The Iraqi J. Vet. Med. Vol. 21, No. (1), 1997.

MICROBIOLOGICAL AND TREATMENT STUDIES OF OVINE FOOTROT INFECTION IN MOSUL AREA

M.SH. RHAYMAH M. L. SAWA, AND Y.A. YOUSIF
College of Veterinary Medicine, University of Mosul Mosul, Iraq.

ABSTRACT

It emerges from bacteriological study that it is possible to isolate many bacterial types and yeasts from the lesions of footrot infection in sheep. Anaerobic cultivation came out with the isolation of Spherophorus spp. (24%), Bacteroides spp. (60%), Corynebacterium spp. (64%), Enterobacteracae (76%), Streptococcus spp. (76%), Staphylococcus spp. (36%), Clostridum sordellii (46%) and Irichosporon cutaneum (4%).

On the other hand aerobic cultivation rendered the isolation of Corynebacterium spp. (100%), Entrobacteracae (100%), Staphylococcus spp. (15%), Penicillium spp., Aspergillus fumigatus and Trichosporon cutaneum.

Five different drug combinations were studied for their efficacy in the treatment of ovine footrot. Their healing rates were as follows : oxytetracycline (59%), oxytetracyclin with formaline (70.9%), Procaine penicillin and streptomycin (72.5%), Procaine penicillin and streptomycin with formaline dipping (80.76%), Formaline alone (63.8%). All kinds of treatment indicated statistically significant differences to exit between the treated (experimental) and untreated (control) groups.

INTRODUCTION

Major microorganisms causing ovine footrot are Bacteroides nodosus and Spherophorus necrophorus (1,2).

Other types of microorganisms are frequently isolated from cases of ovine footrot including Corynebacterium spp., Staphylococcus spp., Streptococcus spp., and Enterobacteracae. Also many other type of yeast and fungus like Penicillium spp., Mucor spp., Geotricum spp., and Aspergillus spp. can be isolated (3,4,5,6). Viruses have also been shared in the aetiology of the diseases (2).

Ovine footrot is very pernicious disease since it causes high economic losses stemming from low productivity and high cost of chemotherapeutic agents used in its combat (8). In Iraq a comprehensive study of ovine footrot is still lacking and the only preliminary study in this respect was conducted in Mosul (9). Therefor, the present study was conducted to clarify the aetialogical agents of footrot. The efficacy of commonly used chemotherapeutic agents for the treatment of this infection was evaluated.

MATERIALS AND METHODS

Samples were collected from the infected foot by using a sterile cotton swab by smearing the swab in-between the necrotic and healthy tissues. The swab was placed in pijou bottles containing thioglycolate broth, then immediately transported to the laboratory and incubated anaerobically at 37oC for 72 hr's using Gaspak (bioMerieux-France) (2).

When this phase was over, Gram - stain smear were made from them ; then these were subcultured on brain - heart infusion agar (oxoid, England) supplemented with 2% ovine hoof powder (10). After anaerobic cultivation for 72 hr's at 37oC, and after many steps of purification by repeating subcultures, isolated bacteria were cultured aerobically to isolate the facultative anaerobic bacteria.

A second sample from the same case was cultured aerobically in brain - heart infusion broth for 24 hr's at 37 °C after making smears and Gram stain, subculture were made in brain heart infusion agar supplemented with 2% hoof powder (10) and reincubated at 37 °C for 48 hr's, multiple steps of subcultures were made for cony purification.

A third sample from the same case was cultured on sabouraud dextrose agar (oxoid, England), and incubated at room temperature (22 °C – 25 °C) for 42 days for fungal isolation.

Some selective media like mannitol salt agar for Staphylococcus spp. Edward media for Streptococcus spp., and Tinsidal media for Corynebacterium spp. were used.

Standard biochemical tests were performed according to (Buchnan and Gibbons) (11).

Five types commonly used Antibiotics were used. A total of 163 moderately infected sheep were grouped into 6 different groups.

Group 1 included 30 ewes having 61 infected feet treated with oxytetracyline (oxycen-10, cenavesa, S.A.Lab) in a dose of 10 mg/kg body weight intramuscular for 3 days.

Group 2 included 33 animals having 55 infected feet treated with oxycen -10, 10gm/kg. body weight for 3 days, and dipping the feet on time in 5% formaline solution for 2 to 3 minute.

Group included 25 animals having 52 infected feet treated once with both Procaine penicillin (S.D.I Iraq) and streptomycin sulphate (Biochemic, Austeria)70.000 1-u/kg body weight and mg/kg body weight respectively.

Group 4 included 25 infected animals having 40 infected feet treated as the 3rd group animals above in addition to their

treatment by dipping either infected feet once in 5% formalin solution.

Group 5 includes 35 animals having 47 infected feet treated only by dipping the feet once in 5% formaline solution.

Group 6 (control) included 20 animals having 32 infected feet. This group of animals was left untreated in the same environmental condition.

All animals were housed in semidry conditions for 3 days after treatment and results were obtained after 8 to 10 days. All the results were analyzed statistically according to Theodore colton (12) and All-Rawi (13).

Chi-square test was used to analyze the results obtained by the different chemotherapeutic agents.

RESULTS

1. Bacterial Isolation :

Anaerobic cultivation of 25 sample was resulted in the isolation of 7 types microorganisms and one type of yeast (Table 1). The microorganisms were classified as Bacteroides nodosus, from 15 samples (60%). Other species of the genius Bacteroides were also isolated including B. fragilis, B. clostridiformis, and B. melaninogenicus.

Spherophorus (Fusibacterium) bacteria were isolated from 6 samples (24%). Spherophorus species identified were Fusibacterium necrophorum, F. equitile, F. cumbiosum and F. glutinasum, Corynebacterium pseudotuberculosis was isolated from 16 sample (64%). Streptococcus were isolated from 19 samples (76%) and were identified as St. faecalis (enterococcus), St. zooepidemicus, and beta haemolytic Streptococcus spp., Enteric bacteria were isolated from 19 samples (76%) including Escherichia coli and Alcaligenes facecalis. Staphylococcus aureus were isolated from 9

samples (36%). Closteridium sordellii were isolated from 16 samples (64%). Only one type of yeast was isolated anaerobically from one sample (4%) and was identified as Trichosporon cutaneum.

Aerobic cultivation of 13 samples resulted in the isolation of Arthobacter globiformis (Corynebacterium) from 13 samples (100%). Enterobacter were also isolated from all 13 samples (100%) and were identified as Escherichia coli, Proteus vulgaris, Pseudomonas aeroginosa and Citrobacter intermedius. Staphylococcus epidermidis were isolated from 2samples (15%). Aerobic cultivation of the same 13 samples on subbouraud media enabled the isolation of the following yeasts Aspergillus spp., Penicillium spp., and Trichosporon cutaneum (Table 2).

Smears made from aerobic cultivation facilitated the identification of Corynebacterium spp., Enterobacterium spp., Gram positive cocci and yeast in all the 20 samples examined.

TREATMENT RESULTS

Five types of treatment were used in this study (Table 3). The first type was treated by use oxytetracyline, only 36 feet from a total of 61 feet were healed after 8 days of treatment (59%), ($P<0.05$).

The second type was treated by the use of oxyteyracycline and the dipping of the affected feet with 5% formaline solution. 39 feet from a total of 55 feet were healed after 8 days of treatment (70.1%) ($P<0.001$).

The third type was treated by the use of procaine penicillin and streptomycin sulphate. 29 feet from a total of 40 feet were healed after 8 days of treatment (72.5%) ($P<0.001$).

The fourth types was treated by the use of penicillin, streptomycin and formaline, 42 feet from a total of 52 feet were healed after 8 days of treatment (80.8%), ($P<0.001$).

The fifth type was treated by the use of formaline only. 30 feet from a total of 47 feet were healed after 8 days of treatment (63.8%) ($P<0.001$).

The sixth group was control group, which was left untreated, 3 feet out of 32 feet were healed after 8 days of treatment (9.4%).

DISCUSSION

The isolated Bacteroides nodosus has been regarded as the primary causative microorganism of footrot in sheep (14,15,16). It has been mentioned that this microorganism is isolated only from footrot lesions (15,16). B. melaninogenicus was also isolated, and it seems that it was present in suppurative lesion (17). Other bacteria which were isolated in the present study act as secondary causes of footrot infection in sheep (10).

The present rate of Bacteroides isolation (60%) is higher than what have been previously reported 35% by Katith and 33% by Volder (15,18) in Yogoslavia and Hessen respectively. However, Marsh and claus (10) isolated Bacteroides nodosus from all footrot cases (100%), It is worth to mention that the difficulty encountered in isolating B. nodosus, sttems out of the presence of rapidly growing microorganisms, (6,19) Many species of Fusibacterium spp. were isolated and they acted as secondary causes of footrot infection. They may also cause other systemic infections (10).

Gupta et al.(3) were able to isolated Spherophorus necrophorus from (47.5%) of footrot cases, other investigators showed variable rates of isolation such as 10% (5), to 24% in

our study. Corynebacterium pseudotubereulosis was isolated from footrot samples in anaerobic cultivation (64%). Other investigators mentioned the presence of Corynebacterium spp. in both aerobic and anaerobic cultivation (7). It is worth to mention that Corynebactenium pyogenes was not isolated from both aerobic and anaerobic cultivation, bur another motile bacteria Arthobacter globiformis was isolated from all the cases in anaerobic cultivation (100%). Its role and importance in footrot infection is unknown since most of the investigators refer only to the secondary role of C. pyogenes (20).

Staphylococcus aureus also plays a role in footrot infection. Langworth (2) found that Staph aureus can produce footrot infection if injected with Spherophorus necrophourus in between the hoof space.

Streptococci were isolated anaerobically (76%) and they seem to play a role in this infection comparable to that played by F. necrophorum (5,18). Isolation results of different genera of entrobacteria which reached 100% in aerobic cultivation and 76% in anaerobic cultivation are comparable to other workers finding with variable rates (2,7). These genera are frequently isolated with B. melaninogenicus from suppurative lesions in sheep (15). The rate of Closyridium sordellii was 64%, which is higher than the rate recorded by Gupta et al., (3) in their study of foot rot in cows (38.7%). However, their role is still vague. The same is applicable to yeasts isolated in the present study as well as other studies (3).

The healing rate of (59%) obtained from using tetracycline in this study conferable to cross's (21) results this rate was increased to 70% - 90% after dipping in formalin which is also comparable to Egerton et al., (22) in which he mentioned that the efficacy of systemic drug was increased when secondary local treatment was resorted to as well. Nearly

equivalent rate of (72.5%) was obtained from using penicillin and streptomycin which seems to be identical with other results (21, 22). We also noticed that using these two antibiotics with formaline dipping increase the healing rate to 80.7% which is the highest healing rate obtained, and comparable to Egerton's (23) results. While using formula alone showed a healing rate of (63.8%) which is comparable to cross's (21) results. The low rate of healing in the control untreated animals (9.3%), enhances the need for systemic and local treatment. Moreover, the healing rate after systemic treatment could be raised significantly when the animals are housed in warm and dry environment, while wet and cold environment minimizes the possibility of reaching their required concentration of antibiotics to the affected feet, therefore, minimizes its efficacy (22). The results of therapeutic treatment of footrot vary in different countries depending on their environmental conditions, and all kinds of treatment give a healing rate of 90-100%, in summer but no more than 75% healing rate in rate in wet seasons (24).

Table (1) : The rate of anaerobic bacteria isolated from ovine footrot lesions.

Type of isolated bacteria	No. of samples	%
Strep. Spp. Str. Zooepideimicus P. haemolytic strept. spp. Peptostreptococcus micros.	19	76
Staph. spp. Staph. aureus	9	36
Cory. Spp. Cory. Pseudotuberculosis	16	64
Bacteroid spp. B. nonsus B. melaninogenicus B. clostridiiformis B. fragilis	15	60
Sherophorus spp. S.necrophorus S. aquitile S.symbiosum S. glutinosum	6	24
Enterobacteracae E.coli, Pseudomonas, Alcaligenes faecalis	19	76
Proteus Clostrid sordelii	16	64
Yeast (Trichosporon cutaenum)	1	6

Table (2) : The rate of aerobic bacteria isolated from ovine footrot lesions.

Type of isolated bacteria	No. of samples	%
Staph. spp.	2	15.3
Staph. epidermidis		
Cory. Spp.	13	100
Arthobacter globiformis		
Enterobacteracea		
E.coli, Pseu. Aeruoginosa	13	100
Citrobacter intermedius		
Proteus vulgaris		
Gram negative bacilli		
Aspergillus fumigatus	1	7.65
Penicillium spp.		
Trichosporon spp.		

Table (3) : The results of types of treatment used to treat ovine footrot.

Group number	Type of treatment	No. of infected sheep	Total No. of treated foot	No. of healing limb	%
1.	Oxytetracycline 10 mg/kg body weight 1/ml, 3 days.	30	61	46	59.06 (P<0.01)
2.	Oxytetracycline 10 mg/kg B.Wt. with soaking affected feet with 5 % formalin.	33	55	39	70.9 (P<.001)
3.	Procaine Penicillin with Streptomycin sulphate at the dose (70000 IU 70 mg/kg respectively). I/M (one injection dose)	25	40	29	72.5 (P<.001)
4.	Procaine Penicillin with Streptomycin and soaking affected feet with 5 % formalin (once)	25	52	42	80.76 (P<.001)
5.	Dipping affected feet with 5 % formalin (once)	30	47	30	63.82 (P<.001)
6.	Control group (without treatment)	20	32	3	9.37

REFERENCES

1. Simon, P.C. and P.L. Stovell (1969). Diseases of animals associated with Spherophours necrophorus (Characteristic of the organism). Vet. Bull. 39 : 311-215.
2. Langworth, B.F. (1977). Fusobacterium necrophorum : Its characteristics and role as on animal pathogen, Bacteriological reviews, 41 : 373-390.
3. Gupta, R.B. ; M.G. Fischer and D.W. Bruner (1964). A study of the etiology of footrot in cattle. Cornell Vet. 54:66-76.

4. Egrton, J.R. and I.M. Parsonson (1966). Isolation of Fusiforms nodosus for cattle. Austs. Ve. J 42 : 425-429.
5. Cottereau P. (1967) Panaritium (Foul - in - the foot) in cattle, Veterinary Medical Review (2/3) : 296 - 305.
6. Richards, R.B. ; L.J. Depizzi ; J.R. Edwards and Wilkinson. (1980). Isolation and Characterization of Bacteroides nodosus from Lesion of cattle in western Australia Aust. Vet. J. 56 : 517 - 521.
7. Soliman, I.A. Shabaan ; A. Elmeligy and Y. Abdl Elgalilk (1984) Management of certain Foot - Problems in sheep and goats. Zagazing Vet. 10 : 295-320.
8. Ivanov, I., (1978). Epidemiology of footrot in sheep, Veterinarnomcditsinski Nauki 14 : 3 - 9. (Vet. Bull 48 Abs. 5274.
9. Yousif A. Y. ; Moaied, I. sawa and B.P. Joshi (1989) infections ovine footrot outbreak in Mosul. Mesopotemia J. of Agric 21 : 105 -112.
10. Marsh, H. and K.D. Claus (1970). The diagnosis of footrot in sheep, the Cornell vet. 1 : 309-317.
11. Buchnan, R.E. and Gibbons, N.E. (1975). Bergey's manual of determinative bacteriology 8th. edit, Williams and Wilkins company, Baltimore.
12. Colton, T.Sc.D. (1974). Statistics in medicine, copyright by little, Brown and company Boston 1st. edit.
13. Al-Rawi, K.M. (1984). Introduction to statistics, Ministry of Higher Education and Scientific Research, University of Mosul. 1st edit.
14. Depiazzi L.J. and Richards R.B. (1979) A degrading proteinase test to distinguish Bening and virulent ovine isolates of Bacteroides nodosus. Aust. Vet. J. 55 : 25-28.
15. Valder, W. A. ; Bechmann, G. ; Reuss, U. (1980). Typing Fusiformis (Bacteroides) nodosus from sheep herds in Hessen. Deutsche Tieraztliche Wochenschrift 87 168-170. (Vet. Bull. 50 : 942 Abst. 7130).

16. Stewart, D.J. ; J.E. Peterson ; J.A. Vaughan ; B.L. Clark ; D.L. Emery ; J.B. Caldwell and AA, Kortt (1986). The pathologenicity and Characteristic of virulent, intermediate and being strain of Bacteriodes nodosus causing ovine footrot. Aust. Vet. J. 63 : 317-326.
17. Biberstein, E.L., Karen England ; A.B. (1968) Bacteroides melaninogenicus in diseases of Domestic animals J.A.V.M.A. 153 : 1045 - 1049.
18. Katitch,RV.(1979)Problems of aetiology and immunoprophylaxiis in footrot of sheep. Comparative immunology, microbiology and infectious Disease (Yugoslavia) 2 : 55-59 (Vet. Bull. 48 (3) Abst. 1407).
19. Bulgin, M.S. (1986). Diagnosis of Lameness in sheep compendium foodanimal F121-128,8 Bonus Continuing Education Article.
20. Barber, D.M.L. (1979). Footrot in sheep. Vet. Rec. (1) : 194 - 195.
21. Cross, R.F. (1978). Influence of environmental factors on Transmission of ovine contagious footrot, J. A. V. M. A., 173 : 1567-1568.
22. Egerton, J.R. ; I.M. Parsonoson ; N.P.H. Graham (1968). Parenteral Chemotherapy of ovine footrot, Aust. Vet. J. 44 : 275 - 282.
23. Egerton, J.R. (1988). Footrot strategic plan, NSW, Farmers NsW Agriculture and Fisheries, Pastures Protection Boards 1-20.
24. Blood,D.C.; O.M. Radostits ; J. A. Henderson (1985). Veterinary Medicine, Sixth edit., Bailliere Tindall, London Philadelphia Toronto : 665-669.

The Iraqi J. Vet. Med. Vol. 21, No. (1), 1997.

دراسة الأحياء المجهرية وعلاجية مرض تعفن الظلف في الأغنام في منطقة الموصل

ساجدة شيال رحيم مؤيد إبراهيم ساوا ، يوسف ابلحد يوسف

كلية الطب البيطري ، جامعة الموصل ، موصل - العراق

الخلاصة

عزلت في هذه الدراسة عدة من الجراثيم والفطريات من أفة مرض تعفن الظلف في الأغنام. في العزل اللاهوائي تم عزل جراثيم من أنواع المغزليات بنسبة ٢٤% والبكترويد بنسبة ٦٠% والوتديات بنسبة ٦٤% والمعويات بنسبة ٧٦% والمكورات السبحية بنسبة ٧٦% والمكزورات العنقودية بنسبة ٣٦% والمطثيات من نوع Cl. sordellii بنسبة ٤٦% واخيرا خميرة الترايكوسبورن (Trichosporon cutaneum) بنسبة ٤%.

اما العزل الهوائي فقد اظهر وجود جراثيم الوتديات والمعويات بنسبة ١٠٠% لكلا الجنسين والمكورات العنقودية الذهنية بنسبة ١٥% ، كذلك عزلت الفطريات من أنواع البثيليوم والرشاشيات الخناء وخميرة Trichosporon cutaneum.

استخدمت خمسة أنواع من العقارات لتقييم كفاءتها في علاج حالات مرض تعفن الظلف في الأغنام وكانت النتائج كالآتي :

الاوكسي تتراسايكلين أعطى نسبة شفاء ٥٩% ،الاوكسي تتراسايكلين مع تغطيس الاقدام المصابة بمحلول الفورمالين ٧٠,٩% ، البروكاين بنسلين والستربتومايسين ٧٢,٥% ، البروكاين بنسلين والستربتومايسين مع تغطيس الاقدام المصابة بالفورمالين ٨٠,٧٦ الفورمالين لوحده ٦٣,٨% ، جميع العقارات المستخدمة اختلفت معنويا مقارنة مع مجموعة حيوانات السيطرة.