

# ON THE PATHOLOGY OF BOVINE ACTINOMYCOSIS<sup>1</sup>.

A PRELIMINARY REPORT.

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### *Introduction.*

ATTENTION has recently been directed to the subject of bovine actinomycosis and its bearing on the public health by the discovery on the part of the health authorities at various ports in this country of large numbers of imported ox tongues showing evidence of actinomycotic infection. A considerable amount of work has been done, both in this country and abroad, upon the pathological condition known as actinomycosis in animals and man, and it has been shown that the disease, in relation to the causal agent, is not a single pathological entity; but in many respects the etiology of the bovine and the human disease and the inter-relationship of the two have not been fully elucidated. With the desire of obtaining fuller information on these matters, the Local Government Board have referred the subject to their Pathological Laboratory for investigation.

<sup>1</sup> Reprinted from *Reports to the Local Government Board on Public Health and Medical Subjects* (n.s. No. 107) 1915, by permission of the Controller of H.M. Stationery Office.—Ed.

This preliminary report deals with the following specimens of bovine actinomycotic lesions which have been sent to the Board's Laboratory for examination between April 8th, 1914, and January 7th, 1915: 50 frozen tongues and lymphatic glands from the lingual region, of which 46 were imported from Argentina, 2 from North America, and 2 from Siberia; 44 fresh specimens from animals slaughtered in this country.

I am greatly indebted to Dr Eastwood for his constant cooperation in this investigation. Acknowledgments are due to the Board of Agriculture and to Professor Woodhead for facilities for certain inoculation experiments; to Dr Litteljohn for organising the collection of pathological material; to Mr Young, Veterinary Inspector for the City of London, Mr Hayhurst, Superintendent, Metropolitan Meat Market, and Mr Cuckney, Brighton Borough Abattoir, for many valuable specimens.

The further stages of this investigation are postponed in view of the emergency work connected with the war.

*Actinomycotic Lesions in Ox Tongues Imported from Foreign Countries.*

As evidence of the frequency of the disease, I may mention that Dr MacFadden, in his report on the work of Inspectors of Foods during the year 1913-14, states that between August 1st and October 28th, 85,445 tongues were examined in the City and Port of London, of which 4949, or 5·8 per cent., were found to be affected with actinomycosis, chiefly in the glands.

With one exception, the forty-six Argentine specimens received at the Laboratory for examination consisted of diseased lymphatic glands which had been removed from forty-five tongues; the remaining specimen was a diseased tongue. The lesions in these glands presented the characters of actinomycosis, and, though individual cases differed in certain macroscopical features, could by summary microscopical examination readily be identified as of similar nature. Occasionally tuberculous lesions were found in lymphatic glands affected also by actinomycosis.

Of the total number of Argentine specimens received and identified as being of an actinomycotic nature, twenty-six were subjected to a more minute examination, which included the isolation of the specific granules under the microscope, the application of various staining

methods, particularly Gram's, and the attempt to recover the causal organism in culture. A histological examination by means of paraffin sections was made of eleven cases.

The following is a summary of the anatomical and microscopical characters of the lesions in the lymphatic glands. In some glands there were single nodules not larger than a small pea, while in others the whole glandular tissue was replaced and there was a considerable increase over the normal size of the gland. The smallest lesions were grey, the largest yellow or reddish. They all consisted of soft moist granulation tissue, occasionally semi-purulent, surrounded by a capsule of fibrous tissue. The abundant formation of fibrous tissue was a characteristic feature, and the central soft tissue appeared to be under pressure, as it usually projected beyond the cut surfaces when a nodule was incised. In the granulation tissue, just visible to the naked eye like minute grains of sand, were numerous orange or yellowish foci. They were fewest in numbers in the early grey lesions and very numerous in the more advanced, to which they gave the characteristic yellow or orange pigmentation. They were generally gritty, and upon the presence or absence of calcareous matter appeared to depend the variation in colour, the soft, non-gritty granules being translucent and of an amber tint. Microscopically these foci or granules were composed of masses of radiating clubs, which either met at a point towards the centre or surrounded a central finely granular area. The clubs did not show a characteristic positive reaction when stained by Gram's method, though they sometimes offered a considerable resistance, most marked in the older lesions, to decolourisation by alcohol, retaining partially and irregularly the violet colouration. The centre of a granule never showed a Gram-staining mycelium, but was usually of a finely granular nature, and therein small aggregations of a minute bacillus could be demonstrated when stained by carbol-fuchsin or methyl violet. The clubs could be well stained in sections by the following methods: (1) carbol-fuchsin, decolourisation by weak hydrochloric acid, counter-staining with methylene blue; (2) Van Gieson's stain; (3) aniline gentian violet, followed by rapid treatment with alcohol.

Numerous attempts have been made to obtain the causal organism in culture from these lymphatic gland lesions, but without definite success. It is possible that the organism had died out on account of the time which had elapsed since the animal was killed, during which the tissues were maintained at a low temperature. Against this assumption, however, there may be mentioned the occurrence in culture

from two of the Argentine cases of an organism morphologically similar to one which has been obtained from fresh cases in this country. It was not grown in pure culture.

Inoculation experiments with emulsions of tissue from the actinomycotic glands into guinea-pigs (subcutaneously and intraperitoneally), a pig (subcutaneously), and a monkey (subcutaneously) have not been successful in the production of lesions.

The following is a description of the single Argentine case examined, in which the tongue itself was infected. Beneath the epithelium of the posterior portion of the dorsum of the tongue were scattered nodules, up to a centimetre in diameter, consisting of fibrous capsules enclosing yellowish gritty tissue which was softening and sometimes semi-purulent. The lymphatic glands in this case were not affected. Histological sections showed granulation tissue containing small masses of radiating clubs which were Gram negative, and surrounded a central granular area staining with eosin. Cultures were unsuccessful.

Two tongues from North America were examined. They showed fibrous nodules beneath the epithelium of the posterior half of the dorsum and sides of the tongue. The nodules were numerous and widely distributed, and ranged up to a pea in size. On section they contained soft granulation tissue beset with minute granules, some yellowish and just visible to the naked eye. On microscopical examination the granules were not gritty, but were of a gummy consistency, translucent, irregular in shape, soft when moist but hard and brittle when allowed to dry. Histological sections showed that the granules consisted of masses of radiating clubs surrounding a finely granular central area, in which no Gram-staining organisms were present. Cultures remained sterile.

From Siberia two frozen tongues were examined. The blades were not affected, but a lymphatic gland at the root in each case contained small nodules of soft yellowish tissue surrounded by fibrous capsules. Histological sections showed the usual masses of radiating clubs and absence of central Gram-staining mycelium.

The disease in the North American and Siberian material appeared to be of the same type as in the Argentine material.

Important contributions to the subject of actinomycosis have been made by Lignières and Spitz. In an article entitled "*L'Actinobacillose*<sup>1</sup>," these authors described an organism which they proved to be the cause of various forms of disease in cattle clinically identical with

<sup>1</sup> *L'Actinobacillose. Bull. Soc. Centr. de Méd. Vétér.* 1902.

actinomycosis, and to which they gave the name of actinobacillus. Their results were unreservedly accepted by Nocard. At the period at which they made the discovery, the disease occurred in the Argentine in epizootic form, attacking 50 to 70 per cent. of the members of a herd. In a second publication<sup>1</sup> they discuss the question of the classification of affections known under the name of actinomycosis, pointing out the impossibility of accepting actinomycosis as a single pathological entity. They describe three distinct groups of actinophytoses, the existence of which their own work has enabled them to establish—(1) *Actinomycosis bovis* (Harz) or *Actinophytosis à Streptothrix actinomyces*, (2) *Streptothrix israeli* or *Actinophytosis à Streptothrix israeli*, (3) *Actinobacillosis of Lignières and Spitz* or *Actinophytosis à Actinobacille*. The second name in each case is in accordance with a method of classification suggested by Lignières and Spitz, to which it is impossible to do justice in a brief summary.

*Actinomycotic Lesions in Cattle from the British Isles.*

Specimens of actinomycosis have been obtained from oxen slaughtered in this country in forty-four instances. It is possible to divide these cases into two groups by the application of Gram's method of staining either to smear preparations of the actinomycotic granules or to histological sections of the pathological tissue. In the first group the clubs are the more prominent feature, and no Gram positive organisms are demonstrable; the second group is characterised by the presence of Gram-staining organisms.

GROUP I.

*Summary of anatomical and histological characters.*

Of the above cases, forty fall in this group. The lesions were found in one or more of the following situations: cheek, floor of mouth, palate, tongue, posterior pharyngeal glands, submaxillary glands, and glands in relation to the tongue. The anatomical and histological features of the lesions were identical with those found associated with the imported tongues. To recapitulate briefly, the lymphatic glands contained circumscribed areas of soft granulomatous tissue, sometimes replacing the whole of the gland. These areas were surrounded by fibrous tissue, and the central portions often contained thick yellowish

<sup>1</sup> Contribution à l'étude, à la classification, et à la nomenclature des affections connues sous le nom d'actinomyose. *Centrabl. Bakt. etc. Abt. Originale*, xxxv. No. 4.

pus. In the pus or the granulation tissue were large numbers of the characteristic granules. The tongues contained varying numbers of nodules similar in structure to those in the glands, situated, in early cases, just beneath the epithelium, and, in the more advanced, extending deeply into the muscle and surrounded by dense fibrous tissue. The lesions in the other situations were similar. Microscopically the specific granules in the tongue nodules, as in the glands and elsewhere, were composed of masses of radiating clubs. By Gram's method the clubs retained irregularly the violet colouration, but no filaments or organisms staining by Gram were present. In the sections stained by carbolfuchsin or methyl violet occasionally small patches of a minute bacillus could be demonstrated between the clubs or in the central portion of the granule.

*Details of cases investigated.*

The following is a brief description of the individual lesions investigated in the above forty cases:

1. One posterior pharyngeal gland contained four nodules up to .5 or .6 cm. in diameter, composed of a yellowish granulation tissue in which were imbedded minute yellowish gritty foci. Cultures positive.
2. A gland at the root of the tongue on one side contained several small lesions up to .4 cm. in diameter, the smallest grey, the larger yellow and showing minute gritty granules. The tongue was not affected. Cultures positive.
3. A gland at the root of the tongue on one side contained a nodule 1 cm. in diameter. This was composed of soft greyish tissue surrounded by a fibrous capsule and subdivided by fibrous strands. In the soft tissue were small yellowish gritty granules. The tongue was not affected. Numerous organisms in culture not identified.
4. The tongue showed just below the epithelium of the dorsum a number of small discrete fibrous nodules up to a pea in size. On section these nodules contained a softer grey tissue in which were imbedded minute irregular yellowish granules not obviously gritty. The lymphatic gland at the root on the same side contained similar grey nodules. Cultures remained sterile.
5. A lymphatic gland at the root of the tongue on one side contained a lesion about 1 cm. in diameter, composed of soft grey tissue with yellowish granules and surrounded by a fibrous tissue capsule. The tongue was not affected. Cultures positive.

6. A lymphatic gland at the root of the tongue on one side, roughly spherical and about 5 cm. in diameter, showed on section a nodule about 3 cm. in diameter. This was composed of a soft translucent tissue which projected beyond the cut surfaces and was contained in a capsule of fibrous tissue. The gland showed smaller areas similar to the above, and where they approached the capsule there were some fibrous adhesions. The characteristic minute granules were found in all. Cultures positive.

7. The head of an ox was examined. The right posterior pharyngeal gland, measuring  $7 \times 5 \times 3.5$  cm., was hard, and on section more than half replaced by two nodules which were similar to that described in No. 6. At the angle of the jaw on the same side was a gland  $6 \times 5.5 \times 3$  cm., almost entirely replaced by similar tissue, the central portion of which had softened and become purulent. The left posterior pharyngeal gland contained a single small nodule in the centre. The tongue and jaw were not apparently affected. Cultures positive.

8, 9. Lymphatic glands at the roots of tongues contained small lesions similar to those described above. Cultures not attempted.

10. The roof of the mouth showed a hard fibrous tumour, and the upper lip was thickened and hard. On section the fibrous tissue was beset with small areas of soft granulation tissue containing minute granules. Numerous organisms in culture not identified.

11. The posterior half of the tongue was infiltrated by numerous sub-epithelial nodules ranging up to 2 cm. in diameter. On section they presented the appearance of soft granulomata surrounded by fibrous tissue and beset with minute granules. A few of the larger nodules were purulent in the centre. Cultures positive.

12. The tongue was affected similarly to the above, but was in a more advanced stage. Several of the nodules projected through the epithelium on the dorsum, forming flat warty elevations, the largest  $7.5 \times 4.5$  cm. The nodules extended also deeply into the muscles of the tongue. A lymphatic gland on one side contained a nodule about 1 cm. in diameter. Minute granules were present in the lesions in both situations. They were very numerous, and the majority were markedly calcareous. Cultures positive.

13. The tongue was affected similarly to No. 12, but slightly less extensively. No lymphatic glands were involved. Cultures negative.

14. The tongue was infiltrated in the posterior half with nodules beneath the epithelium. Granules were numerous, of a gummy consistency and not gritty. Cultures positive.

15. A lymphatic gland at the root of the tongue contained small soft yellow lesions. Cultures not attempted.

16. On the inner side of the cheek of a bullock were a number of nodules up to 1 cm. in diameter, consisting of fibrous tissue enclosing soft yellow substance in which were numerous minute granules. Several of the nodules had broken through, forming fungating ulcers on the buccal surface. Cultures negative.

17. The tongue showed several sub-epithelial nodules similar to those described above. Cultures positive.

18-22. In each case the lymphatic glands at the roots of tongues showed granulomatous areas with the characters above described. Cultures from two were positive; from three they were not attempted.

23<sup>1</sup>. The tongue showed beneath the epithelium of the posterior half a number of small fibrous nodules up to a pea in size, a few extending into the muscle. The lymphatic glands at the root on each side contained soft grey nodules with yellowish granules surrounded by fibrous tissue and subdivided by fibrous strands. Cultures positive.

24. Beneath the epithelium of the hard palate were small grey fibroid nodules presenting the appearance described above, and containing yellowish gritty granules. The tongue was extensively infiltrated by similar nodules. The material was formalised. Cultures not attempted.

25<sup>1</sup>. Beneath the epithelium of the tongue and extending deeply into the muscle were a number of nodules ranging up to a hemp-seed in size. Cultures negative.

26. In the posterior half of the dorsum and sides of the tongue were about fifteen hard sub-epithelial nodules, slightly prominent, the largest extending into the muscle and measuring up to 1 cm. in diameter. They were composed of fibrous tissue with a grey or yellowish granulomatous centre containing numerous granules. A small lymphatic gland on one side contained a yellowish nodule full of gritty granules. Cultures positive.

27. A portion of the posterior part of the tongue was hard, and infiltrated with fibrous tissue and small grey nodules extending deeply into the muscle. Cultures negative.

28. Tongue showed some superficial ulceration of the epithelium. Immediately beneath and in the muscle were fibrous streaks and very small nodules. Cultures positive.

<sup>1</sup> This case and case 25 were obtained from Irish cattle.



29. Cheek of ox thickened, and on section infiltrated with fibrous tissue and small hard nodules. The nodules were close beneath the epithelium of the mouth, and there was ulceration at one point where the teeth came in contact. Cultures positive.

30. Pharyngeal gland containing a yellowish nodule which projected above the cut surfaces of the gland and measured 1.7 cm. Cultures sterile, excepting a few moulds and colonies of cocci.

31. Submaxillary gland containing a collection of small grey nodules separated by fibrous tissue. Numerous granules consisting of masses of clubs with no Gram-staining organisms. Cultures not attempted.

32. Submaxillary gland containing a lesion similar to that in No. 30, measuring  $2.1 \times 1.4$  cm.; granules slightly gritty. Cultures sterile, excepting a few moulds.

33. Lesions in the floor of the mouth consisting of two nodules the size of thrush's eggs. They were composed of soft yellowish tissue, purulent in the central portion and containing numerous granules. The whole was surrounded by fibrous tissue. Two glands at the angle of the jaw each showed small nodules of similar material to the above. There was also a caseo-calcareous area in each gland, from one of which pure cultures of tubercle bacilli were obtained. Cultures positive.

34. The posterior portion of the tongue was infiltrated with fibrous translucent nodules containing soft tissue with numerous granules. The nodules were aggregated at the sides into dense fibrous areas, over which the epithelium was ulcerated, and they extended deeply into the muscle of the blade and roots. A submaxillary gland contained a similar nodule 1 cm. in diameter. Cultures positive.

35. Submaxillary gland showed on section a nodule measuring  $4.2 \times 3 \times 3.5$  cm. This consisted of soft translucent tissue closely beset with opaque yellowish foci; microscopically typical granules. Cultures positive.

36. Pharyngeal gland contained small areas consisting of granulomatous tissue with yellowish granules surrounded by fibrous tissue. Submaxillary gland was almost replaced by a nodule  $4 \times 2$  cm. similar to the above. Cultures positive.

37. Portion of cheek muscle containing numerous nodules of soft tissue beset with yellow foci and surrounded by much fibrous tissue. The largest measured 2.2 cm. Submaxillary gland contained a similar nodule 1.3 cm. in diameter. Cultures positive.

38. Posterior two-thirds of tongue showed beneath the epithelium numerous soft grey nodules with yellowish foci surrounded by fibrous

tissue and ranging up to 1 cm. in diameter. They extended into the muscle, but not deeply. Four had ulcerated through the epithelium, forming projecting tumours. Submaxillary gland showed an area containing three nodules similar to the above, from .4 to .8 cm. in diameter. Cultures positive.

39. Portion of palate showed numerous irregular ulcers up to  $4 \times 1.5$  cm. in area. Beneath the epithelium were numerous soft grey nodules with yellowish granules. The nodules were occasionally purulent in the centre, and were surrounded and separated by fibrous tissue. Submaxillary gland contained similar nodules up to 1 cm. in diameter. A second gland was almost replaced by nodules becoming purulent. Pharyngeal gland contained similar nodules. Cultures from both pharyngeal and submaxillary glands positive.

40. Pharyngeal gland was replaced by a mass, 4 cm. in diameter, of soft, streaky yellowish substance exuding pus when squeezed and containing numerous granules. The gland had ulcerated through the pharyngeal wall, forming a prominent fungating tumour. Cultures positive.

#### *Cultures.*

Cultures have been attempted from all but a few of the forty cases, and have been successful in twenty-three, where apparently identical cultures were obtained. In the unsuccessful cases the cultures have remained sterile or have become overgrown.

An emulsion was made, either in a small test tube or in a mortar, of granulation tissue or pus obtained from the centre of a nodule, after searing the surface in the usual way. This emulsion was spread over a series of plates of agar made with veal broth, reaction + 10 (Eyre's method). After 24 hours' incubation at 37° C. translucent circular colonies with a regular outline were formed, ranging up to 1.5 mm. in diameter, coherent and bluish-grey by transmitted light. Generally the colonies were very numerous, occasionally sparse—only one or two to a plate. In sub-culture on slant agar a non-characteristic growth is produced, resembling somewhat that of *B. typhosus*. The cohesiveness of the primary colonies is quickly lost. Microscopically on this medium the organism grows in the form of a short bacillus or cocco-bacillus, with occasional long forms. The bacillus does not retain the stain by Gram's method; it stains best with weak carbol-fuchsin. It is non-motile. Viability is not great. The numbers of organisms capable of producing colonies rapidly diminish in emulsion of tissue kept in the ice-chest,

and sub-cultures in series must be made every four or five days. In glucose agar shake tubes growth occurs in the depth as minute colonies, but just below the surface of the medium the colonies, when not too numerous, grow much larger and assume a rosette form. Microscopically such colonies are composed of a mass of long, tangled, unbranched filaments, not Gram-staining, with a variable number of smaller curved bacilli and circular bodies. On transplantation to slope agar the ordinary short bacillary form is resumed.

Several strains (Nos. 19, 26, 28, 29, and 35) have been tested on various other culture media, with the following results:

Solidified egg medium: growth sparse, in the form of circular slimy colonies consisting of involution forms. They retain their vitality, and have been transplanted after two or more months.

Broth (+ peptone): uniform turbidity and surface scum.

1 % peptone salt water: slight growth.

10 % peptone salt water: uniform turbidity.

Lactose litmus agar (Würtz): reddened in 48 hours.

Neutral red agar plates + lactose or dextrose: red colonies.

Litmus milk: acidity, no clot.

Glycerin-litmus milk: acidity and clot.

Bile salt agar plates: colonies either moist or coherent.

Potato (acid) pure: discrete moist, slimy, grey colonies after 12 days.

Microscopically either long threads or irregular involution forms.

Potato alkaline to litmus: as above.

Glycerin-agar: moist, slimy translucent layers.

Solidified serum: thin layers as above.

Solidified glycerin serum: thin layers as above.

Veal extract (- peptone): no uniform turbidity; flakes of growth which deposit on the bottom or sides of the tubes, leaving medium clear.

Serum water + litmus (Hiss): no change in reaction. Growth feeble.

#### *Inoculation Experiments.*

On June 16th, 1914, a calf was inoculated subcutaneously on the left side of the neck with the growth from six agar tubes of 24-hours-old culture from No. 11. Total duration of sub-cultivation 33 days. A fluctuating tumour rapidly formed, and there was a slight rise of temperature for a few days. A second inoculation was performed, July 7th, 1914, on the other side of the neck with the 24-hours-old culture from four agar tubes of No. 2. There was a slight rise of temperature and

a local reaction which ultimately disappeared without leaving a trace behind—a result possibly due to the immunising effect of the first inoculation. The animal was killed in good condition on August 8th, 1914. There was a hard subcutaneous tumour on the left side of the neck about the size of a partridge's egg. On section it was composed of a collection of soft greyish nodules with slightly yellowish points surrounded by dense fibrous tissue. The prescapular gland was normal, and the animal was otherwise healthy.

Microscopically the local lesion showed numerous granules similar to those found in the natural disease, but rather smaller, more irregular, and less coherent. Under a higher magnification the granules were composed of masses of minute Gram-negative bacilli, around which were typical small refractile clubs. In histological sections the appearances were identical with those in cases of the natural disease.

Two pigs were each fed and inoculated with emulsions of actinomycotic tissue from Nos. 6 and 7. No disease was produced.

One pig was inoculated subcutaneously with culture; when killed no lesion was found.

A monkey was inoculated subcutaneously with  $1\frac{1}{2}$  agar cultures of No. 11 24 hours old. The animal remained healthy, and no lesion was found.

A monkey was inoculated subcutaneously with culture from eight agar tubes four days old, of No. 19, on November 23rd, 1914. The animal was alive and healthy on January 11th, 1915.

Guinea-pigs inoculated intraperitoneally with  $\frac{1}{4}$  to 1 slant agar growth became ill after the inoculation. They either died in from 1–3 days or after a brief illness recovered and survived. Flakes of pus were found scattered about the peritoneal cavity. No granules or clubs were found.

#### GROUP II.

In this group are four cases, in each of which the lesions were in the inferior maxillary bone. The bone was expanded and occupied by a mass of granulation tissue containing large numbers of the characteristic granules. Microscopically these granules were composed of masses of Gram-staining organisms comprising long branched filaments, chains of coccid bodies, and short bacilli. Around the periphery of the granules were numerous clubs staining with eosin.

The investigation of the biological properties of the organisms concerned in the production of the lesions has not yet been completed.

## SUMMARY.

Actinomycotic lesions in tissues from a consecutive series of forty-four oxen slaughtered in this country have been examined and compared with fifty specimens imported chiefly from Argentina.

The British cases can be divided into two groups, according as the specific granules do not or do contain Gram-staining organisms.

The first group, characterised by the presence of granules consisting of clubs without Gram-staining organisms, contains 40 cases. In these the lesions were situated in the cheek, palate, tongue, or lymphatic glands in relation to the mouth and pharynx. From twenty-three of the forty cases, cultures of a non-Gram-staining organism were obtained, which were identical in their characters on nutrient agar plates and in shake glucose agar tubes; and five of the strains were found identical when tested on a large series of differential media. One of the cultures was inoculated into a calf, and produced a local lesion with the characters of a natural lesion.

This first group shows complete identity in the histological features and in the anatomical distribution of the disease with Actinobacillosis described by Lignières and Spitz in Argentina. The bacillus obtained has the characters of the Actinobacillus, except that the production of typical granules in the peritoneal cavity of guinea-pigs, described by Lignières and Spitz, has not been demonstrated.

All the imported specimens which were examined microscopically exhibited the characters of the lesions of this first group, but the causal organism was not obtained in pure culture.

The second group contains the remaining four British cases. The specific granules were composed of clubs and Gram-staining organisms, including branching filaments. The lesions were situated in each case in the inferior maxilla. The investigation of the biological characters of the organisms concerned is still in progress.

These results show that Actinobacillosis is widespread in the world, and forms a considerable proportion of the cases of disease in oxen known under the name of Actinomycosis.