

Bacterial Flora of Foot Rot: Report of Findings in 100 Cattle

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INTRODUCTION

Foot rot of cattle is a disease of economic importance occurring endemically in Puerto Rico, especially in the northern part of the Island. The disease is prevalent during rainy periods when the mud macerates the feet of cattle, predisposing them to infection. In Puerto Rico, animals of the Holstein breed, extensively used for milk production, are especially susceptible to this condition.

Foot rot is an inflammation of the tissues of the feet which may lead to pus tracts and death of foot tissue, producing lameness, which may become permanent if not attended promptly. When thus affected, the animal loses appetite, and milk production and breeding efficiency are greatly lowered.

REVIEW OF THE LITERATURE

In spite of the economic importance of this disease a bacteriological study of foot rot lesions is lacking. Numerous references in the literature imply that *Spherophorus necrophorus* is the causative agent (1,2,3,4,5,6) ² but definite proof of this is lacking. The evidence lies mostly in the study of direct smears from scrapings of foot rot lesions (7). This is not conclusive since only a small percentage of the smears examined (7) are reported as showing organisms with the morphology of *S. necrophorus* (*Actinomyces necrophorus*).

Since the etiology of the disease is rather obscure, treatment has been undertaken on an empirical basis. There are various reports on the use of sulfa drugs (8,9,10,11,12,13,14,15,16) some being favorable, while other workers report that local treatment is as good as any (17). Emphasis has also been placed on preventive measures (1,18). However, since preventive methods are not always feasible or practical, a study of the bacteria found in foot rot lesions should give a more complete picture of the organisms involved, and therefore indicate the drugs or antibiotics likely to be most useful in treating this condition.

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² Numbers in parentheses refer to Literature Cited, p. 124.

MATERIALS AND METHODS

SPECIMENS

Specimens were taken from diseased cattle at 10 cattle farms located in Hato Rey, Bayamón, Dorado, Carolina, and Canóvanas. The procedure was as follows: The diseased foot was immobilized and washed thoroughly with tapwater to remove as much dirt as possible. The interdigital space was further cleaned with cotton and 70-percent alcohol. A sterile cotton swab was then introduced into the wound or, when the lesion was small, it was just rubbed over the lesion site, a smear was made on a sterile slide, and the specimen was then immersed in a tube of thioglycollate broth enriched with 10-percent beef serum. The smear was stained by Gram's method and examined under the microscope.

Several types of lesions were encountered. The great majority involved swelling of the tissues between the claws, associated with an open wound or scratch.

CULTURE

The thioglycollate-broth culture was incubated at 37°C. and as soon as growth was observed, smears were made and stained by Gram's method. If no growth was observed the inoculated broth culture was further incubated for 7 days, after which it was discarded if still sterile.

The thioglycollate-broth culture was streaked on duplicate plates of rabbit-blood agar. One plate was incubated aerobically while the other was placed under anaerobic conditions in an atmosphere of nitrogen. To insure growth of all anaerobic organisms an additional plate of Brewer anaerobic blood agar was also streaked and incubated. After a 24- to 48-hour incubation, colonies from the various plates were examined and described.

Isolated colonies were picked to tubes of heart-infusion broth, or trypticase-soy broth in the case of aerobic organisms, and to the thioglycollate medium when the colonies grew under anaerobic conditions. Purity plates were performed on rabbit-blood agar or Brewer anaerobic-blood agar, and when a pure culture was obtained, fermentation and other tests were carried out for classification. Such organisms as *Bacillus alkaligenes*, *Proteus vulgaris*, and *B. coli* were encountered irregularly as contaminants and discarded.

PATHOGENICITY TESTS

Pathogenicity tests were carried out on several cultures of pathogenic or potentially pathogenic species of organisms. White mice were injected with 0.1 to 1 cc. of culture by the intraperitoneal or subcutaneous route. A few tests were also carried out on guinea pigs.

SENSITIVITY TESTS

Sensitivity tests were carried out on a number of representative cultures of the more important organisms isolated. An 18- to 24-hour broth culture of the organism was evenly spread on the surface of a blood-agar plate and Bacto sensitivity disks containing aureomycin, bacitracin, chloromycetin, penicillin, dihydro-streptomycin, polymyxin B, terramycin, erythromycin, and magnamycin were placed on the surface with sterile precautions about 4 cm. apart. The plates were incubated aerobically or anaerobically, depending on the organism tested, until growth developed. They were then examined for zones of inhibition of growth.

TABLE 1.—*Bacteria isolated from 108 lesions of foot rot in 58 recent and 50 old cases*

Organism	Recent cases (58)	Old cases (50)	Percentage of total number of specimens (108)
<i>S. necrophorus</i>	7	0	6.4
<i>C. pyogenes</i>	7	4	10
<i>Clostridium</i> spp. ¹	18	11	26.8
Streptococci	33	26	54
Micrococci	9	10	17
<i>B. cereus</i>	33	23	52
<i>Bacteroides melaninogenicus</i>	8	3	10
<i>Ps. aeruginosa</i>	3	0	3

¹ *Cl. sporogenes* and *Cl. bifermentans*, mostly.

RESULTS

A total of 108 specimens was obtained from 100 head of cattle on 10 cattle farms located at Hato Rey, Bayamón, Dorado, Carolina, and Canóvanas. Fifty-eight of the specimens were from recent cases 1 day to 2 weeks in duration, while 50 specimens were taken from cases of more than 2 weeks duration. The results of the cultural studies are shown in table 1. Cases of more than 2 weeks duration are recorded in the table as "old" cases. In this table the number of positive cultures for the species listed is shown in the first two columns under "recent" and "old" cases. The right-hand column gives the percentage of positive cultures for the total number of specimens taken.

It will be seen that *S. necrophorus*, the organism commonly suspected of being the cause of cattle foot rot, was found in only 6.4 percent of the total number of specimens taken. *B. cereus* and Streptococci appeared most frequently of all species in the table. *B. cereus*, however is very abundant in soil and almost all the Streptococci found were nonhemolytic. A mixed bacterial flora was cultured from all cases.

An attempt was made to correlate the presence of Gram-negative bacilli showing the characteristic morphology of *S. necrophorus* in direct smears from foot rot lesions with the number of cultures positive for *S. necrophorus*. It was observed in 11 specimens showing suspicious bacilli in the direct smears that 5 gave a culture positive for *S. necrophorus*. On the other hand, in 2 specimens from which a positive culture was obtained, the smear from the lesion did not disclose any organisms morphologically resembling *S. necrophorus*.

Pathogenicity tests were carried out on a number of strains of *S. necrophorus*, *C. pyogenes*, *Ps. aeruginosa*, and *B. cereus*. The results of these tests are shown in table 2. Although *B. cereus* is one of the more frequent

TABLE 2.—Pathogenicity of 4 bacterial species isolated from cases of foot rot

Species tested ¹	Number of strains tested	Number found pathogenic
<i>S. necrophorus</i>	4	1
<i>C. pyogenes</i>	4	4
<i>Ps. aeruginosa</i>	2	2
<i>B. cereus</i> ²	7	1

¹ Pathogenicity of *S. necrophorus*, *C. pyogenes*, and *B. cereus* was tested on white mice; *Ps. aeruginosa* was tested on guinea pigs.

² Pathogenicity tests were carried out on several strains of *B. cereus* since this species occasionally has been reported as pathogenic (19).

inhabitants of soil and dirt and is commonly regarded as a contaminant, seven strains were tested for pathogenicity since there have been reports (19) of pathogenic effects produced by this organism. However, only one of the strains proved actually pathogenic. In each case the pathology was characteristic for the species tested. All strains of *C. pyogenes* also produced a hemolysin active on rabbit red cells.

A limited number of sensitivity tests were carried out by the paper-disk method using nine different antibiotics and several strains of *C. pyogenes*, *Cl. bifementans*, *Cl. sporogenes*, *S. necrophorus*, *Ps. aeruginosa*, and one pathogenic *B. cereus* strain.

The majority of strains of *C. pyogenes* tested were completely susceptible *in vitro* to aureomycin, bacitracin, terramycin, erythromycin, and magnamycin. All strains of the Clostridia (*Cl. bifementans* or *sporogenes*) were susceptible to aureomycin, chloromycetin, and terramycin. Most strains of *S. necrophorus* were susceptible to aureomycin and chloromycetin. Results with other antibiotics were not as definite. As expected, the wide-spectrum antibiotics were most effective against the several species of bacteria tested.

DISCUSSION

A study of the bacterial flora obtained from cases of cattle foot rot has been lacking to the present. The study here reported was undertaken with the purpose of ascertaining whether a single bacterial species was involved in at least the majority of cases, or whether predominance of a few species occurred in most of the cases studied. A mixed bacterial flora was found in all lesions cultured, whether recent—1 day to 2 weeks' old—or old. Thus no particular species or group of organisms predominated in any case.

The cultivation of material from the diseased feet of cattle is attended by the presence of numerous contaminants, since the feet are continuously exposed to feces, urine, soil, and dirt. Organisms present in these sources have easy access to the interdigital space and to any abrasions or bruises in the feet. In spite of precautions taken in having the feet as clean as possible, and in taking the specimens only from the lesion site, many of the organisms appearing were common contaminants and were discarded as such. Attention was therefore directed to the isolation and cultivation of organisms belonging to genera which most probably would contain the offender or offenders: Spherophorus, Clostridium, Bacteroides, Streptococcus, Staphylococcus, Corynebacterium, and Pseudomonas. Many organisms belonging to these genera cause a variety of diseases in cattle and may be found in materials such as feces, dirt, manure, and urine which come in direct contact with wounds or abrasions on the feet of cattle.

Perhaps the most interesting finding was that *S. necrophorus* was actually isolated from the diseased feet of cattle but only from fresh lesions. It was found in only 6.4 percent of all specimens. It is possible that this organism may have been present in a larger number of specimens and may have been missed in some because its cultivation is difficult, the more so because of overgrowth on the part of the numerous contaminants encountered.

Another interesting finding was the isolation of *C. pyogenes* from 10 percent of all specimens from both recent and old lesions. To our knowledge this is the first time *C. pyogenes* has been connected with foot rot in any way, and it may well play a role in at least some cases.

As mentioned before, *S. necrophorus* has been mainly suspected to be the most common offender in cattle foot rot. The finding of long Gram-negative filaments and pleomorphic bacilli, or short Gram-negative bacilli showing bipolar staining in smears, has been regarded by some as sufficient evidence for incriminating *S. necrophorus*. Actually quite a few species of Gram-negative bacilli, such as *Proteus vulgaris* and *B. coli* occasionally show these long filamentous forms in direct smears from lesions (as stated in a personal observation by J. E. Pérez). Therefore the only way of actu-

ally proving the causative agent to be *S. necrophorus* would be by its occurrence in pure culture, or, at least as a predominant species in mixed culture. In our study the suspicious Gram-negative filaments were found in 11 specimens, but only 5 of these actually yielded *S. necrophorus* and then only in mixed culture.

Bacteroides melaninogenicus was found in 11 percent of all lesions. Dack (20) mentioned this organism as one of the species associated with ulcerative processes in human beings.

The limited number of sensitivity tests carried out *in vitro* and the results from the cultural studies suggest that wide-spectrum antibiotics should be employed in the management of foot rot, to insure proper coverage of most of the organisms involved.

From the results obtained it is evident that a low incidence of pathogens was encountered. It may be that organisms usually regarded as nonpathogens *per se* may exert a pathogenic effect when found associated in certain combinations, but this is a matter of speculation which would have yet to be proven in the particular condition of cattle foot rot.

SUMMARY

One hundred and eight specimens were obtained for bacteriological study from 100 head of cattle affected with foot rot on 10 cattle farms located in Hato Rey, Bayamón, Dorado, Carolina, and Canóvanas. Smears were made on sterile slides and stained by Gram's method. Aerobic and anaerobic cultures were also made with the specimens.

Gram-negative bacilli morphologically resembling *S. necrophorus* were encountered in about 10 percent of the smears, yet the organism itself was cultured from only half of the specimens having positive smears. *Bacillus cereus*, Streptococci, and Clostridia were found in many specimens. *Corynebacterium pyogenes* and *Bacteroides melaninogenicus* each had an incidence of 10 percent of the total number of specimens. *Pseudomonas aeruginosa* was cultured from only 3 percent of all specimens.

It was concluded from limited *in vitro* tests that wide-spectrum antibiotics were most active against these organisms. However, *Ps. aeruginosa* was resistant to all the antibiotics tested.

RESUMEN

Se tomaron 108 muestras de ganado afectado por la podredumbre de la pezuña (mazamorra) para estudios bacteriológicos. Se hicieron frotis, los cuales se tiñeron al Gram. Con las muestras tomadas se hicieron también cultivos para aerobios y anaerobios. Bacilos Gram negativos, morfológicamente similares al *Spherophorus necrophorus*, fueron notados como en un 10 por ciento de los frotis. El *Spherophorus necrophorus*, sin embargo, pudo cultivarse solamente de la mitad de las muestras que dieron frotis positivos.

En muchas muestras se encontraron organismos de los géneros *Streptococcus*, *Clostridium* y el *Bacillus cereus*. Tanto el *Corynebacterium pyogenes* como el *Bacteroides melaninogenicus* tuvieron individualmente la incidencia de un 10 por ciento del número total de muestras. El *Pseudomonas aeruginosa* se encontró sólo en un 3 por ciento de las muestras. Un número limitado de pruebas de sensibilidad mostró que la mayoría de estos organismos fueron susceptibles a los antibióticos de gran radio de acción, como la terramicina, cloromicetina y aureomicina. El *Ps. aeruginosa* fué resistente a todos los antibióticos probados.

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