bacteroides fragilis were highly sensitive to erythromycin, gentamycin, oxtetracycline and penicillin-G. also.

* The number of viable F. cecrophorum in the liver of experimentally inoculated mice were greatly increased than present in spleen. The mean number of viable bacteria in liver and spleen per gram were calculated in details.

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