Foot-Rot in Sheep: A Transmissible Disease due to Infection with *Fusiformis nodosus* (n. sp.)

Studies on Its Cause, Epidemiology, and Control

By

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<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Summary</strong></td>
<td>6</td>
</tr>
<tr>
<td>I INTRODUCTION:</td>
<td></td>
</tr>
<tr>
<td>1. Definition: Synonyms: History.</td>
<td>7</td>
</tr>
<tr>
<td>2. Economic Importance</td>
<td>8</td>
</tr>
<tr>
<td>II CLINICAL STUDIES:</td>
<td></td>
</tr>
<tr>
<td>1. Clinical Description</td>
<td>9</td>
</tr>
<tr>
<td>2. Histopathology</td>
<td>10</td>
</tr>
<tr>
<td>3. Differential Diagnosis</td>
<td>10</td>
</tr>
<tr>
<td>III EPIDEMIOLOGICAL STUDIES:</td>
<td></td>
</tr>
<tr>
<td>1. Geographic Distribution</td>
<td>12</td>
</tr>
<tr>
<td>2. Incidence</td>
<td></td>
</tr>
<tr>
<td>(a) Yearly and seasonal variations</td>
<td>15</td>
</tr>
<tr>
<td>(b) Effect of type of country and pasture</td>
<td>15</td>
</tr>
<tr>
<td>(c) Age and sex</td>
<td>16</td>
</tr>
<tr>
<td>(d) Breed and individual susceptibility</td>
<td>16</td>
</tr>
<tr>
<td>(e) Susceptibility of animals other than sheep</td>
<td>16</td>
</tr>
<tr>
<td>5. Predisposing Factors</td>
<td></td>
</tr>
<tr>
<td>(a) Introduction</td>
<td>15</td>
</tr>
<tr>
<td>(b) Strongyloides larvae</td>
<td>15</td>
</tr>
<tr>
<td>(c) Scalp</td>
<td>16</td>
</tr>
<tr>
<td>(d) Other predisposing factors</td>
<td>17</td>
</tr>
<tr>
<td>4. Contagious and Specific Nature of Foot-rot</td>
<td>17</td>
</tr>
<tr>
<td>5. Survival of the Contagion in Nature</td>
<td>18</td>
</tr>
<tr>
<td>IV BACTERIOLOGICAL STUDIES:</td>
<td></td>
</tr>
<tr>
<td>1. Introduction</td>
<td>20</td>
</tr>
<tr>
<td>2. General</td>
<td></td>
</tr>
<tr>
<td>(a) Miscellaneous bacteria</td>
<td>21</td>
</tr>
<tr>
<td>(b) Tests for filterable virus</td>
<td>22</td>
</tr>
<tr>
<td>(c) Spirochetes melophila</td>
<td>22</td>
</tr>
<tr>
<td>(d) The motile Fusiform</td>
<td>23</td>
</tr>
<tr>
<td>(e) Fusiformis nodosus</td>
<td>23</td>
</tr>
<tr>
<td>(f) Bacteria in histological sections of lesions</td>
<td>24</td>
</tr>
<tr>
<td>(g) Summary of bacterial flora</td>
<td>24</td>
</tr>
<tr>
<td>3. Study of Specific Micro-Organisms</td>
<td></td>
</tr>
<tr>
<td>(a) Description of Fusiformis nodosus (n.sp.)</td>
<td></td>
</tr>
<tr>
<td>(i) Isolation</td>
<td>24</td>
</tr>
<tr>
<td>(ii) Morphology</td>
<td>25</td>
</tr>
<tr>
<td>(iii) Cultural requirements</td>
<td>25</td>
</tr>
<tr>
<td>(iv) Surface cultures on solid media</td>
<td>26</td>
</tr>
<tr>
<td>(v) Cultures in deep agar</td>
<td>27</td>
</tr>
<tr>
<td>(vi) Cultures in fluid media</td>
<td>27</td>
</tr>
<tr>
<td>(vii) Viability and resistance</td>
<td>28</td>
</tr>
</tbody>
</table>
(viii) Biochemical properties 26
(ix) Animal inoculation 28
(x) Agglutination tests 29
(xi) Classification 30

(b) Description of Spirochaeta penworthi
   (i) Isolation 30
   (ii) Morphology 30
   (iii) Cultural characters 31
   (iv) Viability and resistance 22
   (v) Animal inoculation 32
   (vi) Agglutination tests 32

(c) Description of the motile fusiform of foot-rot
   (i) Isolation 33
   (ii) Morphology 33
   (iii) Cultural characters 33
   (iv) Biochemical properties 34
   (v) Animal inoculation 34
   (vi) Agglutination tests 34
   (vii) Classification 35

4. Experimental Reproduction of Foot-rot with pure Cultures
   (a) Experimental procedure 35
   (b) The experiments 36
   (c) Bacterial flora of experimental cases 39
   (d) Analysis of results 40
   (e) Summary 41

5. The Aetiological Significance of various Bacteria in Foot-rot
   (a) Fusiformis necator
   (b) Spirochaeta penworthi
      (i) Experimental cases in which Sp. penworthi
          was not present 42
      (ii) Occurrence of Sp. penworthi in nature 43
      (iii) Summary 45
   (c) The motile fusiform 44
   (d) Miscellaneous bacteria 45
   (e) Conclusions 46

V STUDIES ON CONTROL OF THE DISEASE:
1. Treatment 46
2. Immunisation 46
3. Control by Eradication
   (a) Introduction 48
   (b) Scheme for eradication 49

VI ACKNOWLEDGMENTS 51

VII REFERENCES 52

APPENDIX 54

"Field Trials on Eradication of Foot-rot,"
by W. J. B. Beveridge and T. S. Gregory.
SUMMARY

Foot-rot of sheep occurs over large areas in that portion of southern Australia enjoying a moderately good rainfall; but it is absent from most of the tablelands, although prevalent on the New England tablelands 40 years ago.

The principal lesion is a spreading destruction of the superficial parts of the underlying epithelium leading to detachment of the horn. It is a specific, contagious disease, but its spread in the field is dependent on certain predisposing factors usually associated with lush pasture. Percutaneous infection with larvae of Strongylodes papillosum may possibly be a predisposing factor.

Previous investigators have considered the disease to be caused by Fusiformis necrophorus or Treponema podocyte, but neither of these views could be confirmed in the present investigation.

In lesions at the margin of the healthy tissue, two organisms usually predominate over the miscellaneous bacteria present. One is Spirochaeta penortha and the other is a motile fusiform. There is also present, but in much fewer numbers, a newly discovered bacterium for which the name Fusiformis nodosus (n.sp.) is suggested.

F. nodosus is a large, gram-negative, non-motile, rod-shaped bacterium which usually has enlargements at both ends. It is a strict anaerobe and good growth on most artificial media has been obtained only in the presence of 10 per cent horse serum, but not every batch of horse serum is suitable. Sheep serum has an inhibitory effect. Surface colonies on horse serum agar have the peculiar property of etching themselves into the surface of the medium.

As reported previously, Sp. penortha differs from other spirochetes in that undulations cannot be detected by ordinary staining and microscopic methods. However, it has been found that undulations can usually be made to appear by heating the smear in strong carbol fuchsin.

The motile fusiform closely resembles F. necrophorus morphologically except that it is motile.

The scarified sheep's foot inoculated with cultures of Sp. penortha and the motile fusiform develops no lesions; cultures of F. nodosus produce foot-rot lesions which are usually less severe than in the natural disease. F. nodosus and Sp. penortha used together for the inoculum produce the typical disease. The addition of the motile fusiform to the inoculum does not influence the result obtained with F. nodosus or with F. nodosus and Sp. penortha together. Sp. penortha does not occur commonly, if at all, apart from foot-rot but the motile fusiform does.

F. nodosus is the primary causal agent and it is very probable that Sp. penortha is a specific, accessory, causal agent. The motile fusiform is a constant secondary
invader and possibly plays some part in the pathogenesis of the disease. The continued survival of these non-sporing anaerobes is probably aided by association with the non-specific, aerobic bacteria which are present.

These observations, made originally by the writer in Australia, were confirmed by him in U.S.A. Strains of F. nodosum from these two countries were shown to be related serologically.

No evidence could be found to indicate that the causal agent of foot-rot occurs naturally elsewhere than on the feet of sheep showing some lesions. In this situation it may persist for years, but apart from lesions it has been found incapable of surviving for more than a few days except in artificial culture. A scheme for the control of the disease has been elaborated whereby it can be eradicated by removing all animals carrying the infection during the dry period of the year. These animals may show three different types of lesion, all of which can be detected by close visual examination aided, if necessary, by paring of the horn. Following these principles foot-rot has been eradicated from several large sheep stations which subsequently remained free of the disease when an epizootic occurred in the neighbourhood.
Foot-Rot in Sheep: A Transmissible Disease due to Infection with *Fusiformis nodosus* (n. sp.)

Studies on Its Cause, Epidemiology, and Control

**I. INTRODUCTION.**

1. **Definition: Synonyms: History.**

The disease with which this investigation deals may be defined as a contagious disease of the sheep's foot characterized by separation of a large portion of the hoof from the soft tissues due to a spreading infection immediately beneath the horn and caused primarily by *Fusiformis nodosus*.

The term foot-rot has been applied to this disease for many years, but it is sometimes called contagious foot-rot to distinguish it from certain other foot affections referred to loosely as non-contagious foot-rot. It would be less confusing if the term foot-rot, when used in connection with sheep, were reserved exclusively as the name for the specific disease just defined. Such terms as contagious foot-rot, non-contagious foot-rot, simple foot-rot, and malignant foot-rot have not infrequently been mis-applied and consequently have misled people. It would be wise, therefore, to discontinue their use.

Foreign synonyms are: *Panaritis* (L); *piéte* (French); *Moderbinde* (German); *zoppina* (Italian); *kontartet Kløvesyge* (Danish).

The history of foot-rot of sheep in Europe and the United States of America was earlier reviewed by Yonatt (1857) and later by Mohler and Washburn (1931). The disease was well-established in England during the 18th century; and during the early part of the 19th century it was reported in France, Germany, Italy, and the United States of America, serious outbreaks occurring in U.S.A. in the middle of the century. It was first recognized as a contagious disease by Godwin in France about 1810 and since then its contagious nature has been repeatedly demonstrated, although some have continued to regard it as non-contagious even up to the present day.

Foot-rot was probably introduced into Australia with some of the first importations of sheep. Robertson (1932), in his review of animal diseases in Australia, gives two early references to foot-rot. In a report dated 1802 it was stated that many sheep in New South Wales used to die of foot-
rot when they were not well cared for. Again in 1830 foot-rot was reported to be common in sheep throughout the colony. From the writings of Graham (1870), one infers that at the time the disease was widespread. Graham considered that the disease was not so prevalent after the introduction of fencing and the abandoning of shepherding and folding. He also stated that the Darling Downs in Queensland was free of the disease during the 15 to 17 years after occupation, but that it was prevalent there at the time of writing (1870). In 1896 Sutherland stated that foot-rot had disappeared from Queensland previous to that date but had been again introduced. In 1890 and for several years later the disease was prevalent on the New England tablelands in New South Wales, but that area has now been free of it for 50 to 60 years. Similar changes have occurred on the southern tablelands of New South Wales, except that there have been one or two introduced outbreaks during the last thirty years.

Judging by the literature, serious outbreaks of foot-rot do not seem to have been so common during recent years in Australia and abroad as they were during the last century. It is noteworthy that the disease has disappeared from at least two large areas in Australia without eradication measures having been adopted.

2. Economic Importance.

It is impossible to make even an approximate estimate of the economic losses occasioned by foot-rot in Australia. They vary from almost nil in very dry seasons to a large but indeterminate figure in very wet seasons.

During exceptionally wet seasons serious mortalities have occurred on some properties, mainly among lambs which the affected ewes were unable to suckle, but usually foot-rot does not cause many deaths unless the flock is neglected. The main economic loss is sustained through (a) loss of body condition, which is often severe and accompanied by depreciation in both the quantity and quality of the fleece ("break" may occur), and (b) cost of the laborious treatment of affected sheep. Losses from these sources are very considerable during severe outbreaks, when 50 per cent., or more of the animals in the flock may become affected. The disease occurs in some of our best sheep raising country. On some properties in the affected areas it has been necessary to abandon Merinos for a more resistant breed of sheep and even to give up sheep altogether for cattle.

Foot-rot is much more prevalent on improved pastures than on natural pastures; therefore as pasture improvement becomes more general in Australia, foot-rot will become a much more serious economic problem if it is not properly controlled.

In England foot-rot is regarded as one of the most serious diseases of sheep (Anonymous, 1938).
II. CLINICAL STUDIES.

1. Clinical Description.

The initial lesion is usually a mild inflammation of the skin in the interdigital space. This is soon followed by a break in the skin-horn junction on the axial aspect of one or both digits and then a separation of the adjacent soft horn from the underlying epithelium. Within the next few days this separation extends to the edge of the sole or around the back of the heel. At this stage usually the lesions are still rather mild and there is little or no pus visible macroscopically, but the sheep shows lameness and the foot feels warmer than normal. During the next 5 to 10 days the infection spreads, causing separation of the horn across the whole sole, and the sheep becomes decidedly lame. There is now a little pus with necrotic detritus present, but this is rarely copious. The infection may then extend under the walls so that nearly all the hoof is separated from the soft tissues and only attached to the foot near the corneum. Both digits on the one foot are almost invariably involved.

In the advanced stages the skin between the digits shows a diffuse superficial ulceration. After the infection has spread across the sole there may be a new growth of horn over the sole, but this usually again becomes involved by the destructive process extending from the skin in the interdigital space, where the infection tends to persist for long periods. On feet which have been affected for several weeks or months the hoof becomes long and mishapen. The non-horny portion of the infected sole in advanced cases is covered by soft, necrotic, epithelial tissue, and usually there are several ulcer-like areas with more or less sharply defined edges (see Fig. 4). Where the wall is involved the laminae become partly necrotic and there may be some excessive granulations. The severity of the lesions varies considerably in different animals and in the same foot during the course of the disease.

In advanced cases there is extreme lameness, and if only one foot is affected it is usually "carried". Two or more feet are frequently affected in the one animal, and in this case the animal lies down most of the time, moves with great difficulty, and becomes thin from inability to graze properly. The wool frequently develops "breaks". When the two fore feet are affected the animal adopts a characteristic attitude of kneeling while feeding.

The disease is of long duration. One case at the McMaster Animal Health Laboratory persisted over a period of 3½ years. Some cases heal spontaneously, but usually not until after several months' duration.

On rare occasions the deeper tissues of the foot become infected. Ligaments and tendons become necrotic, and the distal interphalangeal joint becomes infected, with sinuses opening either in the interdigital space or just above the corneum. These may be regarded as complications of foot-rot and are probably not due to its specific causal agent but are the same as in the condition known as digital suppuration.

Such lesions developed in three out of 121 feet which were inoculated by scarification with F. necrophorum without developing foot-rot. These complications probably occur more frequently in crossbreds and in rams than in Merino ewes. According to Hasenkamp (1909) and Murnane (1933) a few sheep affected with foot-rot develop as complications abscesses of the liver, lungs, or spleen.
2. Histopathology.

At the advancing edge of a lesion, inflammatory processes are evident in the more superficial layers of epithelium just beneath the keratinized layers, that is, about or just external to the level of the extremities of the rete pegs. There is usually a considerable degree of infiltration with polymorphonuclear leucocytes and some pus formation and cellular degeneration. There may be haemorrhages. Often there is a progressive accumulation of exudate in the intercellular spaces, producing cavities between the cells and leading to pressure atrophy of the surrounding cells (see Figs. 2 and 3). In some cases the inflammatory processes are mild but sufficient to cause detachment of the keratinized horn from the unkeratinized epithelium.

About the base of the rete mucosum the epidermis is usually normal. The cutie vera usually shows a moderate degree of reactive inflammation with plasma cells and sometimes round cells and some polymorphonuclear leucocytes in evidence, and there may be severe inflammation at the extremities of the long rete pegs. Occasionally small abscesses are seen in the cutis vera.

Since the active lesions of foot-rot are mainly situated in the layers of epithelium just beneath the zone where keratinization takes place, the disease may be regarded as a superficial ulceration. That the rete mucosum is not ordinarily destroyed is also evident clinically, for, after surgical treatment, a thin layer of new horn covers the previously diseased surfaces, often within 24 hours.

The location and type of bacteria found in sections are described later in Section 2 of the Bacteriological Studies.

3. Differential Diagnosis.

Foot-rot can usually be diagnosed on clinical examination alone when there are large numbers of the flock affected, but this may not be so easy when only a few cases occur. A definite diagnosis may be made by the demonstration of F. nocardiae and Sp. penortha in smears, or by transmission experiments. In foot-rot, smears of material carefully collected from an active lesion at the margin of the healthy tissue usually reveal some F. nocardiae and many Sp. penortha, both of which are recognizable by their distinctive morphology. The presence of Sp. penortha only does not justify a positive diagnosis but may give rise to suspicion. Failure to find F. nocardiae in only one or two smears does not justify a definite negative diagnosis. However, if neither F. nocardiae nor Sp. penortha can be found in several carefully prepared smears a negative diagnosis is justified. Where a definite conclusion cannot be drawn from the examination of smears, a transmission experiment should be done. For a positive diagnosis the disease produced in the inoculated sheep should show typical lesions, which should not heal in less than a month and contain F. nocardiae. Material sent to the laboratory from the field for diagnosis should consist of several smears and some material from the lesions. The latter must reach the laboratory within 24 hours.

In the following paragraphs are mentioned the various affections of the sheep's foot with which foot-rot might be confused.
Other "Types of Foot-Rot". — Many writers (Stanley 1891, Sinclair and Archer 1896, Sutherland 1896, Mohler and Washburn 1909, Hunsaker 1909, Oppermann 1921, Belechner 1931 and 1939, Wurman 1933, Stiles 1936) describe two or more "types of foot-rot". The descriptions and classifications of these "types" vary with the author and thus some confusion has resulted. In addition to the true disease most of them describe a "non-contagious type" consisting of a non-specific, purulent infection of the foot following injury caused by stubble, thorns, sharp stones, dried mud between the toes, long continued exposure to wet mud, or occlusion of the interdigital duct. Some of these writers also describe a "malignant type" in which the ligaments and tendons of the pastern and fetlock joints are infected, causing swelling above the hoof, which is itself not affected.

Now that foot-rot has been clearly defined and can be differentiated by demonstration of the causal organism from other foot affections, it is possible to define these allied conditions more accurately. Gregory (1939a) describes adequately for the first time the condition which he terms digital suppuration or "foot abscess" and which has previously been referred to as "non-contagious foot-rot" by some and as "malignant foot-rot" by others.

Digital Suppuration. — Usually the first sign of the infection is an acute lameness. Examination may show no obvious lesion, but one hoof may be found warmer than normal and mantles may be young. Careful peeling may reveal imprisoned pus. In slightly older cases the pus may have undermined quite a large part of the horn and finally produced an abscess at the coronet or between the digits. In other cases the infection commences on the skin between the heels where a definite inflammation, sometimes with formation of granulation tissue, occurs.

In many cases of digital suppuration the infection invades the joints, ligaments, and tendons of the foot, causing extensive damage. Abscesses form, and when these discharge tissues remain for over two months.

In digital suppuration there is usually much pus, which is in contrast to foot-rot. Other differential features are (a) that it is largely confined to adult sheep whereas foot-rot attacks young animals readily, and (b) that it is usually confined to one foot on an animal and usually to one digit only. Foot-rot almost invariably affects both digits on the one foot and often more than one foot.

In one outbreak of digital suppuration seen by the present writer, the lesions commenced as ulcers in the interdigital space. These gradually penetrated into the distal interphalangeal joints. The infection then gave rise to abscesses around the coronet, sinuses, and extensive necrosis of ligaments and tendons, as described by Gregory. All four feet on the one sheep were sometimes affected.

Suppurative cellulitis. — This condition is described by Mohler and Washburn (1909) and Gregory (1939a). It commences as patches of dermatitis, usually on the back of the pastern. It extends rapidly and the skin as high as the "knee" or hock may be involved. If untreated, suppurative cellulitis ensues.

Contagious ecthyma. — Vesico-pustular lesions may occur around the coronet as well as around the mouth.
Foot and Mouth Disease. - In the past there was some confusion between foot-rot and foot and mouth disease in Europe.

Furulent wounds of the feet sometimes resemble foot-rot clinically.

"Seals". - This condition is described under the Section on predisposing Factors. Some writers include it under the term "non-contagious foot-rot".

Laminitis may occur in sheep causing severe lameness (Stiles, 1934).

Thaler (1911) described a foot disease in which ulcers occurred usually around the coronet and sometimes extended under the horn. The condition was apparently contagious but clinically different from foot-rot.

III EPIDEMIOLOGICAL STUDIES.

1. Geographic Distribution.

At the date of the latest references, foot-rot of sheep was prevalent in parts of France (Mourea 1923), Germany (Oppermann 1921), Italy (Arru 1934), Denmark (Hansen 1936), England (Anonymous 1938), Hungary (Scharnbeck 1934), U.S.A., Australia and New Zealand. Oral reports have come to hand that it occurs in China. No information is available regarding its occurrence in South Africa or Argentina. In U.S.A. it occurs to some extent in most sheep raising states.

In Australia foot-rot is enzootic over large areas in central and southern New South Wales, Victoria, Tasmania, and the higher rainfall areas of South Australia and Western Australia. It does not occur in Queensland. The approximate distribution of the disease in Australia is shown in a map (see Fig. 1a), which was drawn from data obtained through the courtesy of the Chief Veterinary Surgeons of the various State Departments of Agriculture. This map is merely an approximation and there are undoubtedly many properties in the shaded areas free of the disease. In some areas where the disease is endemic its distribution tends to follow river or creek valleys, probably owing to the richer pastures there. The extension of the disease inland is probably limited by low rainfall. Its absence from most of the northern portion of the State might be associated with the fact that a higher proportion of the rainfall occurs there during the summer and this country carries a less dense pasture.

It is noteworthy that the tablelands, except for one small area in southern New South Wales are free of the disease. The rainfall in most of the tablelands is higher than in more inland areas where the disease is endemic. Foot-rot was very prevalent in the New England and Southern Tablelands in the last decade of the last century and the reason for its absence from there now makes interesting conjecture. Several reports have been received that foot-rot soon disappears if introduced into some of the tableland districts. One pastoralist informed the writer that it
disappeared from New England in 1928 when there was a severe drought and most of the sheep in that area died. However, this does not explain why it has not become re-established, for it has almost certainly been introduced a number of times since then. The clearing of the trees and the effect of grazing animals has probably altered the type of pasture and the soil texture, and the change may have made conditions not so favourable to the spread of the disease. Information was obtained on the pH of the soil in different districts from the Soil Chemist of the New South Wales Department of Agriculture, who informed the writer that the soils of the tablelands of New South Wales are acid in reaction and, broadly speaking, the reaction becomes neutral or alkaline as one proceeds further west. It seemed that this might help to explain the absence of foot-rot from the tablelands, but when pH determinations were done on soils from some properties in southern New South Wales and western Victoria, where foot-rot is endemic, many of these showed an acid reaction (pH 4.5 - 5.2). Whatever the explanation may be, and it is possibly a combination of the factors mentioned above, together with improved methods of husbandry and treatment, certain tableland districts where foot-rot was prevalent 40 years ago are now apparently not favourable for the spread of the disease. In France foot-rot is endemic in the mountain areas and plateaus (Moussu, 1923).

2. Incidence.

Data on the incidence of foot-rot have been obtained by personal observations and from information given by pastoralists during visits to 15 properties in Australia where the disease occurs. These data are in general agreement with the observations of other writers both in Australia and abroad.

(a) Yearly and seasonal variations.

The incidence of foot-rot is largely determined by the condition of the pasture. On irrigated pastures foot-rot occurs at all times, but under all other conditions in Australia the incidence of the disease varies greatly from year to year and at different times of the year. During dry years in most districts very few cases occur, and during a succession of dry years the disease may not be observed for several years on many properties. On the other hand in very wet years as many as 75 per cent. or even more of the sheep may be affected in the endemic areas, and the disease extends to districts where it is not usually observed.

Outbreaks of foot-rot usually occur when there is lush pasture, dampness, and fairly warm weather. Thus an outbreak may occur in the late autumn when there have been good early autumn rains, but the greatest incidence is usually in the spring. During the winter the disease does not usually spread to any great extent, although a few pastoralists report having had outbreaks during frosty weather. During the summer the pasture ordinarily dries off and becomes brown in the southern portion of Australia and the disease does not usually spread, although sheep already infected may remain so. However, the writer saw an outbreak in late spring when the pasture was drying off and seeding.

(b) Effect of type of country and pasture.

Foot-rot is, broadly speaking, more prevalent on rich pasture land than on country of lighter carrying capacity,
and it presents a more serious problem on improved pastures as well as in naturally rich river and creek valleys. However, on heavy pastures on hilly country it is often just as prevalent as on nearby flat country, this also being the case in Italy (Arn, 1931). On the same property the incidence often varies considerably in different paddocks, but there is usually no apparent explanation. Where there is surface water lying on the ground the disease is usually not prevalent. Some types of soil are more conducive to the disease than others, but this may be due to the soil type being associated with the type of pasture. The disease is most prevalent on dense, green pastures consisting of barley grass (Hordeum murinum), clovers, and what is popularly termed "herbage" (various plants other than grasses).

(c) **Age and sex.**

Sheep of all ages are susceptible. Some pastoralists consider that aged sheep are rather more susceptible than younger animals and that lambs, although often attacked when only a few weeks old, usually develop mild lesions which are easily cured. In the writer's experience sheep 4 to 12 months old seem to be somewhat less readily infected experimentally than adults, and in these animals the lesions are usually less severe.

Ewes are more susceptible than are ewes or wethers, possibly because their greater weight places more strain on their feet.

(d) **Breed and individual susceptibility.**

The Merino is the most susceptible breed of sheep. Crosses between the Merino and British breeds are appreciably less susceptible, and pure British breeds are probably still less susceptible though by no means entirely resistant. The Romney Marsh is said to be more resistant than most other British breeds. The relative resistance of British breeds and their crosses resides in their resistance to initial infection; once infection is established, however, the lesions are as severe as in Merinos.

Observations carried out at the Victorian State Research Farm by Mr. T. E. Berulaen (personal communication) suggests that some individual crossbred sheep are much more resistant than others of the same breeding and apparently are entirely resistant to natural infection. Working mostly with Merinos, the writer has found all animals susceptible to experimental infection although the severity of lesions has varied in different individuals.

(e) **Susceptibility of animals other than sheep.**

Goats are susceptible to foot-rot in the field according to Washburn (1904) and Aitken (1932). The writer infected one by inoculation and it showed rather mild lesions for three months. After that time the infection was successfully transferred back to a sheep.

Bovines are not susceptible to natural attack so far as the writer has been able to ascertain. The disease popularly known as foot-rot in cattle or "foul in the foot" is a different disease and more closely resembles "digital suppuration" or "foot abscess" of sheep, which term would be more appropriate. However, the writer infected a calf with foot-rot of sheep, inoculating the skin between the digits after scarification. The skin became ulcerated over all the interdigital space but the
infection did not spread under the horn. After eleven weeks, material was inoculated back on to a sheep and gave rise to typical foot-rot.

A hare was inoculated on the skin of the pad with material from foot-rot and the part bandaged. Six days later suppuration was still active, but when the pus was used to inoculate a sheep it did not set up foot-rot.

Blot and Lamarre (1932) consider canker of horses to be due to the same causal agent as foot-rot of sheep which they considered was a spirochaetal infection. The present writer has had no opportunity to carry out any investigations on canker. However, no association of the two diseases in the field in Australia has been observed.

2. Predisposing Factors.

(a) Introduction.

Since foot-rot has a fairly well-defined seasonal incidence and geographic distribution, it is evident that something more than mere contact with infected sheep in the same paddock is necessary to bring about transmission of the disease; or, in other words, some predisposing factor is required. Experimentally, mere application of infective material to the healthy foot usually does not set up the disease. Some injury to the skin or lowering of its resistance is necessary to provide a portal of entry, the intact, healthy skin being resistant to infection. The most generally held belief is that maceration caused by continued wetness is the main factor rendering sheep susceptible.

(b) Strongyloides larvae.

However, the theory of water maceration does not account for all the facts; for instance, swammy areas are often unfavourable for the spread of the disease and warm weather is more favourable than cold; the disease usually not spreading readily on wet pastures in winter. It therefore seems likely that some factor other than mere wetting is frequently involved. In a previous paper (Beveridge, 1934b) the writer put forward a hypothesis that percutaneous infection with larvae of the nematode Strongyloides papillosus may possibly be a common predisposing factor. Conditions conducive to the spread of foot-rot are also those favourable to the development of large numbers of Strongyloides larvae. Larvae of this parasite, when applied to the skin between the digits, caused an appreciable degree of damage. Application of infective material along with larvae to eight feet resulted in foot-rot in six, whereas five feet to which only infective material was applied remained normal.

A few further observations in this connection have been carried out. In the experiments reported previously the sheep were kept on a dry floor. In each of three experiments carried out since then, a sheep was placed in a cage the floor of which was covered with sanding and wetted three times daily. Infective material from a case of foot-rot was applied to all feet daily over a period of two to three weeks, and at the same time about 5,000 Strongyloides larvae were applied to both right feet on each sheep. In all three experiments foot-rot developed in the right hind foot after 2 weeks and in the right front foot and left hind foot after 3 weeks,
while the left front foot remained normal. Thus the larvae apparently rendered the feet more susceptible than witness alone, but the left hind feet to which no larvae were applied also developed the disease. No precautions were taken to prevent larvae dislodged from the feet to which they were applied from contaminating other feet.*

Whether or not *S. papillosus* is an important predisposing factor in foot-rot in the field is difficult to determine definitely, but it probably plays at least some part.

(c) Scald.

Most pastoralists in Australia report that outbreaks of foot-rot usually are preceded by the condition which they term "scald". The writer has not had an opportunity to observe this condition. The descriptions given by pastoralists vary somewhat. The more usual description is that there is an inflamed, "scalded" appearance of the skin between the digits, with some exudation of serous fluid and often some separation of the horn from the soft tissue, especially around the heels, but usually not extending very far. There is often lameness, which may be severe and is usually worse in the early morning and wears off during the day. Scald occurs usually on lush, damp pasture in warm weather, but sometimes after frosts, and may appear suddenly in a large number of the flock. It is said to heal spontaneously on dry pasture and respond readily to treatment with copper sulphate solution.

A specimen received from a pastoralist in the Western District of Victoria was said to be typical of scald as known to him. He said the condition was quite resistant to treatment. The skin and connective tissue between the digits were broken by a cleft separating the digits for 1/2 inch higher than normally, the exposed surface being covered with necrotic material and pus. The condition was not reproduced in a sheep which was inoculated between the digits with the pus by scarification.

Attempts have been made to reproduce scald by depasturing Merino sheep in an irrigated clover pasture at this laboratory. They developed no abnormality; however, conditions were not ideal. Sheep free of foot-rot infection kept for more than 10 months on irrigated pasture at the Victorian State Research Farm, Werribee, did not develop lameness. Sheep kept standing in water for 3 months at this laboratory developed no lesions resembling scald.

Scald occurs on some properties where foot-rot does not, so there is little doubt that it is a distinct condition, although probably sometimes it is confused with the early lesions of foot-rot. The actual nature and cause of scald are at present unknown. It may possibly be due to maceration and mild trauma caused by pasture dragging between the digits, or to percutaneous infection with *S. papillosus* larvae or to some other cause altogether. Scald is probably identical with "non-contagious foot-rot" as defined by some of the writers using this term.

*Another experiment was set up to observe the spread of the disease by contact in lambs standing in faeces containing *Strongyloides* larvae, but the lambs died 23 days after the commencement of the experiment. It is usually thought that *S. papillosus* has little, if any, pathogenicity, but there is little doubt that it caused the death of these two lambs. The carcasses were emaciated, the lungs contained small haemorrhages, and there were haemorrhagic areas in the small intestines. The intestines of one lamb contained 30,000 *S. papillosus* and the other 40,000.*
(a) Other predisposing factors.

Anything causing injury to the skin of the foot may provide a portal of entry for the infection, and scarification serves well for experimental transmission. For instance, grass seed infestation and travelling over stony ground are sometimes associated with outbreaks. Whether or not water maceration alone is sufficient it is not possible to state definitely.

4. The Contagious and Specific Nature of Foot-rot.

Foot-rot has been shown to be transmissible experimentally by a number of investigators. Youatt (1837) quoted the experiments of several workers in the early part of the 19th century and since then Brown (1892), Mohler and Wawburn (1901), Hesekamp (1909), Marsch and Tannicoff (1934) and, in this country, Murmane (1933), have confirmed this finding. Nevertheless, when this investigation commenced, most pastoralists in New South Wales, and some veterinarians, regarded the foot-rot occurring in their flocks as a non-contagous disease caused by lush, wet pastures per se. It was therefore necessary at the outset to determine if the disease under investigation was transmissible and similar to that studied by other investigators.

Material from natural lesions of foot-rot was inoculated on a healthy sheep's feet by applying it to the skin between the digits after deep scarification. The inoculated sheep developed lesions of foot-rot which corresponded in all respects with the disease seen in the field. During the course of the investigation a total of 107 feet of Merino sheep were inoculated with material from foot-rot lesions and the disease developed in 105 of these feet. In all, 42 different batches of material from lesions were tested separately and each proved infective. Of the two feet which failed to develop the disease after inoculation, one was in a test in which 16 feet were inoculated with a limited amount of material, and the failure was probably due to insufficient dosage. The other failure occurred in one of the inoculated feet of a lamb four months old (this and other experiments of the writer suggest that sheep 4 to 12 months of age are possibly somewhat more resistant than are adults). Strains of the disease from 5 different districts in Australia and one in U.S.A. all behaved similarly in transmission experiments.

That the disease produced by inoculation was caused by an infective agent in the inoculum, and not by the scarification and contamination from the environment, was shown by the fact that foot-rot developed in none of over 100 feet scarified and left uninoculated or inoculated with cultures of Staphylococcus aureus. These feet were contaminated with sheep faeces and, in many cases, with soil. Also five sheep each with two feet scarified were kept a week in a pen on the floor of which were spread fresh faeces from several horses. No lesions resembling foot-rot developed in these sheep. On another occasion horse faeces were applied to the feet of three sheep which had been scarified, without setting up any lesions. Several sheep's feet have been inoculated with pus from wounds in sheep, without developing foot-rot.

Cross R.
Sheep have been subjected to standing for long periods in water or mud by Brown (1892), Murmans (1933), and Carne (unpublished experiment) and did not develop foot-rot. Therefore, foot-rot cannot be set up by water maceration. Observations in the field show that flocks will remain free of foot-rot when pastured for long periods on lush, wet pasture provided the infective agent of the disease is absent.

It may be concluded therefore that the common foot-rot occurring in Australia, like that in other countries, is a contagious and specific disease and that the infective agent is present in lesions at all stages of the disease and is not, or at least not commonly, present in soil or faeces of sheep or horses in the absence of contamination from diseased sheep. The diseased foot of the sheep or goat appears to be the only natural habitat of the causal agent.

Natural transmission of the disease no doubt occurs through the medium of the soil or pasture. Discharges from the feet of diseased sheep are deposited on the soil and pasture and these contaminate the feet of healthy sheep. In an experiment carried out by Brown (1892), sheep developed the disease when placed in a pen from which diseased sheep had been removed 2 days previously, and in one of three experiments carried out by the writer sheep developed the disease when placed in a pen one day after the removal of infected sheep. In another experiment carried out by Gregory (1938b), sheep became infected when placed in a pen 5 days after the removal of infected sheep. The sheep's habit of walking in single file and along "pads" no doubt contributes to the natural spread of the infection.


As described above, outbreaks of foot-rot are, under ordinary conditions in Australia, separated by periods of many months and even years when the disease does not spread and may not be in evidence at all. The question arises as to how the infective agent survives during periods when the disease is not in evidence.

The first investigation of the longevity of the infective agent apart from sheep was conducted by Brown (1892), who found it survived in a muddy pen for at least 2 days. More recently March and Tunnicliff (1938) found that the infection did not live in dry soil more than 15 days or in mud more than 30 days, but it apparently survived in an "attenuated form" for 9 months in a swamp.

The writer conducted a rather extensive series of observations on the longevity of the infective agent. These have been reported fully (Beveridge, 1938b), and will be briefly recapitulated here.

1. Eight lots of material from lesions of foot-rot were either kept moist, air-dried, or suspended in water, and tested for infectivity on sheep's feet after various intervals. On the day of collection 22 out of 23 feet treated developed foot-rot; after keeping 24 hours, 24 out of 24 feet were infected; after 4 to 8 days, none of 9 feet developed foot-rot.
II. Ten lots of material from lesions were mixed with mud prepared from soil collected on properties where foot-rot is endemic. The soils had a pH range of from 4.7 to 7.1. When the mixtures were used to inoculate sheep's feet 2 hours after collection, 11 out of 12 feet became infected, after 3 days 5 out of 8, after 7 days 2 out of 16, after 14 days 4 out of 12, after 21 days none out of 6. The one infection which occurred after the material was kept 14 days was quite probably due to accidental contamination from other experimental sheep, as also may have been one of the two which occurred after 7 days, because adequate precautions were not taken in these two tests.

III. Two lots of material were mixed with sheep faeces. After an interval of one week one of the two was infective and after two weeks both were non-infective.

IV. A small muddy yard was contaminated by placing three infected sheep in it for a week. These were removed and 24 hours later test sheep with scarified feet were placed in it. In one of these experiments the test sheep developed foot-rot.

V. Six experiments were conducted in two quarter-acre plots with sheep pasture. The plots were contaminated with diseased sheep and left vacant for periods of 2 weeks, 9 days, 24 hours, 24 hours, 24 hours, and 9 hours. Test sheep with scarified feet placed in the plots failed to become infected in each instance. The reaction of the soil in these plots varied from pH 5.2 to 7.1. In three of the six experiments control sheep were placed in the plots together with the contaminating ones and they became infected.

VI. Observations on the viability of the infective agent in association with sheep showed: (a) sheep may remain affected with foot-rot for 3½ years or more and are still infective for other sheep; (b) some sheep after apparent recovery may continue to harbour the infective agent for at least 5 months in small foci of infection over which the horn has healed; (c) occasionally after recovery there remains a moist hairless area on the skin between the digits and in one sheep the infective agent survived 7 months in such a lesion; (d) on the other hand, the infective agent could not be demonstrated on 32 feet which had recovered for from 14 weeks to 4 months and appeared normal. Sheep with enclosed foci of infection as described under (b) are not lame and the feet appear healthy until pared except for some departure from the normal shape. Such a case recently observed at the laboratory carried the infection for five months and then relapsed into frank foot-rot.

VII. In two sheep, foot-rot material was applied to skin on the side of the body after scarification and in a third it was inoculated intradermally. None from these lesions failed to set up foot-rot in feet inoculated with it. This suggests that the causal organism does not grow readily in situations other than the natural habitat, the sheep's foot.
Field observations show that flocks in which the disease is endemic contain at all times of the year a few animals with chronic open lesions and a few with foci of infection over which the horn has grown.

More recently Gregory (1939b) has also investigated the longevity of infection in moist soil and faeces and on pasture. Test sheep did not become infected when moved on successive days through a series of muddy yards which had been left vacant for seven days after being heavily contaminated. However, they did become infected when the yards had been left vacant only 5 days. Gregory also conducted trials in 4-acre plots on irrigated pasture at the Victorian State Research Farm, Woorinen. Six sheep with foot-rot and two healthy sheep were run in these plots in rotation, being moved every 7 days. Each plot as it was vacated was left unstocked for 7 days and then five healthy sheep, constituting a test group, were placed in it for 7 days. The test group was rotated through the plots following the infected group on six occasions. Both of the healthy sheep running with the infected group developed foot-rot, but none of the test group did. The experiment was repeated, except that the period that the plots were left vacant was reduced to five days. The test sheep followed five days behind the infected group on eleven occasions. They remained free of foot-rot in spite of this intensive and repeated exposure to pasture heavily contaminated 5 days previously.

Conclusions: It is certain that in areas where foot-rot is endemic and breaks out periodically, the infection does not survive the inter-epidemic periods in the soil and pasture but either survives on the feet of some members of the flocks, or else is re-introduced. The maximum period of survival in the environment, even under the most favourable conditions, is not more than 2 weeks, and after only one week insufficient of the infection survives to produce foot-rot in sheep exposed to it naturally.

IV. BACTERIOLOGICAL STUDIES.

1. Introduction.

Mohler and Washburn (1904), Hasenkamp (1909), Murbane (1933) and March and Tunnillieff (1934), isolated _Pseudomonas necrophorus_ from lesions of foot-rot and considered it to be the causal agent, although March and Tunnillieff qualified this conclusion by stating 'there is another factor involved which we have been unable to discover'.

Mohler and Washburn, Hasenkamp, and Murbane, claimed to have reproduced the disease with pure cultures of _P. necrophorus_, but March and Tunnillieff failed to do so. However, Mohler and Washburn reported that the disease produced by inoculation of pure cultures of _P. necrophorus_ differed somewhat from the natural disease, being less severe, which was thought to be due to there being more secondary invaders in the natural disease. Murbane did not reproduce the disease consistently in his experiments with pure cultures.
As a result of these reports it has been fairly generally believed that foot-rot is caused by *F. necrophorus*. However, in 1926 Ludovic and Blainot described a spirochaete, *Treponema podotis*, which they believed was the cause of foot-rot owing to its prevalence in lesions. They were unable to isolate it. Howarth (1930a, 1930b), Rat (1932), and Hult and Lampe (1932) also reported finding this spirochaete in foot-rot and believed it to be the causal agent, although later Howarth (1934) stated that *F. necrophorus* had been inactivated. Marsh and Tunnicliff searched in lesions of foot-rot for organisms resembling the description of *T. podotis* but failed to find them, and Murman found them there only occasionally.

Our investigations into the cause of the disease were first made in an attempt to confirm the claims made regarding *F. necrophorus* and *T. podotis*.

*F. necrophorus* was isolated from eight cases of the disease. Pure cultures of these strains were inoculated on 121 feet of sheep after scarification. A purulent reaction was usually produced which was sometimes rather severe for about a week. Usually there was no separation of the horn from the soft tissues, and where this did occur the lesions were not extensive and healed spontaneously in one or two weeks. In no case did lesions resembling typical foot-rot develop. Similar purulent reactions were produced by the inoculation of cultures of *Staphyloococcus aureus* or of a pyogonic corynebacterium. Furthermore, *F. necrophorus* was isolated from a purulent wound in a sheep's foot not resembling foot-rot. These results, considered together with the fact that *F. necrophorus* is frequently found as a secondary invader in lesions caused by other organisms, for instance, in contagious chlamydia (Marsh and Tunnicliff, 1937), led us to conclude that *F. necrophorus* is not the primary causal agent of foot-rot in sheep.

*T. podotis* was described by all the aforementioned authors who observed it as a typical treponema presenting 3 to 5 undulations. We have been able to find organisms of this type in only a few of over a hundred cases of foot-rot examined repeatedly, and they were only numerous in two cases. This accorded with the experience of Marsh and Tunnicliff and Murman. Therefore it was concluded that *T. podotis* is not the cause of foot-rot and that the causal agent remained to be discovered.

2. General.

(a) Miscellaneous bacteria.

Lesions of foot-rot, being exposed to gross contamination, always contain a large variety of bacteria.

Cocci and corynebacteria are always abundant in lesions, and various rod-shaped organisms are also present. A number of these miscellaneous bacteria have been isolated culturally under aerobic and anaerobic conditions. Sixteen feet of sheep were inoculated with these organisms after scarification, none developed foot-rot.

As stated above, *F. necrophorus* is frequently, and perhaps constantly, present in lesions of foot-rot, but it is not capable of reproducing the disease experimentally.
In all of six cases of the disease which were being investigated by cultural methods at one stage of the investigation, the most prevalent organism was a pleuropneumonia-like, cultivable, organism similar to that described by Laidlaw and Elford (1936). It formed pin-point, convex colonies on blood agar after 3 days' incubation. Smears from colonies showed irregularly shaped coccal bodies varying in diameter from 0.5μ to the limit of visibility with the ordinary microscope. Cultures of this organism caused no lesions on eleven feet of sheep when inoculated after scarification.

On several occasions typical treponema- and leptospira-types of spirochaetes have been seen in material from foot-rot lesions, but they are not commonly present and also were found in a wound on a sheep's foot.

None of these miscellaneous bacteria can be regarded as the causal agent.

(b) Test for filterable virus.

Several batches of material from lesions of foot-rot were suspended in broth. In one test the suspension was passed through a Berkefeld N filter and in three tests through Gradloc membranes of pore diameter approximately 0.5 μ to 0.8 μ, whereas in three other tests the suspension was centrifuged at 5,000 r.p.m. for 30 minutes. The filtrates and supernatant fluids were tested on 15 feet, in some cases together with F. necrophorus and Sp. penorhtha. In no case were lesions resembling foot-rot produced, although the original suspensions were proved infective. Thus no evidence could be obtained that a filterable virus was involved in the aetiology of foot-rot.

(c) Spirochaeta penorhtha.

Smears of material collected carefully from recently invaded areas in foot-rot lesions usually show two organisms predominating over the miscellaneous bacteria (see Figs. 5 and 6). One of these is Sp. penorhtha and the other is a motile filamentous resembling F. necrophorus except that it is motile. These may be present in about equal numbers but one or the other may predominate.

In over 100 feet affected with foot-rot at the laboratory, Sp. penorhtha has been consistently present in all (excepting certain experimental cases to be discussed later). It has usually been present in large numbers in smears taken from active lesions adjacent to healthy tissue and less numerous in smears from more superficial necrotic detritus. It is usually numerous in smears taken from the inflamed skin between the digits in cases of foot-rot. Occasionally a smear from foot-rot was obtained in which it could not be found but further smears from the same foot revealed it.

Sp. penorhtha was found in 55 out of 65 smears from lesions of foot-rot obtained in the field on 16 properties in 14 districts in New South Wales and Victoria (Wagga, Gundagai, Young, Forbes, Junee, Canowindra, Corowa, Holbrook, Yooma, Werrito and Campbelltown). It was also found in all of 28 smears from New Zealand*, all of 4 from Northumberland, England* and in the one smear obtained in the field in New Jersey, U.S.A. Thus in smears from 94 cases

* The smears from New Zealand and England were kindly obtained for the writer by W. B. Pitch and W. L. Lyde Stewart respectively.
in the field, *Sp. penorhosa* could be found in all but 12 and its absence from these smears does not necessarily mean that it was not present elsewhere in the lesions on those feet. It is probably significant that 9 of the 12 negative smears were among those obtained by the writer at the commencement of the investigation when not so much stress was laid on the necessity for collecting smears from active areas in the lesions. The spirochaete was found in at least some of the smears from each of the properties excepting one, where only two smears were obtained.

This organism proved very difficult to isolate in artificial culture media, but eventually a means was devised, and pure cultures of several different strains were inoculated on 54 feet of sheep after scarification, sometimes alone and sometimes together with the motile fusiform, *P. necrophorus*, and *Staphylococcus aureus*. In several tests the spirochaete was isolated in less than a week and immediately inoculated. In two tests it was inoculated together with the motile fusiform and the mixture passed through sheep's feet 5 times and 11 times. *Sp. penorhosa* could be demonstrated on all inoculated feet for at least several days. In no case were lesions resembling foot-rot produced and the spirochaete alone caused no reaction at all.

(a) The motile fusiform.

In nearly 100 feet affected with foot-rot examined at the laboratory since the motile fusiform has been recognized, it has always been present in great numbers in the active portions of the lesions. In the smears obtained in the field, it was probably present in all. In many of them it was numerous and definitely recognizable, but in some in which it was not numerous it was not possible to identify it with any degree of certainty as its morphology is not sufficiently distinctive to differentiate it from other rod-shaped organisms.

The motile fusiform was isolated and cultures of a number of different strains were inoculated on 37 feet of sheep, often together with other organisms. It caused no lesions.

(e) Fusiformis nodosus

Smears carefully prepared from active areas in lesions of foot-rot at the laboratory nearly always revealed a large, Gram-negative, rod-shaped organism with enlarged ends, for which the name *Fusiformis nodosus* is suggested (see Figs. 5 and 7). This organism was present in quite small numbers as compared with *Sp. penorhosa* and the motile fusiform. Occasionally it could not be found in an individual smear from lesions in which its presence was demonstrated by subsequent smears. It has been found in all forty affected feet examined at the laboratory since its significance has been recognized and a proper search made for it.

It was searched for in smears collected in the field from foot-rot lesions on seven properties in New South Wales and Victoria, and found in at least some smears from each property. It could not be found in a few of these smears and in many of them it required a long search before occasional ones were found. It was found in most of 24 smears from foot-rot in New Zealand, in 2 out of 4 from England, and in one in New Jersey, U.S.A.
Difficulty was experienced in isolating this organism at first but eventually a method was devised. When cultures were inoculated on sheep's foot after scarification, lesions of foot-rot usually developed which were less severe than the natural disease. Simultaneous inoculation of F. nodosus and Sp. penortha produced lesions of typical foot-rot. These experiments are described in detail in a later section.

(f) Bacteria in histological sections of lesions.

Sections stained with Giemsa, or by silver impregnation, usually show that the organisms which penetrate furthest into the tissues are rods and filaments which are sometimes fairly definitely recognisable as the motile fusiform, although it is difficult to differentiate this organism from F. neorophorous in sections. Often there is a dense, salted mass of these organisms penetrating along fissures in the epidermis ahead of other bacteria. Somewhat less frequently Sp. penortha is found penetrating the epidermis in large numbers, either alone or together with the motile fusiform (see Fig. 17). The difficulty of demonstrating the spirochaete satisfactorily in sections may account for its not being seen more frequently. A few F. nodosus have been seen in about half the sections examined, although, as one would expect from examination of smears, this organism is never seen in large numbers. It is usually only present near the more superficial portion of the lesion, although occasionally a few elements are found penetrating the epidermis with the motile fusiform or the spirochaete ahead of other bacteria. No bacteria are usually found below the epidermis. On the surface of the lesion there are always many coccil and sometimes various other bacteria.

(g) Summary of bacterial flora.

Lesions of foot-rot always contain a gross mixture of bacteria comprised largely of cocci and spirillum-like organisms near the surface of the lesion. F. neorophorous is frequently present and there may be large numbers of a pleuropneumonia-like organism. Spirochaetes with typical morphology are sometimes, but not frequently, present. In the active areas of lesions the most prevalent organisms usually are Sp. penortha and the motile fusiform, these being constantly present. F. nodosus is constantly present but in relatively small numbers.


(a) Description of Fusiformis nodosus

The isolation of this organism was first reported in a preliminary note (Beveridge 1938a) where it was referred to as "organism F", and later it was briefly described under the name F. nodosus (Beveridge, 1940). Several strains isolated in Australia and one in U.S.A. have been studied, and a full description of the organism is now given. The only natural habitat of the organism appears to be the diseased foot of the sheep or goat.

(1) Isolation. The technique for isolation is essentially the same as that to be described for the isolation of Sp. penortha, except that it is essential to incorporate 10 per cent. of horse serum in the medium. The further addition of blood to the medium renders the colonies more
readily detectable. The typical colonies are usually found after two days' incubation. *F. nodosus* can usually be isolated without difficulty at the first or second attempt if good seeding material has been obtained.

(ii) Morphology. *F. nodosus* is a large, rod-shaped bacterium characterized by the presence of terminal enlargements, usually at both ends. These enlargements are most pronounced in organisms in natural material from lesions of foot-rot (see Figs. 5 and 7). In cultures they are frequently present though less pronounced (see Fig. 6), but they may be entirely absent in cultures on media not very favourable to growth or in strains isolated several months previously. When the terminal enlargements are absent the sides are usually parallel but occasionally there is a convexity in the centre of the rod. The axis of the rods may be either straight or slightly curved. Organisms as seen in smears from lesions of foot-rot measure from 0.6 to 0.8 μ wide in the centre portion of the rod and from 0.8 to 1.2 μ, at the ends, and from 3 to 10 μ long, though not often longer than 6 μ. In cultures the dimensions are somewhat less, usually 0.6 to 0.8 μ wide by 2 to 4 μ long, and one strain after 9 months' artificial cultivation appeared as rods little bigger than 0.6 μ with a few almost cocoid forms. The organisms are usually arranged singly but occasionally two are joined end to end. In smears from lesions, *F. nodosus* is sometimes seen with other organisms, most of which are probably motile fusiforms, clumped around it in radial arrangement (see Fig. 6). The organism is non-motile and does not form spores or capsules.

*F. nodosus* stains readily with gentian violet, dilute carbol fuchsin, or methylene blue and is Gram-negative and non-acid-fast. Organisms stained with Loeffler's methylene blue show one, two, or more, prominent, red-staining granules, either at the ends or along the rod. The terminal enlargements do not have the staining properties of spores. Films from lesions of foot-rot stained with strong carbol fuchsin, decolorized for 20 seconds with 1 per cent. aqueous solution of sulphuric acid and counter-stained with 0.5 per cent. methylene blue may show pink staining of the terminal portion of *F. nodosus*, but this effect has not been consistently produced. More vigorous treatment completely decolorizes.

(iii) Cultural requirements. *F. nodosus* is an obligate anaerobe. Growth is enhanced in an atmosphere containing 5 to 10 per cent. CO₂, and even 80 per cent. of this gas is not inhibitory. McIntosh and Pildes' jars have been employed for all our anaerobic cultural work, except that cooked heart medium cultures and deep agar tube cultures have been incubated in air. Growth is more rapid at 37°C than at 34°C, whereas, at 25°C, only slight growth is visible after 10 days. The optimal pH for growth is 7.4 to 7.6. My colleague, Mr. T. S. Gregory, found that no growth takes place at pH 4, pH 5, or pH 6, but that at pH 7 and pH 9, growth is only slightly less abundant than at pH 7.

Practically no surface growth is obtained on *V-P* agar plates unless serum is incorporated in the medium. Horse serum is the most suitable of the various sera tested, and 10 per cent is the optimal concentration. All of several batches of horse serum of various ages obtained by allowing the blood to clot proved satisfactory in this respect, but

* "V-P" plates are prepared from a peptic digest of ox muscle and liver, as described by Turner (1930)."
when the serum was obtained by allowing the cells to sediment in defibrinated blood, 3 out of 5 batches failed to promote growth. Sheep serum obtained from clotted blood (from a sheep not affected with foot-rot) not only failed to promote growth but inhibited it in the presence of horse serum. Several different batches of sheep blood also failed to promote growth. No growth occurred with one batch of rabbit serum obtained from defibrinated blood. Ox serum obtained from clotted blood promoted some growth but considerably less than horse serum.

The addition of 0.1 per cent. cysteine hydrochloride further enhances growth on horse serum "V-P" agar, but glucose does not, either in the presence or in the absence of horse serum. Autoclaved potato extract, as described by Elamets and Retger (1933) as favouring growth of certain fusiforms, does not enhance the growth of this organism. Mr. T. S. Gregory has found that gelatin can replace horse serum as a growth-promoting substance in "V-P" broth and "V-P" agar surface cultures.

(iv) Surface cultures on solid media. Surface cultures grow well on "V-P" agar containing 70 per cent. horse serum and 0.1 per cent. cysteine hydrochloride. After 48 hours, smooth convex colonies develop which are round with an entire edge, but grow in consistency, easily emulsified, and are up to 1 mm. in diameter. They are semi-opaque and colourless but appear translucent on medium containing blood. The colonies sink into the surface of the medium, producing a sunken appearance, which is characteristic. Variation in appearance is more easily observed in media containing blood. The more common form is a smooth convex colony lying in a depression which extends just beyond its edge (see Fig. 9). Occasionally the depression does not extend beyond the edge and is revealed only by scraping off the colony. Where the colonies are numerous and close together, the only evidence of them to the naked eye is "pock marks" in the medium 0.5 mm. or less in diameter, each containing a small colony often visible only with difficulty with the aid of low magnification (see Fig. 9). When the growth is confluent there is usually no stolching. There is usually no stolching on media giving poor growth, as when unsuitable serum is used. These different colonial appearances do not represent variants.

When plates are incubated for five days, or sometimes less, the characteristic colony usually develops a flat extension beyond the surrounding depression, about 0.5 mm. wide, without causing further stolching. Sub-cultures from this non-stolching extension give rise to the characteristic stolching colonies. Sometimes in place of the flat extension one or two concentric rings of very small subsidiary colonies appear (see Fig. 10).

Colonies usually have a smooth surface, but some have been seen with a ground-glass or definitely rough surface, and these are flatter and about twice the diameter of the smooth colonies. These may represent R variants, although some of them gave rise to a mixture of both types, so that if variation had taken place it was not complete.

Plate cultures have a rather faint, characteristic odour which might be described as acrid.

Veal infusion peptone agar plates containing 10 per cent. horse serum and 0.1 per cent. cysteine hydrochloride give poor growth and colonies may not show stolching.
Growth does not take place on inactivated horse serum or egg medium.

(v) Cultures in deep agar. Shake cultures grow poorly or not at all in "V-P" 2 per cent. agar medium but well when 10 per cent. horse serum has been added. After 2 days' incubation small colonies are visible in a narrow band 1 cm. below the surface; after 3 days they extend down to the bottom of the tube. The colonies are convex, creamy-white with entire edges, and are up to 0.5 mm. in diameter. Optimal growth, as evidenced by greatest concentration and size of colonies and earliest appearance, occurs in a band about 0.3 cm. deep situated about 1.5 cm. below the surface (see Fig. 11).

Shake culture in semi-solid "V-P" 0.2 per cent. agar medium form roughly spherical colonies which later develop a downward extension of more diffuse growth giving them the appearance of small feathers. Growth is better when serum is incorporated in the medium, but good growth may occur in its absence. After about 7 days' incubation an opaque band 1 mm. or less in depth occurs about 1 cm. below the surface; below this there is a further clear zone about 0.5 to 1 cm. deep and then the zone of optimal growth. Some cultures develop a series of these opaque bands separated by about 1 mm. of clear medium. Tubes of similar medium inoculated with heat-killed cultures and showing no growth do not develop these bands. The bands consist of a layer of crystals probably composed of amino acids. The heavier the inoculum and the more rapidly growth develops, the nearer these bands are to the surface.

There is no growth in semi-solid agar made from veal infusion peptone medium.

Stab cultures in horse serum "V-P" agar sown liberally produce an even, granular growth throughout the length of the stab except near the surface, in horse serum veal infusion peptone agar, growth is slower and less abundant.

(vi) Cultures in fluid media. Using fairly heavy inocula, very slight growth or none at all occurs in "V-P" broth, whereas, with 10 per cent. horse serum added, a rather poor growth ensues which produces a light turbidity and small, dirty white, granular deposit.

There is no growth in peptone water with 10 per cent. horse serum added.

In a medium consisting of "V-P" broth with minced cooked heart added, growth takes place rather slowly. Recently isolated strains produce after 3 or 4 days a darkening of the fluid and of the meat. On further incubation the meat is partly digested and occupies about 25 per cent. less volume in the tube and many small fragments are formed from the breaking up of the larger particles. Strains which have been isolated for several weeks produce in 2 or 3 days a slight white deposit and light turbidity which clears after a further 3 or 4 days' incubation, and they darken and digest the meat more slowly. There is no gas formation and no odour. Serum does not enhance growth in this medium. After incubation for from four to six weeks, cultures develop white clumps or small needle crystals of tyrosine.
(vii) Viability and resistance. Shake cultures in "V-P" agar and cultures in "V-P" cooked heart medium covered with liquid paraffin remain viable at room temperature for five weeks. Cultures dried in vacuo at low temperatures survive at room temperature for over two years, but subcultures may show a lag period of as long as 14 days.

Five-day cultures in cooked heart medium exposed to air in Petri dishes in 5 mL amounts containing only a few small meat particles and mixed with an equal volume of broth survive at room temperature 24 hours but not 3 days. When mixed with an equal quantity of Staph. aureus broth culture in place of the broth they live under the same conditions six days but not ten days. Colonies on surface cultures survive exposure to air 24 hours but plates which have been inoculated will not grow subsequently after exposure for 24 hours to even very low percentages of oxygen, as in an anaerobic jar not functioning efficiently.

F. nodosus is killed at 50°C in 10 minutes and sometimes in 5 minutes.

(viii) Biochemical properties. Plate cultures on media containing sheep or horse blood show no haemolysis. Hydrogen sulphide is produced. Nitrates are not reduced to nitrates. In litmus milk there is no change in reaction but with heavy inocula a soft clot is formed after several days and this is later digested. There is no growth on inosinate serum; in cooked heart medium the meat particles are partly digested, and after four to six weeks' incubation white clumps of tyrosine crystals are formed. As there is no growth in peptone water on indole test cannot be carried out in this medium, but no indole can be detected in cooked heart medium cultures. No growth occurs in peptone water plus serum as in Hia's serum water, but fermentation tests may be carried out in a sugar-free digest medium to which is added horse serum, 0.1 per cent, cysteine hydrochloride, and brom-cresol purple. Sucrose, maltose, galactose, lactose, dextrose, and mannite, are not fermented.

(ix) Animal inoculation. Subcutaneous inoculation of sheep, rabbits, guinea pigs, and mice with from 0.5 to 5 mL of cultures in cooked heart medium produce no local reaction or only a slight swelling which subsides in a few days. When calcium chloride is included in the inoculum a small nodule develops which may persist several weeks but gradually subsides. Male rabbits may develop a rather severe oedema of the scrotum after subcutaneous inoculation in the thigh but this subsides in a few days. Intradermal inoculation produces only a slight transient swelling.

Subcutaneous or intradermal inoculation of F. nodosus mixed with Sp. pyogenes in sheep, guinea pigs, and mice produce a small nodule which may persist several weeks but finally subsides.

Intravenous inoculation of large doses of cultures may have a sudden fatal effect. Inoculation of 4.5 mL of 6-day culture in cooked heart medium killed one rabbit within one minute of inoculation. The animal threw itself about violently and died with the heart continuing to beat after respiration ceased. Two other rabbits similarly inoculated showed no symptoms. One out of three mice inoculated intravenously with 1 mL of culture died within one minute of inoculation; the other two showed no symptoms. These fatalities were probably not caused by toxin.
(x) **Agglutination tests.** Specific antisera were prepared against an Australian strain and an American strain by inoculating rabbits intravenously four times at intervals of 4 to 7 days and bleeding out 6 days after the last inoculation. The antigens used for inoculation consisted of a thrice-washed suspension of growth from horse serum agar plates, standardized to an opacity equal to a barium sulphate suspension made by adding 3 ml. of 1 per cent. barium chloride to 97 ml. of 1 per cent. sulphuric acid. These were administered in doses of 4 to 6 ml. The suspensions were used either freshly prepared on the day of inoculation and not killed, or else preserved in 0.1 per cent. formalin.

Antigens for agglutination were prepared from growth on ox serum medium, were thrice washed in saline, and were standardized as those for inoculation.

The tests were carried out at 55°C., for 1 hour and the results were read after standing at room temperature for 2½ hours, which was found to be the best time to read them.

The "American" antiserum gave homologous agglutination at a final dilution of 1 in 3,800 and heterologous agglutination at 1 in 1,600. The "Australian" antiserum gave homologous agglutination at 1 in 6,400 and heterologous at 1 in 100. Serum from an un inoculated rabbit gave no agglutination at 1 in 100. These results show a definite relationship between the strains. They suggest, however, that the Australian strain lacked some antigenic component present in the American and this may have been lost on artificial cultivation for at the time the antisera were prepared the Australian strain had been artificially cultivated over a much longer period than had the American.

The same agglutination test was carried out with sera of seven sheep affected with foot-rot and the homologous strain of *F. nodosus*. No agglutination occurred at dilutions of 1 in 50, 1 in 100, and 1 in 200. This result is not surprising when one considers the relatively superficial nature of lesions of foot-rot and that in sections of living tissue *F. nodosus* rarely found near the living tissue.

Mr. T. S. Gregory carried out cross-agglutination tests with four strains from New South Wales, Victoria, and Tasmania, and found no, or only slight, interrelationship.

(xi) **Classification.** We have been unable to identify this bacterium with any previously described and therefore regard it as a new species. It appears to be most closely related to the group of organisms isolated from human fomites by Eggert and Gagnon (1933) and by Weiss and Betiger (1937) and classified by them as belonging to the genus Bacteroides. The genus Bacteroides, however, has not been generally recognized as valid. Also the definition of this genus by Bergay (1934) includes "good growth on ordinary culture media" and this does not apply to the present organism.

Under the more conservative system of classification used by Topley and Wilson (1937) the organism falls into genus Fusiformis, and it is thought wiser to place it in this genus, at least for the present. It is unfortunate that the generic name implies fusiform shape whereas this organism usually has enlarged ends. However, some of the organisms placed in the genus Fusiformis by Topley and Wilson do not have pointed ends, so this feature does not seem to warrant its exclusion, since its other characteristics
are well covered by the definition of the genus. Its parasitic habit, the type of lesion in which it occurs, and its association with a spirochaete may be regarded as supporting evidence in favour of this classification.

The name F. nodosus is proposed. (L. nodosus = knobbled).

(b) Description of Spirochaeta penortha.

A detailed description of this organism was published earlier (Beveridge, 1936). It is described more briefly here, with the addition of some recent observations.

(i) Isolation. This is a difficult organism to isolate, therefore the procedure with which the writer has had most success will be described in detail. The most important step is the collection of the material from the lesions. After removal of the overlying horn and grossly contaminated material, a recently invaded area of the sole is selected. The more superficial pus and necrotic tissue is lightly scraped off and then with sterile scalpel a little of the infected tissue is scraped up and deposited in a Petri dish in the lid of which has been placed some wet filter paper. Several lots of material are collected in this way from different feet and then a loopful of broth is added to each and smeared made. Material is selected for culturing which shows a high proportion of spirochaetes. Three serial dilutions are made in boiled "V-P" broth and a drop from each is inoculated on to two or three plates and spread with a glass spreader. The plates consist of "V-P" agar containing 5 per cent. sheep blood and 0.1 per cent. cystein hydrochloride (10 per cent. horse serum is also added if it is desired to isolate F. nodosus simultaneously). The plates are placed in an anaerobic atmosphere without delay. Carbon dioxide, which enhances growth of the spirochaete, is provided by contaminating bacteria, but it is probably an advantage to place 60 per cent. CO₂ in the jar, as this exerts an inhibitory effect on some bacteria but not on the spirochaete. Colonies of the spirochaete may on rare occasions be found after two days' incubation but more often after 4 or 6 days. Colonies are searched for with the aid of a hand lens, and, when found, are subcultured on to similar plates until a plentiful, pure, growth is obtained which may be inoculated into semi-solid agar containing a piece of raw potato or into "V-P" cooked heart medium.

Several attempts are frequently necessary before the spirochaete can be isolated, but if painstaking care is taken in selecting the seeding material and a little patience in exercised the method meets with success.

(ii) Morphology. Sp. penortha stains well in a few seconds with amiline gentian violet or concentrated carbol-fuchsin. It is Gram-negative.

It is a filamentous organism in which neither spore formation nor branching has been observed. Flagella and spirochaetal "terminal filaments" are lacking. The dimensions are usually 0.5 to 0.5 μ wide by 6 to 10 μ long, but may vary slightly beyond these limits. The organism has a peculiar type of motility, progressing in a slow even manner, usually without showing any flexion. However, it is flexible and able to flex itself actively.

Roughly spherical bodies, 0.5 to 1.5 μ in diameter, are sometimes seen in cultures and may be very numerous in old cultures.
In smears made by spreading unmoistened material from foot-rot lesions and drying before fixing, the filaments are irregularly curved and many bend sharply, giving various twisted forms, while sometimes a few show more or less regular waves. In smears made from cultures, or from foot-rot material mixed with a drop of fluid on the slide, and fixed while wet with osmic acid vapour, the filaments are usually only slightly curved, or straight, but occasionally show very shallow waves (see Fig. 12). Living organisms examined by dark ground are usually straight or slightly curved, and do not show the regular waves of typical spirochaetes.

However, it was found recently that *Sp. ponorha* usually shows distinct regular waves in smears which have been air-dried and fixed by heat or methyl alcohol and then stained with concentrated carbol fuchsin while heating over the Bunsen pilot for 3 minutes (see Figs. 13 and 14). No waves are to be seen in duplicate smears fixed with heat, methyl alcohol, or osmic acid and stained with gentian violet, or in dark ground preparations of living organisms from the same source. We have not been able to reproduce this waviness with absolute consistency and repeatedly failed to do so with a strain isolated in U.S.A. using American stain.

(ii) Cultural characteristics. *Sp. ponorha* is a strict anaerobe, and surface growth is enhanced by the addition of about 10 per cent. carbon dioxide. The optimum temperature for growth is 37°C. In all media, growth is accompanied by fairly copious gas formation in the case of five Australian strains, but gas is not produced by a sixth Australian strain or by one strain isolated in U.S.A. Vigorous cultures have a faint putrescative smell.

There is no haemolysin produced.

Litmus milk sown heavily is clotted in from 2 to 3 weeks without acid formation.

Surface growth does not take place very readily, but discrete colonies are usually obtained after two days' incubation using the following technique: Plates are made of *U-P®* agar containing 5 per cent. blood (sheep, horse, or rabbit) and 0.1 per cent. yeast hydrochloride, and the inoculum is spread with a glass spreader. Incubation is carried out in an anaerobic atmosphere consisting of 5 to 10 per cent. CO2 in hydrogen, or illuminating gas. The colonies are usually convex, smooth, semi-opaque or water-clear, and may be white, green, or pink. Those over 1 mm. in diameter nearly always are a characteristic pale green or bluish colour which, under a hand lens, is seen to be situated in fluorescent granules within a clear matrix. The colonies are butyrous but slightly viscid, and when emulsified with water form an emulsion which has a marked "watered silk" appearance. After 3 days' incubation colonies may be surrounded by a flat spreading growth extending about 2 mm. from the colony. The edge of the spreading growth is fimbriated and its surface may be smooth or slightly rough. It has a "shot silk" appearance, being bronzy or green at different angles to the light. This flat growth occasionally occurs without any well-defined central colony and it is in this form that the strain isolated in U.S.A. usually grows.

In poured plate cultures in *U-P®* agar containing 5 per cent. haemolysed blood, small biconvex colonies develop.
after six days. The largest of these may show the characteristic green flecks seen in surface colonies, but the smallest ones are translucent.

In tubes of "V-F" agar or "V-F" semi-solid agar, with or without serum, growth either fails to take place or is scanty and slow. The addition of a piece of raw potato with all except one of the seven strains studied stimulates growth. In semi-solid "V-F" agar containing raw potato, growth is visible 24 hours after liberal inoculation. Rather bulky irregular masses of growth form and later the growth travels up the channels formed by rising gas bubbles. No growth takes place in "V-F" broth, but the addition of a piece of raw potato provides a liquid medium in which growth will ensue if it is fairly liberally inoculated. The medium becomes turbid and a loose, finely floccular deposit forms.

In a medium consisting of "V-F" broth with minced cooked heart added, good growth occurs after liberal inoculation, producing turbidity.

(iv) Viability and resistance. Pure cultures exposed in thin layers to the air are killed in one to three days, but if mixed with a culture of Staphylococcus aureus they survive from 3 to 11 days.

Cultures live longer at 37°C than at 4°C. Cultures in "V-F" agar with raw potato live from 1 to 3 months at 37°C. Cultures dried in vacuo at low temperatures survive at least 8 months.

Addition of sufficient hydrochloric acid to bring the pH to between 4.1 and 4.9 killed cultures within 2 hours, and to pH 5.0 to 5.6 killed cultures within 24 hours. At pH 6.0 to 6.3 the organisms survived 8 days but showed no growth.

Cultures at pH 7.6 survived 53°C, for 6 minutes and 55°C, for 2 minutes, but succumbed to 53°C, in 9 minutes and 55°C, in 3 minutes.

(v) Animal inoculation. All strains tested are non-pathogenic for sheep, guinea pigs, rabbits, and mice. Subcutaneous and intradermal inoculation of sheep, guinea pigs, and mice with a mixture of S. pensinsulae and F. nodosum produces a small nodule which subsides after several weeks.

(vi) Agglutination tests. Cross-agglutination tests previously reported showed three Australian strains to belong to one group. Antisera were also prepared against a strain isolated in U.S.A. and another Australian strain. In the case of the American strain the antigen for inoculation was obtained from surface cultures on rabbit blood agar and, in the case of the Australian, from culture in cooked heart medium. These antigens were inoculated either freshly prepared and untreated or after preservation with 0.1 per cent. formalin. The antigens for the agglutination tests were prepared from surface cultures on horse blood agar and standardized to an opacity equal to a suspension of barium sulphate made by adding 3 ml. of 1 per cent. barium chloride to 97 ml. of 1 per cent. sulphuric acid. The tests were carried out at 55°C. for 1 hour and read after half an hour at room temperature. The "American" antiserum gave homologous agglutination at a final dilution of 1 in 2,400 but none with the Australian strain at 1 in 100. Antiserum from the Australian strain gave homologous
agglutination at 1 in 1,200 and with the American strain at 1 in 400. Thus there is but a slight antigenic relationship between these strains.

c) Description of the motile fusiform of foot-rot.

This organism was briefly reported in a preliminary note (Beveridge 1938a) where it was referred to as the fusiform of foot-rot. It will now be referred to as the motile fusiform to distinguish it from F. nodosum.

(1) Isolation. Material is sown on blood agar plates which have not been dried, and after 2 or 4 days' incubation in an anaerobic atmosphere a thin spreading growth is found. Subcultures from the advancing edge usually prove pure. The organism may also be isolated in shake cultures when it is very prevalent in the seeding material.

(11) Morphology. In foot-rot lesions and in most cultures the organism occurs as short rods, but in some cultures long filaments are formed (see Figs. 6 and 15). The shorter forms, which are from 2 to 7μ long, are usually straight but occasionally are slightly curved. The thickness varies considerably in different strains, ranging from 0.1 to 0.7μ. As with many other fusiforms, two organisms are frequently seen joined end to end. Most organisms in smears from lesions and somewhat fewer in smears from cultures show tapering at the ends, which may be pointed. This feature, however, is not constantly present and the sides may be parallel and the ends rounded. With the same strain and even in the same culture some organisms may show tapering while others have parallel sides. The long filaments are usually found in those surface cultures which show colony formation but they are also formed by some strains in fluid or semi-solid agar cultures. These filaments are usually even throughout and show gradual curves but occasionally are divided by constrictions into chains of bacilli and are bent sharply at the constrictions. Round or slightly fusiform enlargements about 1μ in thickness are sometimes seen in filaments. The organism is actively motile by means of peritrichous flagella. Spores are not formed.

The organism stains readily with ordinary stains. It is Gram-negative but does not decolourize as readily as most Gram-negative bacteria. If the smear is dried after removal of the iodine and then decolourized with absolute alcohol, the organism appears Gram-positive. It is not acid-fast. Staining is usually even, but is sometimes beaded and sometimes the ends stain more deeply than the rest of the rod. In dark-ground preparations the ends of the rods often show a different shade of lighting from the rest of the organism.

(iii) Cultural characters. The organism is a strict anaerobe, and the presence of 5 per cent. carbon dioxide probably favours growth whereas 80 per cent. is not inhibitory. Growth is more rapid at 37°C., whereas at 26°C. it proceeds only very slowly.

In TVF™ broth with minced cooked heart added the organism grows fairly well. Some strains produce abundant gas but others produce none, although growing well. Most strains produce moderate turbidity but some leave the fluid clear. Most strains redren the meat particles but a few do not. There is no odour.

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In semi-solid agar some strains produce abundant gas, others none. The former spread throughout the medium, except within 2 cm. of the surface, along the channels formed by the gas, producing an uneven, floccular turbidity. Of the non-gas producers, some diffuse through the medium producing in 2 or 3 days an even, light turbidity of all the medium, except that within 2 cm. of the surface where there is no growth and immediately beneath this there is a band about 2 mm. deep where the turbidity is denser. Other non-gas producing strains develop a dense cloud-like growth where the inoculum is deposited and this spreads slowly and does not grow throughout the medium.

In shake cultures in serum "V-8" agar the bioprex colonies are about 0.5 mm. in diameter after 4 days and do not grow near the surface. In stab cultures in serum agar an even granular growth occurs along the needle tract.

Good surface growth occurs on 5 per cent. blood "V-8" agar in anaerobic atmosphere containing 5 per cent. carbon dioxide. The plates should not be dried and it is an advantage to have some damp cotton wool in the bottom of the jar. The growth usually takes the form of an inconspicuous, thin film which eventually spreads over the whole plate. The thickness of the film varies, but often is so thin as to be difficult to detect except when one holds the plate so that the light is reflected from the surface at the margin of the growth. In this case the growth imparts only a ground-glass appearance to the surface of the plate, but sometimes the growth is much thicker and is definitely raised at the edges, and then the surface presents a beaten-copper appearance. When the growth is thick, it is semi-opaque and dirty-white. The edge of the growth may be entire, frambriate, or present long finger-like processes, these different forms perhaps being due to variation in the amount of moisture on the plate. Occasionally there are two or more layers of growth, the edges of which form contour lines. Sometimes colonies are formed on plates; this is usually so when the plate has been dried, but some strains, especially after being subcultured for several months, have a tendency to form colonies instead of spreading growth, although the latter may subsequently extend from the colonies. The colonies are usually very small, approximately 0.1 mm. in diameter, but are sometimes as much as 1 mm. in diameter, and are usually irregularly stellate in shape with rough surfaces (see Fig. 16). Smears from the colonies usually show long filaments whereas smears from the spreading growth show only short elements.

(iv) Biochemical properties. The following limited observations have been made on four strains: Litmus milk is not clotting and shows no change in reaction; neither H2S nor indole is produced; there is no haemolysis on horse blood or sheep blood agar plates.

(v) Animal Inoculation. Subcutaneous inoculation of rabbits and guinea pigs with 1.0 ml. of culture in cooked heart medium produces transient diffuse swellings and after these subsides a nodule 1 to 2 cm. in diameter persists for a month.

(vi) Agglutination tests. A suspension was prepared from one strain and an agglutination test put up against sera from four sheep affected with foot-rot and two normal sheep. After 24 hours at 37°C, a slight deposit had occurred
in all tubes including the control. Only two of the sera from affected sheep showed agglutination at a final dilution of 1 in 60, and none at 1 in 120.

(vii) Classification. These strains resemble in many respects some of the fusiforms which have been described by other workers (Vanney 1927, Blaxter and Betiger 1933, Spaniding and Betiger 1937; Hino and Barry 1937) but they differ in being frankly motile and in producing a spreading surface growth. They are therefore regarded as hitherto-undescribed fusiforms.

Several variations are shown by different strains in their cultural characters in cooked heart medium and in semi-solid agar, and there is some variation in morphology. Because the organisms do not appear to play a specific role in the aetiology of foot-rot, and owing to the complexity introduced by the differences in cultural characters, a complete study has not been made. Possibly more than one species is represented in the more than 30 strains which have been isolated, but the strains cannot be easily grouped on the basis of the features studied. Motility and spreading surface growth are features common to all strains and it seems permissible, for the time being, to regard all strains as belonging to one species and to group them under the general term "motile fusiform of foot-rot".

4. Experimental Reproduction of Foot-rot with pure Cultures.

(a) Experimental procedure.

In these experiments, as in the previously described attempts to reproduce the disease with cultures, the method of inoculation was to apply the inoculum to the skin between the digits after scarification. In scarifying the area, the skin and adjacent soft horn was abraded by several deep scratches with a scalpel until a little blood exuded. The feet were scarified and inoculated on two successive days except where otherwise mentioned. No attempt was made to prevent contamination with organisms from the skin, the faces, or the floor of the cage. Two feet were inoculated on each sheep, one fore foot and one hind foot. Scarified but uninoculated negative controls were considered to be unnecessary and were not usually done because over 200 feet had been scarified and inoculated with cultures of various bacteria and in not a single instance were lesions resembling foot-rot produced.

So far as possible only adult Merino sheep were employed, but in Experiments 2(b) and 3, Merino "weaners," 6 to 12 months of age, were used, and in Experiment 7 crossbreds (mostly Dorset and Suffolk "blood") were used. We have found all adult Merino sheep susceptible to the disease when inoculated with material from lesions of foot-rot, but Merinos under one year of age and crossbreds are apparently somewhat less susceptible.

All experimental sheep were isolated so far as the facilities available permitted, but strict isolation was possible only in Experiment 7. In Experiments 1 to 6 each sheep or each test group was kept separate, usually in wooden cages, but these were often not sufficiently far apart to
exclude entirely the possibility of contaminating material being splashed from one cage to the other when they were being housed out, and flies were not excluded. These arrangements proved satisfactory in previous experiments in which only transient lesions caused by searification or else frank foot-rot resulted, the results being consistent in a large series of tests. However, in experiments in which lesions developed and were observed during several weeks to determine whether or not certain bacteria made their appearance in lesions, the significance of these observations was sometimes subject to question. Further reference will be made to this point in describing the observations which might have been affected by contamination. Precautions were taken to prevent contamination being carried on instruments as well as on hands and feet of attendants.

The wooden cages in which the sheep were kept in most of the tests had raised wooden floors with spaces between the battens. To avoid excessive drying of the foot, a sack which was wetted once a day was placed on the floor to cover about 1/3 of its area.

The organisms used for the inoculations had been isolated not longer than one to three weeks, although no evidence has been obtained that their virulence altered on artificial cultivation. The purity of the cultures was ensured by subculturing two or three times before use. Plate cultures were usually used for the inoculations and liberal applications were made. Where foot-rot developed, the lesions are described as "mild", "moderate", or "severe". "Mild" lesions were those in which an active infection was set up on the skin between the digits and extended under at least a portion of one sole causing separation of the horn but less tissue damage than usual was present. "Moderate" lesions were rather more extensive and showed slightly more tissue damage. Lesions were described as "severe" only when there was rather severe inflammation of the skin between the digits, of all the soles, and sometimes of a portion of the outer wall with separation of the horn over these areas. "Severe" lesions are the type usually seen in the natural disease in Merinos. This classification was of course purely empirical and was rendered more difficult by the fact that often lesions on the same foot varied in severity during the period of observation, some developing more rapidly than others and some tending to retrogress while others became more severe. It is important to note that lesions described as foot-rot were shown to be transmissible excepting in a few cases in which "weaners" or crossbreds were used as test animals. The lesions were observed over a period of approximately a month after inoculation to ensure that they would persist as does foot-rot.

(b) The experiments.

Experiment 1.

(a) Two feet were inoculated on four successive days with cultures of P. nodosus, Sp. penorths, the motile fusiform, and the pleuropneumonia-like organism. "Severe" lesions of foot-rot were produced and were successfully transferred to a third foot on the same sheep.
(b) Two feet were inoculated on four successive days with the same cultures as in 1(a), but in addition with P. neocophorus and several other organisms isolated from foot-rot. Severe foot-rot developed in both feet but the lesions were no more severe than in the sheep in Experiment 1(a).

Experiment 2.

(a) Two feet were inoculated with cultures of P. nodosus and developed severe foot-rot which was successfully transferred to a third foot on the same sheep. Sp. penortha appeared in the lesions.

(b) Two feet of a Merino wether were inoculated with P. nodosus, Sp. penortha, and the pleuroneumonia-like organism. Rather mild lesions of foot-rot developed, and when material from these was used for inoculating a third foot, mild transitory lesions developed.

(c) Cultures of P. nodosus and the motile fusiform were used for the inoculation of two feet, one of which developed severe foot-rot and the other mild lesions of foot-rot. Sp. penortha appeared in the lesions. Material from these lesions was used for the inoculation of a third foot, and lesions resembling early foot-rot were developing when the experiment was brought to a conclusion.

(d) Cultures of P. nodosus, Sp. penortha, the motile fusiform, and the pleuroneumonia-like organism were used for the inoculation of two feet. Severe foot-rot developed and after six weeks had partly healed. A third foot infected from the first two developed severe lesions of foot-rot which healed spontaneously after four weeks.

Experiment 3.

In this experiment Merino wethers were used. Difficulty was experienced in getting the cultures to 'take', and four inoculations were made on most feet. The same cultures of P. nodosus and Sp. penortha produced typical foot-rot in an adult in Experiment 1.

(a) Two feet on each of two wethers were inoculated with cultures of P. nodosus and developed moderate lesions of foot-rot which were successfully transferred to a third foot on each animal.

(b) Cultures of P. nodosus and the motile fusiform were used for inoculation of two feet of each of two wethers and produced moderate lesions of foot-rot in two feet and mild lesions in the other two.

(c) Cultures of P. nodosus and Sp. penortha were used for the inoculation of two feet of each of two wethers. Two feet developed mild lesions, one healed after showing mild lesions for two weeks, and one developed no lesions.

(a) Cultures of P. nodosus, Sp. penortha, and the motile fusiform were used for the inoculation of two feet of a wether. Both developed mild lesions of foot-rot.
38.

(c) Cultures of *F. nodosus*, *Sp. penortha*, the motile fusiform, and the pleuropneumonia-like organism were used for the inoculation of two feet of each of two wemara. Two feet developed mild lesions of foot-rot and two feet developed no lesions.

Experiment 4.

Cultures of *F. nodosus*, *Sp. penortha*, and the motile fusiform were used for the inoculation of two feet of an adult Merino. Severe foot-rot developed in one foot and moderate lesions in the other. Foot-rot developed in a third foot inoculated with material from the former.

Experiment 5.

(a) Cultures of *F. nodosus* were used for the inoculation of two feet. Moderate lesions developed which were successfully transferred to a third foot.

(b) Cultures of *F. nodosus* and *Sp. penortha* were used for the inoculation of two feet on each of two sheep. Severe foot-rot developed in all four feet and in two other feet on the same sheep inoculated with material from the former.

(c) Cultures of *F. nodosus* and the motile fusiform were used for the inoculation of two feet on each of two sheep. These four feet developed quite mild lesions of foot-rot, even more mild than those produced by *F. nodosus* alone in Experiment 5 (a). Similar mild lesions developed in the other two feet inoculated with material from the former.

(d) Cultures of *F. nodosus*, *Sp. penortha*, and the motile fusiform were used for the inoculation of two feet on each of two sheep. Severe foot-rot developed in these four feet and in two other feet infected from them.

(e) Two feet on each of two sheep were inoculated with material from natural foot-rot lesions as positive controls. On one sheep the two feet developed lesions exactly comparable with those produced by *F. nodosus* and *Sp. penortha* in Experiment 5 (b) and (d), except that they developed more rapidly. The other sheep's feet developed lesions which were not quite so severe as those in Experiment 5 (b) and (d).

(f) Two feet on each of two sheep were scarified but not inoculated. The scarification lesions healed completely in a week.

Experiment 5.

Cultures of *F. nodosus* were used for the inoculation of two feet of each of 2 sheep. Mild lesions developed but after two weeks the lesions on the feet of one sheep had healed. In the other sheep severe foot-rot developed in one foot and mild lesions in the other and these were successfully transferred to a third foot. The lesions were observed over a period of four months without healing. Several other sheep were infected from this animal.
Experiment 7

This experiment was conducted at the laboratories of the Rockefeller Institute of Medical Research, Division of Animal Pathology, Princeton, N. J., U.S.A., using cultures isolated from a case of foot-rot occurring in a flock in New Jersey. Four groups were maintained under conditions of strict isolation. Experiment 7 also differed from the previous experiments in that the experimental animals were crossbred ewes (principally Dorset and Suffolk "blood"). These sheep appeared to be somewhat less susceptible to foot-rot, when inoculated with material from lesions of foot-rot or with cultures, than the Merinos used previously. In each of four groups there were two sheep, each inoculated in two feet.

(a) Culture of \( F. nobiles \) was used as inoculum. Two feet developed moderate lesions of foot-rot, one developed mild lesions which healed 4 weeks after inoculation, and the fourth foot developed only transient lesions which healed 2 weeks after inoculation and could not be described as foot-rot. When material from these feet was used for the inoculation of two other feet on these sheep, only mild transient lesions developed.

(b) Cultures of \( F. nobiles \) and \( Sp. penorrhys \) were used as inoculum. Two feet developed severe lesions of foot-rot and one foot moderate lesions. Two and three weeks after inoculation these lesions were more severe than those produced by \( F. nobiles \) alone in Experiment 7 (a), and quite as severe as those produced after inoculation of material from foot-rot in Experiment 7 (d). Five weeks after inoculation the lesions were still severe in one foot but in the other two feet they had regressed and only mild lesions remained. In the fourth foot only mild lesions developed and these had completely healed five weeks after inoculation. Material from the first four feet inoculated on two others on the same sheep produced mild lesions of foot-rot in one foot and no lesions in the other.

(c) Culture of \( Sp. penorrhys \) was used as inoculum. No lesions developed. The necrification wounds had healed one week after inoculation.

(d) Material from natural foot-rot was used as inoculum. Before lesions of foot-rot developed in three feet, moderate lesions in one foot. Five weeks after inoculation the lesions were still severe in two feet but were only mild in one foot, while the other had healed. (When two other sheep of the same breeding were inoculated with material from natural lesions, one developed severe foot-rot in both feet but the other developed only slight lesions which healed in two or three weeks).

(c) Bacterial flora of experimental cases.

Smears were examined from all experimentally produced lesions at least twice a week during the period of observation. Miscellaneous cocci and corynebacteria were always numerous as in natural foot-rot, \( F. nobiles \) could always be found in lesions when it had been deliberately introduced. Likewise \( Sp. penorrhys \) and the motile fusiform were numerous in all lesions in feet inoculated with them. Thus smears from lesions produced by inoculation of feet with these
three organisms gave the same appearance as in natural foot-rot.

The motile fusiform was also found in lesions on all 26 feet not inoculated with it. It could usually be found a week or ten days after inoculation and was usually in sufficient numbers to be definitely recognizable morphologically, though in a few cases it was not possible to be sure of it.

In the four feet in Experiments 2 (a) and 2 (c), Sph. penortha was not present in the inoculum but was found in swarms from lesions, possibly as a result of contamination from other experimental animals. It was also found in one foot in Experiment 5 (c) four weeks after inoculation, just before the termination of the experiment. It could not be found in the lesions on the other 14 feet not inoculated with it, two of these being observed over a period of four months whilst the sheep was running on pasture.

(d) Analysis of results.

Experiments 1, 2, and 3, which were of a preliminary nature, are complicated (i) by the fact that organisms other than the three principal ones under consideration were used for inoculation in some cases, (ii) by the use of weaners in Experiments 2 and 3, and (iii) by Sph. penortha making its appearance in lesions in Experiment 2 when it had not been in the inoculum. Therefore the results of only Experiments 4 to 7 will be analysed.

According to the nature of the inoculum the experiments can be grouped and discussed under four heads, A, B, C, and D.

**Group A.** - *F. nodosus,* Sph. penortha, and the motile fusiform were used together on 6 feet in Experiments 4 and 5 (d). Five feet developed severe foot-rot and one moderate foot-rot.

**Group B.** - *F. nodosus* and Sph. penortha were used on 8 feet in Experiments 5 (b) and 7 (b). Six developed severe, one moderate, and one mild foot-rot.

**Group C.** - *F. nodosus* and the motile fusiform were used on 4 feet in Experiment 5 (a) and all developed mild foot-rot.

**Group D.** - *F. nodosus* was used alone on 10 feet in Experiments 5 (a), 6, and 7 (a). One developed severe, four moderate, and two mild foot-rot, and three no foot-rot. (The lesions which healed within 2 weeks of inoculation are not regarded as foot-rot).

By comparing the lesions produced in group A with those in B, and those in C with those in D, no suggestion is obtained that the inclusion of the motile fusiform in the inoculum had any effect on the resultant lesions either in the presence or absence of Sph. penortha.

Since the motile fusiform apparently had no effect when included in the inoculum, and, what is more important, since it was present in all lesions shortly after inoculation whether included in the inoculum or not, this organism may be disregarded, and groups A and B added together and groups C and D added together.
GROUPS A AND B. — Fourteen feet were inoculated with
F. noduleus and Sp. penorhca: 11 developed severe, 2 moderate,
and 1 mild foot-rot.

GROUPS C AND D. — Fourteen feet were inoculated with
F. noduleus without Sp. penorhca: 1 developed severe, 4
moderate, and 6 mild foot-rot, and 3 no foot-rot.

The numbers in the groups are small, the classification
of lesions purely arbitrary and subject to the personal
factor, and several different experiments have been grouped
together; it does not seem justifiable therefore to apply
statistical methods or to draw definite conclusions.
Nevertheless the results indicate that it is very probable
that the addition of the spirochaete to the inoculum enhanced
the severity of the lesions.

(e) Summary

1. Inoculation with F. noduleus alone usually produced
foot-rot which was not severe, but together with Sp. penorhca
it usually produced typical severe foot-rot.

2. Inoculation with Sp. penorhca alone produced no
lesions, but together with F. noduleus it probably enhanced
the severity of lesions caused by the latter.

3. Inoculation with the motile fusiform alone was
without effect. Its addition to an inoculum of F. noduleus
or F. noduleus and Sp. penorhca was without modifying effect.
This organism made its appearance naturally in all lesions
where it had not been deliberately introduced.

THE AETIOLOGICAL SIGNIFICANCE OF VARIOUS
BACTERIA IN FOOT-ROT.

(a) F. noduleus.

The data obtained regarding this organism are summarized
as follows:

1. It has been found in all cases of the disease which
we have been able to examine adequately and has been present
in the majority of smears taken in the field. Its absence
from some smears from the field does not necessarily mean
that it was not present in the lesions from which they were
taken, for it is seldom numerous in smears and sometimes it
may not be found in a single smear from a lesion in which
its presence is revealed by subsequent smears.

2. Foot-rot comparable in all respects with the natural
disease has been reproduced by inoculation of sheep with
cultures of F. noduleus. The "severe", typical disease
usually has been produced only when F. noduleus has been shown
introduced together with Sp. penorhca, but the latter has
been shown to be incapable of producing lesions when intro-
duced without F. noduleus.

It might be argued that the production of foot-rot by
F. noduleus under the conditions obtaining in the experiments
does not prove that this organism is the primary cause of
foot-rot, because there is contamination with environmental
bacteria. However, in a large series of tests it has been
shown that the common organisms in the environment are not
capable of producing foot-rot in the absence of F. noduleus,
and while it is not contended that they may not play some secondary role, this does not affect the conclusion that F. nodosus is the primary cause. There are many precedents to support this view. For instance, if an organism sets up a respiratory disease when inoculated intranasally, or an enteric disease when ingested, it is accepted as being the causal agent although it becomes mixed with other organisms when inoculated.

3. F. nodosus is consistently present in and recoverable from lesions of the disease produced by using cultures as the inoculum.

4. After a prolonged search we have been unable to discover any other infective agent capable of producing the disease, or anything to suggest that such an agent exists. Although this evidence is of a negative nature, and of little value in itself, it lends greater significance to the fact that F. nodosus is capable of reproducing lesions of typical foot-rot.

It is therefore concluded that F. nodosus is the primary causal agent of foot-rot in sheep, but other bacteria probably also play a part in the pathogenesis of this disease which always occurs as a mixed infection.

(b) *Sp. penortha*.

*Sp. penortha* is probably always present in lesions of the disease occurring naturally (see Section 2(c) of Bacteriological Studies).

1. Experimental cases in which *Sp. penortha* was not present. In 6 feet on 3 sheep we produced foot-rot in which the spirochaete did not appear by inoculation with natural material exposed to air in a thin layer for 5 to 10 hours before use. One of these sheep was observed for 12 weeks, and for over a month there were lesions between the digits with an unusual amount of tissue damage but no extension under the sole. Later the soles became involved and showed more tissue damage than has been seen in ordinary cases of foot-rot. Two attempts to transfer the disease from these feet to another adult Merino failed, although the latter was subsequently shown to be susceptible. On a later occasion material from these lesions was inoculated onto a Merino "samer" and an equal amount of the same material was mixed with culture of *Sp. penortha* and inoculated onto another weaner. The latter weaner developed foot-rot in both inoculated feet, the former in neither. The other two sheep in which foot-rot was produced in the absence of the spirochaete showed typical, rather severe lesions of foot-rot, but an attempt to transfer the disease from one of them to another sheep failed.

These observations demonstrated that, in the absence of *Sp. penortha*, lesions of typical and severe foot-rot may develop, though in one of the three sheep the lesions took an unusual form. There is a definite indication that in the absence of *Sp. penortha* the disease is less readily transferred by inoculation to other sheep. This is not altogether in accordance with the results of experimental reproduction of the disease with pure cultures, where in the absence of the spirochaete the lesions were usually less severe but more transferable. It does, however, provide further evidence that the spirochaete plays a part in the pathogenesis of foot-rot.
(i) Occurrence of Sp. penortha in nature. The following observations were conducted to determine whether Sp. penortha is a common inhabitant of faeces, soil, or the skin of sheep in the absence of foot-rot.

A hundred feet of sheep were scarified; some were inoculated with F. necrophorus culture, and some with Staph. aureus culture, and some were left uninoculated. Smears were made from these feet on several occasions until the lesions healed. Approximately half of these feet had been affected with foot-rot but had recovered from 4 weeks to 4 months previously. While under observation most of the sheep were on pasture and some were in pens with concrete floors. In smears from 97 of the feet Sp. penortha was consistently absent, but in the other 3 feet it made its appearance. Two of these 3 feet had previously been inoculated with culture of the spirochaete, 4 weeks and 3 months respectively, without developing lesions. Possibly some of the inoculated organisms had survived on the feet, but this does not seem probable. The appearance of the spirochaete in the third foot was probably due to contamination from an infected sheep which accidentally gained access to the same enclosure a few days previously.

In experiments on the longevity of the infective agent in material from lesions of foot-rot, over 100 feet of test animals were inoculated with material at various periods after it had been taken from lesions. Sp. penortha was consistently absent from the feet which did not develop foot-rot, and was present in all those developing foot-rot except the 3 sheep described earlier in this section.

Smears from four sheep in the field with wounds in the feet showed no Sp. penortha. An experimental sheep at the laboratory which had been on a severely calcium-deficient diet for some months developed lesions in one foot which were indistinguishable clinically from foot-rot. Smears showed many Sp. penortha and motile filiforms, but no F. necrophorus. The lesions were not transferable by inoculation. Sp. penortha was absent from 6 smears from sheep’s teeth where other spirochaetes with regular waves were present.

These observations indicate at least that Sp. penortha does not occur commonly in sheep faeces, soil, or on the skin of sheep in the absence of foot-rot, and possibly not at all. We may recall that it made its appearance in 5 out of 19 feet inoculated with F. necrophorus, but the possibility of contamination from other experimental animals could not be excluded.

(iii) Summary. The evidence regarding the role of Sp. penortha in foot-rot may be summarized as follows:

1. It is probably always present in naturally occurring lesions of foot-rot, usually in large numbers at the margin of the healthy tissue.

2. It does not occur commonly and possibly not at all in the faeces of sheep, in soil, or on the skin of sheep in the absence of foot-rot.

3. Inoculated on sheep’s feet in the absence of F. necrophorus it produces no lesions.

4. Inoculation with Sp. penortha and F. necrophorus together probably causes more severe lesions than inoculation with F. necrophorus alone.
5. Foot-rot has been more consistently produced when Sp. penortha is included in the inoculum whether this consisted of cultures or material from lesions.

It is concluded that it is very probable that Sp. penortha plays a specific role as an accessory causal agent in foot-rot in sheep.

(c) The motile fusiform.

This organism is probably always present in lesions of foot-rot occurring naturally (see Section 2(d) of Bacteriological Studies).

In the experimental production of foot-rot with pure cultures in which it was not included, this organism made its appearance in lesions on all 25 feet. This may sometimes have been due to contamination from other experimental sheep, but the fact that it made its appearance in all instances, including Experiment 7 where there was no reasonable possibility of contamination, indicates that it commonly occurred in the environment, possibly in the faeces. Smears from feet sacrificed and inoculated with cultures of F. necrophorus or Staph. aureus sometimes showed organisms resembling the motile fusiform, but its morphology is not sufficiently distinctive to make its recognition easy on morphological grounds except when present in large numbers, which was not so in these smears.

As we have not been able to produce foot-rot without the appearance of this organism in lesions, little direct evidence has been obtained as to whether or not it plays a part in the pathogenesis of the disease, as is suggested by the study of histological sections of lesions. However, the disease could be reproduced consistently by inoculation with cultures of F. necrosue and Sp. penortha, although in smears from these lesions the motile fusiform could not usually be recognized until a week or more after inoculation. Therefore, the absence of this organism does not affect the infectivity of inocula or the development of early lesions. It was present in abundance in those three cases of foot-rot in which Sp. penortha was absent and which could not be transferred to other sheep.

The available evidence regarding the role of the motile fusiform in foot-rot may be summarized as follows:

1. It is probably always present in lesions of foot-rot, usually in large numbers adjacent to the healthy tissue, and penetrating ahead of other bacteria.

2. It occurs commonly apart from lesions of foot-rot.

3. It has no effect when inoculated on sheep’s feet in pure culture, nor does its additional presence affect the lesions artificially produced by F. necrosue or by F. necrosue and Sp. penortha.

Therefore the motile fusiform is regarded as a constant secondary invader in foot-rot. There is no definite evidence that it plays a significant part in the pathogenesis of the disease.
45.

(d) Miscellaneous bacteria.

F. necrophorous is commonly present in lesions of foot-rot, but we have not produced any experimental evidence that it plays a part in the pathogenesis of the disease. Typical foot-rot has been reproduced with inocula not containing F. necrophorous and in Experiment I where it was present in the inoculum along with F. necrophorous and Sp. penortha, there was no indication that it had any influence on the resultant lesions. The effect of its introduction with only F. necrophorous has not been studied. F. necrophorous is widespread in nature and is frequently found in various lesions as a secondary invader, and apparently that is the role it plays in foot-rot. Nevertheless it may cause some damage in the lesions, and may be responsible for complications such as infections of joints, tendons, and ligaments of the foot and abscesses in internal organs (the latter have been described by Hazenkamp, 1909, and Murnane, 1933).

There is no evidence that the pleuro pneumonia-like organism mentioned previously plays any part in the pathogenesis of foot-rot.

The miscellaneous cocci, oxyrobacteria, and other bacteria which are also present in foot-rot are probably all non-specific contaminants. Many if not all of these commonly present have been isolated and found by inoculation not to be capable of producing lesions of foot-rot. Nevertheless it is probable that non-specific aerobic organisms play a part by protecting the non-sporing, anaerobic F. necrophorous and Sp. penortha from destruction by the air. It has been shown that F. necrophorous and Sp. penortha are destroyed by exposure to air but survive longer in the presence of Staph. aureus. This protective effect was studied more fully with F. necrophorous on which also foot-rot exerts a similar effect (Beveridge, 1934a). Probably many other aerobes have a protective effect on anaerobes which are destroyed by air.

(a) Conclusions.

Foot-rot as it occurs in nature and as it has been studied experimentally is a mixed infection.

F. necrophorous is the primary causal agent and it is very probable that Sp. penortha is a specific, accessory, causal agent. The motile fusiform is a constant secondary invader and possibly plays some part in the pathogenesis of the disease. The continued survival of these non-sporing anaerobes is probably aided by association with the non-specific, aerobic bacteria which are present.

F. necrophorous has little invasive power, seldom being found in living tissues in sections. However, it produces a strongly proteolytic enzyme and the action of this on the epithelial tissue apparently enables Sp. penortha and the motile fusiform to invade the tissue.

Foot-rot may be regarded as a fusco-spirochaetoid disease since it is a complex infection with a fusiform and a spirochaete. However, it should be borne in mind that foot-rot differs from the diseases to which this term is usually applied in that it can be reproduced with cultures and in that both the fusiform and the spirochaete involved differ considerably from those described in the usual fusco-spirochaetal diseases.
V. STUDIES ON CONTROL OF THE DISEASE

1. Treatment.

Nearly all pastoralists with experience in treating foot-rot and nearly all writers on the subject emphasise that thorough surgical preparation of the foot is the most important part of treatment. With this we are in entire agreement. Also, it is a distinct advantage to treat cases as early as possible, while the lesions are confined to the interdigital surfaces and the soles, because then the whole extent of the infected tissue can be adequately exposed, also the tissue here responds well to treatment. When the infection involves the long laminae of the wall it responds less readily to treatment. It is important that treatment be repeated at intervals of not more than two or three days until a complete cure is effected. When a foot is extensively involved, it is frequently not possible to expose every infected pocket at the first treatment, especially when the field becomes masked with blood.

In dealing with an outbreak of foot-rot, vigorous treatment should be instituted as soon as the outbreak commences. Where practicable, affected animals should be isolated to check the spread of the infection to some extent. All sheep in the flock should be passed through a foot-bath containing 10 per cent. copper sulphate or 2 per cent. formalin every week as a preventive measure. By these means the disease can be kept in check, but if neglected it may get out of hand and a serious outbreak ensue.

For the surgical preparation of the foot suitable instruments are essential and these should be kept sharp. A sharp-pointed strong knife with a wooden handle and a pair of secateurs with two cutting blades are suitable. The essential operation was well described 100 years ago by Youatt: "every portion of horn that is the slightest degree separated from the parts underneath must be cut away."

Many pastoralists and some writers emphasise the need for caution to avoid making the foot bleed and especially cutting the artery in the point of the toe. Bleeding is undesirable in that it masks the field and interferes with the action of the medicament, but it is preferable to cause bleeding rather than risk missing a pocket of infection. Some pastoralists state that cutting the artery at the point of the toe produces a permanent deformity. The writer deliberately cut this artery in several affected sheep; bleeding continued for an hour in some cases but the total loss of blood was negligible. These feet later became perfectly normal in shape. However, it has been observed that when the living tissue has been badly cut, healing is retarded considerably. This is undesirable, especially because such wounds provide a ready portal for re-infection.

In applying the medicament the individual foot may be dipped into a pot containing it or the sheep may be walked through a foot-bath. A 30 per cent. solution of copper sulphate or 10 per cent. solution of formalin is recommended.

If the foot is immersed for about 10 seconds in strong solutions such as those recommended, the exposed surface gets thoroughly wetted and sufficient remains there to exert an effect for a considerable period. Probably the curative
action takes place subsequent to immersion. Although, generally speaking, it is perhaps advantageous to treat infective processes by prolonged applications with a dilute solution, this is often not practicable with animals, especially flock sheep. After a brief application of a concentrated solution, the medicament remaining on the tissues continues to exert its effect for a considerable period until it is removed or diluted beyond its effective concentration. The brief application of a strong solution, provided it is not harmful, is often the method of choice with animals and may not be appreciably less effective than prolonged application of a weak solution.

For these reasons it is felt that it is open to question whether Murmane's (1933) method of standing sheep in a large foot-bath for at least an hour would give sufficiently better results to warrant the additional time and expenditure involved. Moreover, when only one foot is affected, the sheep usually holds it out of the solution nearly all the time. Until this method is demonstrated by controlled experiments to be distinctly superior the writer favours the simpler procedure, laying emphasis on the need for treatments being repeated every 2 or 3 days.

Treatment as outlined above cures the large majority of cases in one, or at most, two, weeks. Repeated treatments at intervals of two or three days may involve more work and handling of sheep than many pastoralists are willing to undertake when large numbers are involved, but by this means cures are effected, whereas infrequent treatments are often not effective.

Various medicaments tested.

No opportunity has occurred for testing medicaments on an adequate experimental scale, but some tests have been made on about 100 feet and the general impressions were as follows:

Copper sulphate in 30 per cent. solutions had a good curative action but even with careful paring two or three treatments were often required, probably because it was often not possible to expose every infected area at the first treatment. Repeated treatments hardened the horn and rendered subsequent paring more difficult.

Formalin in 5 per cent. and 10 per cent. solution had a good curative effect, 10 per cent. being probably better than 5 per cent., and probably better than 30 per cent. copper sulphate. Feet of an adult Merino ewe and woeher were dipped in 10 per cent. formalin three times a week for a month. At the end of this period the hooves were not hardened and the skin appeared normal except for some epithelial exfoliation and slight crust formation.

Sodium arsenite in 2 per cent. solution caused severe pain lasting over 24 hours and the lesions did not heal well. A 1 per cent. solution caused severe pain but had a fairly good curative effect.

Tartar emetic and lancing, equal parts, as recommended by Howarth (1930b) had a good curative effect but in some feet the skin between the digits healed very slowly, probably owing to damage from the medicament.
Zinc sulphate in 10 per cent. solution had a moderately good curative effect but is probably inferior to copper sulphate.

Novarsenobillon inoculated intravenously in doses of 0.9 g. on six occasions at intervals of 2 to 5 days had no curative action in one sheep tested, and Sp. penorhëa did not disappear from the lesions.

Lactic acid in 25 per cent. solution had a good curative effect on most feet but did not give consistently good results.

2. Immunisation.

An attack of foot-rot does not confer immunity to a subsequent attack, and a sheep with one or two feet affected can be infected in another foot naturally or by inoculation. The writer has infected a number of sheep a second time and usually they developed the typical disease, though in a few cases the lesions developed slowly and were mild.

Several sheep were vaccinated with either F. necrophorus Sp. penorhëa, or the motile pusiform and developed foot-rot later when inoculated. One sheep was inoculated three times with killed cultures of F. nodosus subcutaneously and later when inoculated with infective material from foot-rot it developed lesions of the disease which, however, were definitely milder than usual. Vaccination with a mixed vaccine containing F. nodosus, Sp. penorhëa and the motile pusiform may possibly be found to be more effective. However, from consideration of the chronic and relatively superficial nature of the lesions, foot-rot is not the type of disease in which the prospects of successful immunisation would be considered good.

Since it is very probable that the disease can be completely and easily controlled by eradication, no serious investigation has been made with vaccination, but if eradication should prove impracticable under certain conditions, further consideration might be given to prophylactic vaccination.

3. Control by Eradication

(a) Introduction.

In 1857 Youatt recommended that sheep infected with foot-rot should be removed from the flock and not returned until the lesions had healed, and this recommendation is made by many subsequent writers on the disease. In 1890 Nett recommended avoidance of introduction of affected sheep into flocks, and in 1904 Mohler and Washburn repeated this advice and stated that healthy sheep may be safely departed on land previously occupied by infected sheep provided that a winter's frosts had intervened. In 1905 Bauman stated that foot-rot seems very difficult to eradicate from a farm, as it keeps recurring although the farmer is positive he has got rid of it, and a farm once infected seems to retain the infection for years.
Nevertheless, he maintained that it is possible for a farmer to rid his farm of the disease, and he advised that healthy sheep should not be placed on contaminated pasture until it has been unstocked for 10 months. In Denmark, striking success has recently been reported following attempts at control by slaughter of affected animals, but control has been found very difficult in marshy areas affected for many years, apparently owing to the difficulty of enforcing rigid quarantine (Hansen, 1936). In Australia no attempt had been made to deal with foot-rot by eradication or quarantine until this was proposed by the writer in 1935 after finding that the disease was not caused by F. necrophorus but behaved as a specific contagious disease.

The fact that until recently there have been no attempts made to control foot-rot on a large scale by eradicating it from districts where it is endemic has probably been due to: (i) the belief that the causal agent is F. necrophorus which is considered to be a normal intestinal inhabitant of herbivores; (ii) the lack of information on the longevity of the infection in the soil or in association with sheep; (iii) the belief held by many pastoralists and some veterinarians, at least in Australia, that the disease is caused by lush wet pastures and non-specific infection and can arise sporadically, and (iv) the lack of a satisfactory means of differentiating foot-rot from other foot infections.

Now that it has been shown that foot-rot has a specific causal agent which probably only occurs in association with the disease, a rational basis for complete and permanent eradication is established, also a satisfactory means of diagnosis is provided.

Epidemiological studies reported in an earlier section of this monograph revealed that under the conditions usually prevailing in Australia the contagion of foot-rot survived during the dry summers on the feet of some members of the flock but not on the soil. Furthermore, those sheep capable of carrying the infection for more than one or two weeks can be detected on close examination. A scheme for the eradication of foot-rot based on these principles was proposed by the writer in 1935 and discussed in detail in 1938 (Beveridge, 1935 and 1938a).

(b) Scheme for eradication.

The basis of the scheme is the removal during the summer of all animals which carry the infection in their feet. In most districts in Australia where sheep are subject to foot-rot, during the summer the pasture becomes dry and brown and foot-rot does not spread. Where these conditions do not obtain, the control of foot-rot presents a slightly different problem which will be discussed later.

If the disease has been prevalent in the flock, efforts should first be made to reduce the number of affected animals during November and December by intensive treatment. Unless the number infected is reduced to 5 per cent, at most - preferably much lower - the subsequent procedure is not only more difficult but also more liable to failure.

During January and February every sheep on the property should be caught and its feet carefully examined by a responsible and experienced person. It is advisable for at least two weeks to elapse between the last occasion on which treatment was carried out and the general examination for removal of "carriers" commenced. The hooves should be
well pared if they are at all overgrown, have bulging walls, or depart in any way from a perfectly normal shape. The following sheep should be searched for and removed: (a) those with foot-rot; (b) those in which a pocket of infection exists under the horn; (c) those with hairless moist skin between the digita. It is the second of these categories which requires most attention. In flocks which have recently been subjected to an outbreak of foot-rot a certain number of sheep are found with hooves appearing healthy but slightly misshapen which after careful paring are found to harbour a pocket of infection, over which the horn has healed (see Figs. 18 a, b, c, and d). Unless feet are pared conscientiously and carefully, some of these are likely to escape detection.

On some properties quite a large proportion of the sheep show what might be termed "dry, shelly wall". A section of the outside wall, usually shaped like a half-moon, is separated from the underlying tissues and the crevice thus formed is packed with soil and faeces. When the detached horn is removed and the soil scraped away the underlying structures are dry and healthy, there being no pus, exudate, or other evidence of infection. These sheep may be passed as clean after they have been pared and examined to make sure that there is only dry dirt and no infection present. It is wisest to regard any other lesion as suspicious and necessitating removal from the flock.

Those sheep passed as healthy should be put through a foot-bath containing copper sulphate solution, 5 or 10 per cent., or formalin, 2 per cent., and then returned to their paddock.

The infected sheep and suspected ones must be kept in quarantine in a well-fenced paddock or sold for slaughter. They may be treated but must not be returned to the flock until they have been passed as healthy at two examinations with a month's interval between. Two examinations a month apart are necessary because this group often contains a number of very chronic cases with a tendency to relapse and because sheep which have recovered may carry the infection on the feet for 1 or 2 weeks but not four weeks. Further, if some of this group are still infected, the recovered ones among them should not be returned to the flock once the autumn rains have set in because they may then be in the incubation stage.

On irrigated pasture or where the climate is such that the pasture remains green during the summer and foot-rot may spread at that time of the year, the measures advocated for control on any pasture may be carried out with the modification that the healthy sheep are returned to a paddock which has been unstocked for two weeks. Where a large proportion of the flock is affected as often happens on irrigated pasture, even during the summer, intensive treatment of affected sheep every 2 or 3 days is first necessary. Furthermore, it may be necessary to pare the cured ones and not affected ones through a foot-bath every 2 or 3 days to avoid relapses or infection developing in sheep passed as healthy during the incubation stage. The spacing period of 2 weeks allows an extra margin for safety, and, if it causes inconvenience, could be reduced to one week without appreciable risk. Where even this period is impracticable it could perhaps be omitted altogether and instead the healthy sheep passed through a foot-bath every 2 or 3 days for one to two weeks, although there is no direct evidence
that this method is effective. An alternative method which is sometimes practicable with small flocks on irrigated pasture is to dispose of all the infected flock and restock with a healthy flock after spelling the pasture for 2 weeks.

When elimination of infected animals is carried out during the summer on dry pasture there is no need for leaving paddocks unstocked for them to become decontaminated. Under these conditions the sheep are not susceptible and will not become infected from any infection which may remain on the pasture for a few days.

Any goats which there may be on the property should be included in the flock for consideration during the eliminating of carriers. Cattle in all probability need not be considered. An experiment described in the section on epidemiology shows that it is possible for cattle to carry the infection, but, as far as is known, they do not become infected naturally.

Once the property has been freed of the disease, precautions must be taken to avoid its re-introduction by examination of the feet of introduced sheep. The most serious difficulty here is the danger from travelling stock when unfenced stock routes pass through a property. Roads, yards, etc., may remain contaminated for 5 to 7 days, though after a day the risk is probably negligible. Tasmania is the first, and so far the only, State in Australia to bring in regulations prohibiting the travelling of sheep affected with foot-rot. Until the other States also offer this protection to the progressive grazier who eradicates the disease he will remain exposed to a serious risk of becoming re-infected.

However, the objective of animal disease control authorities should go further than protecting the progressive grazier and aim at eradicating the disease from the country. Once this is accomplished the only control measures necessary will be examination of imported sheep at quarantine stations.

VI ACKNOWLEDGMENTS

The writer wishes to express his thanks to his colleagues, particularly Dr. J. Clunies Ross, Dr. R. R. Carne, and Mr. L. A. Gill, for much helpful advice, and to certain pastoralists who have given assistance with the field work.

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APPENDIX.

FIELD TRIALS ON ERADICATION OF FOOT-ROT.

By

W. I. B. Baveridge, D.V.Sc.

and


Field trials on the eradication of foot-rot were commenced by one of us at the beginning of 1936. However, owing to a succession of abnormally dry seasons and consequent very low incidence of foot-rot, it was not until 1938 and 1939 that conditions in the field were such that an adequate test of the scheme was obtained. Those trials which were fruitless, owing to the absence of foot-rot in control mobs, need not be described here.

Trial No. 1.

This trial was conducted at the Victorian State Research Farm, Werribee, on a paddock of highly improved, irrigated pasture, Block 12a. During 1935 and 1936, 60 to 70 per cent. of sheep grazed on this paddock had been affected with foot-rot at all times of the year. In February, 1937, the infected flock was disposed of and the pasture left unstocked for two weeks. Two hundred sheep were obtained from a district believed to be free of foot-rot and their feet were examined and found to be healthy before they were placed on this block of irrigated pasture.

They were taken from their paddock for routine procedures such as shearing and dipping and when returned they were passed through a foot-bath of either copper sulphate solution or solution of formalin. There were four such occasions in the first ten months. During the 22 months that they were kept under observation they remained entirely free of foot-rot although they were grazing on highly improved irrigated pasture at the high rate of stocking of 11 ewes and their lambs to the acre, and under seasonal conditions which favoured the persistence and spread of foot-rot in sheep in adjacent paddocks. Lambs dropped during the course of the trial also remained healthy.

Trial No. 2.

This was conducted in the Western District of Victoria, on a property specially chosen because over a period of many years foot-rot infection continually occurred in sheep depastured on it. The infection rate had been so heavy that it was claimed that foot-rot would develop in any flock of sheep introduced there. In other words, it was considered that the favourable environmental conditions on the property when the soil was wet would invariably lead to an outbreak of foot-rot among any sheep grazing upon it.

The trial was commenced in mid-January, 1938. In a paddock of about 170 acres, an area of approximately 15 acres was fenced off so as to include typical pasture, a dam with a
muddy bank, and portion of a small pine plantation, all of which were features of the property which were considered to provide a suitable environment for the initiation of infection.

The test paddock was kept free of sheep for a period of six weeks to ensure that the specific micro-organisms on the soil or pasture had died before new sheep were introduced. However, in view of experiments already described, some of which were conducted subsequent to this experiment, it is apparent that six weeks is an unnecessarily long period and that two weeks would have sufficed.

After this period had elapsed, 73 crossbred wether weaners were transported by motor truck from a property free from foot-rot, and 50 were placed in the "test paddock." All feet of these sheep were carefully inspected and were found to be free from foot-rot; as an extra precaution they were transferred from the motor truck to the "test paddock" through a foot-bath of bluestone solution. To serve as controls, the remaining 23 sheep were similarly treated but were placed outside the "test paddock," and were grazed with the original infected flock on presumably contaminated pasture.

At mid-July some of the control weaners were reported to be affected with foot-rot. One of us (T.S.G.) inspected the control group at the beginning of September, and at that time six of the 23 appeared to be affected with typical foot-rot, and the diagnosis was confirmed by microscopic examination of smeared of material taken from the lesions. It was considered that this adequately proved the susceptibility to foot-rot of the clean sheep introduced into the experiment, and, as the owner was desirous of preventing further infection in the control group, they were removed from the experiment for treatment.

During the time that had elapsed since the commencement of the trial, the 50 sheep in the "test paddock" were separated only by a wire fence from the surrounding paddock containing infected sheep. They were constantly observed by the District Veterinary Research Officer and less frequently by ourselves, but foot-rot was never detected. They were kept in the same paddock for a period of eighteen months, including the rainy months of two successive years, and remained free from infection. During the wetter periods of both these years the seasonal conditions favoured the development of foot-rot, which was prevalent on other parts of this property and on other properties in the district.

Trials Nos. 3, 4, and 5.

In the district in which Trial No. 2 was conducted, trials were commenced on three properties in January, 1936 and 1937. These properties carried 12,000, 14,000 and 20,000 sheep respectively. On each a large portion of the pasture had been improved. The average annual rainfall is between 22 and 24 inches. For at least four or five years prior to 1936 there had been outbreaks of foot-rot every year; on an average from 10 to 15 per cent. were affected but at one time 75 per cent. of the sheep on one property were infected.

The trials initiated by us were fruitless as the seasons were very dry in 1936 and 1937 and there was no outbreak of foot-rot. However, the owners took it on themselves to eradicate the disease from the whole of the flocks, along the
lines demonstrated in the trials. In this they were aided by the long dry period. In 1938 and 1939 seasonal conditions again favoured outbreaks on neighbouring properties in the district but these three large flocks remained free of the disease.

**Trial No. 6.**

This trial was of the same type as Nos. 3, 4, and 5, but was conducted in southern New South Wales. About 2,000 out of a total 26,000 acres on the property are improved and the average annual rainfall is 25 inches. Approximately 30,000 sheep are carried and about 10 per cent. are affected with foot-rot in an average year and very many more in an unusually wet season. For several years prior to the commencement of trials in January, 1936 and 1937, outbreaks had occurred every year, but during these two years the rainfall was abnormally low and there was no outbreak. The manager completed the elimination of carriers from the whole flock during 1937.

From that time until the autumn of 1939, the rainfall was so low and the pastures so dry that foot-rot seemed to have disappeared from that portion of the State.

In the autumn of 1939 the rainfall was excessive, an amount equal to the annual average being received in a period of eight weeks. Lesions was soon apparent in the flock but when we visited the property we found that it was due to digital suppuration and foot-rot was not present. On neighbouring properties the sheep were observed to be suffering from both diseases. During the long dry period the number of carriers on the neighbouring properties had been reduced to a low figure with the result that it took several weeks for the epidemic to gain momentum and involve large numbers. During the autumn, winter, and spring of 1939, foot-rot was very prevalent throughout the district, but on the trial property no case was found. The outbreak of digital suppuration subsided towards the end of the autumn.

**Conclusions:**

1. Flocks of sheep free of foot-rot infection will remain free of the disease indefinitely even when grazing on lush wet pasture which in the past has been regarded as most favourable for the development of the disease. Our a priori reasoning that the disease cannot arise spontaneously has been fully substantiated.

2. The eradication of foot-rot from several large flocks has been successfully carried out by stockowners during dry seasons.

**ACKNOWLEDGMENT.**

We have pleasure in making grateful acknowledgment to those pastoralists whose whole-hearted co-operation enabled these trials to be conducted successfully. Our thanks are also due to Mr. H. A. Muller, Director of Agriculture, Victoria, and to Mr. H. C. Wilson, Manager of the State Research Farm, Werrine, for the facilities afforded there, and to the Senior Irrigation Officer, Mr. T. E. Berulden, for controlling these trials.
Fig. 1 (above).—Map showing distribution of foot-rot in Australia.
Fig. 1 (below).—Map showing average annual rainfall and the areas where there are no sheep.

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PLATE 2.

Fig. 2 (above).—Section of foot rot lesion. Hem. and Eosin. X 65.

Fig. 3 (below).—Section of foot rot lesion. Hem. and Eosin. X 30 (ux.).
Fig. 4 (above).—Foot-rot. Horn removed from sole showing infected epidermis.

Fig. 5 (below).—Section from foot-rot showing F. nadorum (A), S. peloroba, and the mobile fusiform, unusually free from other organisms. Gentian violet. × 1250.
PLATE 4.

Fig. 6 (top).—Smear from foot-rot showing mobile fusiforms and one F. nodosum with other organisms clumped around H. Gentian violet. × 1200.

Fig. 7 (centre).—Smear from foot-rot showing many F. nodosum. Gentian violet. × 1200.

Fig. 8 (bottom).—F. nodosum from 2-day surface culture. Gentian violet. × 1200.
PLATE 5.

Fig. 9 (top, left).—Colonies of *F. melone*, 2 days on horse serum-blood-"V-E." agar. × 20.

Fig. 10 (top, right).—Culture of *F. melone*, 5 days on horse serum-blood-"V-E." agar, showing large colonies surrounded by secondary colonies. × 20.

Fig. 11 (bottom).—Culture of *F. melone*, 4 days in 10 per cent. horse serum-"V-E." agar.
PLATE 8.

Fig. 12 (top, left).—*Sy. pseudonana* from surface culture. Osmic acid. Gentian violet. X 1,000.

Fig. 13 (top, right).—*Sy. pseudonana* from culture. Heated carbol-fuchsin and subsequently Gentian violet. X 900.

Fig. 14 (bottom, left).—*Sy. pseudonana* from culture. Heated carbol-fuchsin. X 900.

Fig. 15 (bottom, right).—The motile fusiform from culture. Gentian violet. X 900.
PLATE 7

Fig. 16 (above).—Surface colonies of the motile fusiform, 2 days. × 65.

Fig. 17 (below).—Section of foot-rot lesion showing Sp. porcatha and the motile fusiform. Muray and Fielding's silver impregnation. × 750.
Fig. 18.—A misshapen hoof of the type which often carries a focus of infection under the horn. 

(a) (top, left)—side view, showing wall overgrown and anterior edge budding. 

(b) (top, right)—antero view showing both hooves wider than normal. 

(c) (bottom, left)—view of soles showing cracks and departure from normal shape. 

(d) (bottom, right)—same foot properly pared and found healthy.