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BOVINE INTERDIGITAL DERMATITIS IN FAYOUM GOVERNORATE AND ITS RELATIONSHIP TO OBLIGATE ANAEROBIC MICROORGANISMS

I. A. RADWAN.
Department of Microbiology, Fac. Vet. Med.
(Beni-Suef, Cairo Univ.)

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SUMMARY

F. necrophorum (62.0%), B. melaninogenicus (43.0%), P. anaerobius (34.0%), C. sporogenes (18.0%), F. mortiferum(18.0%), B. fragilis (12.0%) and C. perfringens (12.0%) were the most prevalent obligate anaerobic organisms isolated from bovine interdigital dermatitis . The pathogenicity of F. necrophorum in mice was related to the route of infection . Intrahepatic route was the most virulent route with mortality rate (86.7%) . Mortalities with s/c or i/p routes reached 53.3%and 73.3% respectively . The clinical signs and gross lesions were discussed in details . The in vitro sensitivity of 20 strains represented each of F. necrophorum , B. melaninogenicus and P. anaerobius to 16 chemotherapeutic agents were described in details . Oxytetracycline, enerofloxacine and danofloxacine were the most effective drugs against the majority of tested anaerobic microorganisms.

INTRODUCTION

Foot-rot is an infectious syndrome, specific to sheep and goats, although it may be also affect cattle, and is caused by synergic action of certain microbial species (Piriz duran et al., 1990). Little antimortum diagnosis are made because these infections are difficult and not routinely economical to be diagnosed clinically. Practitioners do not have many opportunities to culture bacteriological samples, anaerobically, and to test the in vitro antibiotic susceptibility, although the full course of bacteriological examination is required to confirm and treat a case of foot-rot caused by obligatory anaerobic bacteria (Ikawa et al. 1987). Hepatic abscesses, caudal vena caval thrombosis and multiple abscesses in various organs results from Fusobacterium necroforum infection in cattle. (Jensen and Mackey, 1979) . Virulent foot-rot is a mixed bacterial infection and the essential transmitting bacteria is Bacteriodes nodosus and its

elimenation from the flock by chemotherapy or culling of the affected animal can results in permanent eradication of the disease (Beveridge, 1941).

The present work concerning foot-rot aims to identify the species of obligatory anaerobic bacteria associated with bovine foot-rot; to compare the efficiency and selectivity of four culture media used in their isolation, application of experimental infection of F. necrophorum in mice and to detect the most suitable chemotherapeutic agents used for treatment.

MATERIAL AND METHODS

Samples :

One hundred sample were taken from 75 Holstein-Friesian dairy cows suffering from foot-rot in Fayoum Governorate . All animals showed typical foot-rot lesions at various stages of development ranging from interdigital dermatitis to complete separation of the hooves.

Collection of Samples :

Prior to sample collection from the hooves of clinically affected animals, the surfaces of the lesions were cleansed with cotton wool soaked in sterile physiological saline. Samples were taken from the affected areas using sterile bacteriological swabs soaked in modified Stewart(s medium (Lennette et al., 1985). Where the nature of the lesion allowed part of the infected tissues to be collected, tissue was excised and quickly transferred to test tubes containig Thorley's transport medium (Thorley, 1976). Collected samples were rapidly transported to the laboratory in isothermal containers at a temperature between 4°C and 6°C as recommended by Lennette et al., (1985).

Laboratory animals :

Fifty Swiss white mice with average of weight about 20 g were used for experimental purpose.

Isolation and identification :

All samples were grown on to each of the four different culture media : Viz. the Centers for Disease Control (CDC) anaerobic blood agar medium (Koneman et al., 1992) anaerobic kanamycin-Vancomycin blood agar (KV) (Dowell et al., 1977), Agar Brucella (BR) (Sutter et al., 1985) and Thorley's medium (THO) (Thorley, 1976).

Inoculated plates were incubated in anaerobic Jars (BBL, Cockeysville, MD, USA) at 37°C using Gas-pak Sachets (Oxoid Ltd, Hampshire, UK) for 3 days.

The suspected colonies were identified according to (Koneman et al., 1992) as follow : On blood agar : Haemolysis, pigments, pitting of agar, cellular motility (wet mount), inhibition by penicillin, rifampin and kanamycin.

In enriched thioglycolate medium : Appearance of growth, rate of growth, gas production odour and

cellular morphology .

The following biochemical tests were applied according to Koneman et al., 1992) : Including growth on thioglycolate broth, catalase, lecithinase, lipase, reaction on milk medium, production of indole, hydrolysis of starch, aesculin and gelatin, reduction of nitrate, fermentation of glucose, mannitol, lactose and rhamnose, growth in the presence of bile, Penicillin, rifampin, kanamycin, vancomycin and colistin disks (Oxoid).

Experimental infection of F. necrophorum in mice :

A total of fifty male mice used for experimental infection and they divided into 3 groups each of 15 mice and a fourth group comprised 5 mice were kept as a control group . 0.1 ml of 18 hours culture of F. necrophorum grown in BM broth after the adjustment of bacterial count to be $1x10^6$ bacterial cell/ml by the addition of sterile BM broth (Smith et al., 1989). The first group of mice was injected s/c in the right flank, the second group i/p and the third group intrahepatically according to the procedure adopted by Maestrone et al. (1975). The artificially infected and control mice were observed twice daily for morbidity and mortalities . All animals which died during or at the end of the experiment i.e. 4 weeks post infection were observed for gross lesions.

The in vitro disc and agar diffusion method described by Collee et al., (1989) was performed under strict anaerobic conditions using the following chemotherapeutic discs:- Danofloxacin (10) ug, norfloxacin (10) ug, enrofloxacin (10) ug, lincospectin (10) ug, gentamicin (10) ug, erythromicin(15) ug, ampicillin (10) ug, amoxycillin (25) ug, oxytetracycline (30) ug, sulfamethoxazole with Trimethoprim (1.25 + 23.75) ug, colistin sulphate (10) ug, rifampin (30) ug, vancomycin (10) ug, kanamycin (10) ug and Ceftiofur (30) ug.

The interpretation of the results was carried out according to Koneman et al., (1992).

RESULTS

The frequency of isolating strictly anaerobic genera in one hundred samples taken from 75 clinical cases of bovine foot-rot is shown in table (1) . The anaerobic species most frequently isolated belonged to the following genera : Fusobacterium; Bacteroides; Peptostreptococcus; Clostridium; Eubacterium and Peptococcus.

The following species, as shown in table (1), being isolated : Fusobacterium necrophorum, Fusobacterium mortiferum; Bacteroides melaninogenicus, Bacteroides nodosus, Bacteroides fragilis, Peptostreptococcus anaerobicus, Peptostreptococcus magenus, Peptostreptococcus prevotii; Clos-

tridium perfringens, Clostridium sporogenes; Eubacterium plexicaudatum, Eubacterium nitritogenes and Peptococcus niger in order of their frequencies.

The efficiency of the culture media used in the present work revealed that both Agar Brucella (BR) and the Centers for Disease Control anaerobic blood agar (CDC) media were the most efficient specific culture media used for isolation . On the contrary anaerobic Kanamycin-Vancomycin blood agar medium (KV) and Thorley`s medium (THO) showed scanty growth or even no growth could be detected within 3 days post-inoculation.

From table (2), it is evident that the pathogenicity of F. necrophorum in mice were related to the route of inoculation . F. necrophorum injected s/c and i/p caused mortality rates reached 53.3% and 73.3% respectively . As regards to s/c infection, clinical signs were observed only on 8 out of 15 inoculated mice . Necropurulent subcutaneous lesions were developed after few days post infection, followed by skin necrosis and rupture of abscesses in 5 cases . The underling muscular tissues appeared clinically normal and covered by

Table (1): Prevalence rate of obligatory anaerobic organisms obtained from bovine foot-rot:

ANAEROBIC ISOLATES	NO. OF ISOLATES	PERCENTAGE*
Genus Fusobacterium:		
F. necrophorum	62	62
F. mortiferum	18	18
Genus Bacteroides:		
B. melaninogenicus	43	43
B. nodosus	5	5
B. fragelis	12	12
Genus Peptostreptococcus:		
P. anaerobicus	34	34
P. magenus	8	8
P. prevotii	8	8
Genus Clostridium:		
C. perfringens	12	12
C. sporogens	18	18
Genus Eubacterium:		
E. plexicaudatum	6	6
E. nitritogenes	4	4
Genus Peptococcus:		
P. niger	3	3

***The percentage was calvulated according to the total number of examined samples.**

Table (2): Experimental infection of F. necrophorum to mice by using different routes:

Route of infection	No. of mice infected in each group	Clinical symptoms post-infection	Gross lesions	No. of mice died/ No. inoculated	Mortality ra
Subcutaneously	15	Loss of weight, sinus formation, depression, restriction of activity	Purulence and necrosis of the subcutaneous tissues, thick fibrotic tissues of the muscles.	8/15	53.3%
Intraperitonealy	15	Loss of weight, abdominal swelling, depression, ruffled coats	Focal abscesses were formed in the liver, cogulative necrosis to true abscesses with encapsulated pus, peritoneal cavities containing pus.	11/15	73.3%
Intrahepaticaly	15	Loss of weight, arched back, abdominal pain.	Liver abscesses, diaphragmatic adhesions	13/15	86.7%
Control	5	No signs.	-	0/5	0.0%

Table (3): Results of antimicrobial sensitivity testing of the prevalent stricitly anaerobic bacteria isolated:

Organism / Antimicrobial drug	Fusobacterium necrophorum				Bacteroides melaninogenicus				Peptostreptococcus anaerobicus			
	S		R		S		R		S		R	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Oxytetracycline	20	100	0	0	18	90	2	10	19	95	1	5
Danofloxacine	18	90	2	10	18	90	2	10	17	85	3	15
Enrofloxacine	16	80	4	20	16	80	4	20	16	80	4	20
Norfloxacine	5	25	15	75	6	30	14	70	5	25	15	75
Lincospectin	2	10	18	90	2	10	18	90	2	10	18	90
Gentamicin	2	10	18	90	1	5	19	95	3	0	17	85
Erythromycin	0	0	20	100	0	0	20	100	0	10	20	100
Ampicillin	2	10	18	90	2	10	18	90	2	15	18	90
Amoxycillin,	3	15	17	85	2	10	18	90	3	10	17	85
Sulfamethoxazol e+Trimethoprim	0	0	20	100	0	0	20	100	2	0	18	90
Colistin sulphate	0	0	20	100	0	0	20	100	0	5	20	100
Rifampin	13	65	7	35	15	75	5	25	1	5	19	90
Vancomycin	2	10	18	90	1	5	19	95	1	15	19	95
Kanamycin	19	95	1	5	0	0	20	100	3	15	17	85
Ceftiofur	2	10	18	90	2	10	18	90	3	15	17	85
Penicillin	19	95	1	5	1	5	19	95	2	10	18	90

S = Sensitive R = Resistant

thick fibrotic tissues formation . On the other hand, 3-4 days post infection with F. necrophorum i/p resulting in inflammation of the peritoneal wall with purulent exudate . A thick cheesy foetid materials covered most of the visceral organs, focal abscesses in the liver, exudates accumulates in the abdominal cavity causing visible abdominal distention and deaths of 11 out of 15 mice were occurred during the first 10-13 days post inoculation. On the other hand, mice infected intrahepatically revealed loss of weight, anorexia, abdominal pain manifested by arching of the back . Postmortum examination showed enlarged hepatic abscesses, diaphragmatic adhesions were also observed. Organs other than the liver in the digestive system were grossly normal with mortality rate reached 86.7%.

For the treatment of bovine foot-rot, parentral chemotherapy has the advantage of not requiring the extensive paring which is a prerequisite for effective topical treatment . As shown in table (3), all isolates of F. necrophorum, B. melaninogenicus and P. anaerobicus were highly resistant to oxytetracycline, danofloxacin and enrofloxacin . On the other hand, rifampin was effective against Fusobacterium and Bacteroides isolates but not effective against Peptostreptococcus species . Kanamycin and penicillin were highly effective against Fusobacterium species, meanwhile the Bacteroides species were highly resistant . All isolates of strictly anaerobic Gram negative bacteria under study were highly resistant against the other tested chemotherapeutic agents.

DISCUSSION

Regarding the aetiology of foot-rot in ruminants, Katitch and Matitch (1977) and Katitch (1979) had suggested that foot-rot is a multifactorial process resulting from a prior hoof lesions invaded by soil bacteria such as Fusobacterium necrophorum, Clostridium perferingens and Bacteroides nodosus . The results of the present study partially agree with those of Katitch (1979), in that 6 different anaerobic genera were isolated, as shown in table (1) .

As regards to the species of the genus Fusobacterium, Fusobacterium necrophorum was the most common species with the isolation rate of 62% . These findings nearly in agreement with those obtained by Jang and Hirsh (1994), who reported that Fusobacterium necrophorum was the most commonly isolated species from cases of foot-rot in cattle . Also Cook and Cutto (1995) isolated Fusobacterium necrophorum from the feet of 24 Holstein-Friesian dairy cows . Meanwhile Togoe et al. (1995) isolated Fasubacterium necrophorum from 33 out of 40 cows suffering from foot-rot.
The most commonly isolated species of the genus Bacteroides was Bacteroides melaninogenicus with the isolation rate of 43% . This finding support the statement of El-naenaeey et al. (1994) and Togoe et al. (1995) who isolated B. melaninogenicus from 17 samples of necrotic tissues from

the feet of cows with an incidence of 42.5 % .

Peptostreptococcus anaerobius was isolated with a rate of 34% Brunner et al (1982 and 1989) isolated P. anaerobius in 29 cases of bovine interdigital dermatitis . Also Samitz et al (1996) isolated P. anaerobius as a selected obligate anaerobic bacteria from bovine sources .

BR and CDC media were proved to be highly efficient for isolating the studied microorganisms .These findings nearly agree with those obtained by Dowell et al (1977) and by Piriz duran et al., (1990) .

Little is known about the pathogenesis of F. necrophorum of bovine origin . An adult mouse model of the disease would facilitate studies on F. necrophorum virulence properties . A more significant mortality rate have been observed in mice injected intrahepatically (86.7%) than those injected s/c or i/p (53.3%) and (73.3%) respectively . These findings nearly coincide with the results obtained by Smith et al. (1989) . The clinical signs and post-mortum lesions were varied according to the route of injection of F. necrophorum . Focal abscesses, coagulative necrosis to a true abscesses with encapsulated pus and sever pus in the peritoneal cavities were noticed among mice infected by i/p routes . Also, purulent and necrosis of the subcutaneous tissues and thick fibrotic muscles were recorded in mice infected s/c with F. necrophorum . This seems to coincide with the observations of Scanlan and Berg (1986) and Smith et al. (1991) .

Oxytetracycline, enrofloxacin and danofloxacin were the most efficient chemotherapeutic agents used, at the same time, all isolates of Fusobacterium necrophorum were highly sensitive to kanamycin and penicillin . These findings partially agree with those obtained by Berg and Scanlan (1982), Brizza (1990), Piromalli et al., (1994) and Cook et al., (1995) .

The combination of topical treatment with antibacterial compounds like formalin or zinc sulfate together with systemic treatment with more selective antimicrobial drug proved to be more effective in the treatment of bovine foot-rot . The effectiveness of parenteral chemotherapy of foot-rot is equivalent to or better than achieved by topical treatment (Scanlan and Berg, 1986)

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