THE DETECTION OF ANTHRAX SPORES IN EAST INDIA WOOL AND IN YARN MANUFACTURED THEREFROM.

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The following investigation was undertaken during November and December 1911 with the object of determining the source of infection in a series of cases of anthrax which occurred in the same yarn mill. The investigation resulted in proving that anthrax spores were present in a variety of East India wool and in the yarn manufactured therefrom.

Cases of Anthrax.

Case	Sex	Age	Occupation	Situation of pustule	Date
1. F. B.	Female	34	Condenser minder	Forearm	26. 5. 09
2. E. M.	,,	18	Hanker	Face	18. 9. 09
3. A. H.	,,	15	,,	Arm	28. 6. 10
4. A. M. B.	,,	21	,,	Arm	7. 9. 11
5. A. S.	,,	13	,,	Elbow	27. 10. 11

The cases, five in number, occurred between May 26th, 1909, and October 27th, 1911, while one blend of wool was undergoing manipulation. With one exception they occurred among hankers, three of whom were employed at machines in the same room. In each case the diagnosis was confirmed by bacteriological examination. It seems remarkable that not a single case occurred among the workers engaged in the earlier and more dusty processes of shaking, blending and willeying.

Materials Examined and Result of Examination.

As the cases occurred chiefly among the hankers samples were in the first place obtained of the materials with which such workers would be most likely to come into close contact.

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The specimens examined in the first instance consisted of:

Nos. 1 and 2. Specimens of wool dust from ring-twisters, taken from the machines in a room in which three cases of anthrax had occurred.

No. 3. Sample of unscoured yarn made from the same blend and identical with that on which the hankers were employed when the cases occurred.

No. 4. Wool dust and oil scraped up from the floor.

No. 5. Wool blend from which the yarn was spun.

The examination, details of which are given below, showed that anthrax spores were present in the sample of yarn No. 3. This yarn had undergone the complete process of manufacture except the final scouring in soap liquor. As far as could be ascertained this is the first recorded instance of a sample of manufactured wool having been found to contain anthrax spores.

The result of the examination of the other specimens was negative.

The following further specimens were next obtained and examined with negative result:

No. 6. Unscoured yarn (same source as No. 3).

No. 7. Scoured yarn (same source as No. 3).

No. 8. Soap liquor in which the hanks of finished yarn were scoured.

It was suspected that a particular variety of wool composing the blend might be infected and it was therefore decided to examine separately each class of wool employed in blending. The blend was composed of eight different varieties of wool which were as follows:

No. 9.	Egyptian Wool 1.	No. 13.	East India Wool 5.
No. 10.	East India „ 2.	No. 14.	East India „ 6.
No. 11.	French „ 3.	No. 15.	Cape „ 7.
No. 12.	East India „ 4.	No. 16.	East India „ 8.

Samples of each of these wools were examined and No. 14 East India Wool 6 was found to contain anthrax spores. In order to confirm this result a further sample No. 17, of East India Wool 6, was obtained from the same source. This was divided into three portions A, B, and C, each of which was examined separately. Portions A and C were found to contain the anthrax spores, but in the case of portion B the examination proved negative.

As two consecutive samples taken without selection gave positive results, it is probable that the spores were present in large numbers and were widely disseminated throughout this variety of wool. The

samples of wool were not obviously blood-stained, but most of them were contaminated with dried faecal material and all of them were very dusty.

The table shows the materials examined with the result of the examination in each case.

Method of Examination.

The specimens with the exception of No. 4 (wool dust and oil) and No. 8 (soap liquor) were placed in large flasks containing 250 c.c. to 500 c.c. of sterile saline solution. The quantity of solution, depending on the bulk of the sample, was in each case sufficient for complete immersion of the material. The flasks were thoroughly shaken and after standing 24 hours the whole of the washings were poured off and centrifuged. On centrifuging, a considerable quantity of compact brownish deposit collected at the bottom of the tube leaving a more or less clear supernatant fluid the bulk of which was withdrawn, only about 3 or 4 c.c. being allowed to remain. Slight agitation of the tube caused the upper layer of the deposit to mix with the remaining fluid forming an emulsion; from this emulsion cultures were made and guinea-pigs were inoculated.

Specimen No. 4 which consisted of masses of caked dirt, wool and oil was first ground up then thoroughly mixed with saline solution and centrifuged. The upper layer of the deposit was mixed with a little saline solution and examined.

Specimen No. 8 consisting of soap liquor in which the hanks of yarn were scoured, was centrifuged and the deposit obtained was repeatedly washed in saline solution to remove the soap. A small quantity of the washed deposit was examined.

Cultures.

Cultures were made on agar, two plates being used for each specimen. Several drops of emulsion were placed on the first plate and were spread over the surface by means of a platinum loop which was then rubbed over the surface of the second plate. In the case of specimen No. 17 portion C, no cultures were made. The anthrax bacillus was not obtained in any of the cultures. The isolation of the bacillus even although present was rendered impracticable owing to the presence of large numbers of organisms which produced colonies closely resembling, and sometimes almost identical with, those of anthrax. The organisms were probably the bacillus anthracoides of Bainbridge and

the varieties of anthrax-like organisms described by Page (1909) as occurring in abundance in wool and preventing the detection of anthrax colonies by their profuse growth.

Animal Inoculations.

In each case a guinea-pig was inoculated subcutaneously in the left thigh with one cubic centimetre of emulsion. Inoculations made from the following specimens gave positive results:

No. 3. Unscoured yarn.

No. 14. East India Wool (1st specimen).

No. 17. East India Wool (2nd specimen, portions A and C).

The guinea-pigs inoculated from these specimens died and all showed the typical post-mortem appearances of death from anthrax, viz. gelatinous oedematous exudate spreading subcutaneously from the seat of the inoculation over the abdominal wall; injection of the vessels and haemorrhages at the seat of inoculation; spleen dark in colour, enlarged, soft and friable; blood in the heart and great vessels dark in colour and fluid. The bacillus could be demonstrated microscopically in large numbers in the blood and tissues. Cultures were made on agar from the heart-blood, spleen and local exudate of each of the animals (except that inoculated with portion C of specimen No. 17) and abundant growths of anthrax bacilli were obtained.

The time elapsing between the date of inoculation and the death of the guinea-pig was longer than is usual after inoculation with pathological material obtained direct from cases of anthrax. One of the guinea-pigs died in three days, two in four days and one in six days. Glynn and Lewis (1912) found that the average duration of life in 40 animals dying from anthrax after inoculation with extracts of similar materials was 4·2 days and three survived 8, 7 and 7 days respectively. The average duration of life of guinea-pigs inoculated with materials from cases of anthrax is about two days. Of 26 animals recently inoculated in the West Riding Bacteriological Laboratory from cases, both human and bovine, 20 died within two days of inoculation and the remainder within three days.

The guinea-pigs inoculated from the following five specimens died of anaerobic infection.

No. 4. Wool dust and oil.

No. 6. Unscoured yarn.

No. 8. Soap liquor.

No. 12. East India Wool, 4.

No. 15. Cape Wool, 7.

Two of the guinea-pigs died in two days and three in four, three, and one day respectively.

The post-mortem appearances in some of these cases closely resembled anthrax, but instead of the gelatinous oedema the subcutaneous tissues were markedly bloodstained over the whole abdomen and thorax, the spleen was not enlarged or only very slightly so, and the blood in the heart and great vessels was clotted. Large Gram-positive bacilli were found in the exudate by microscopic examination, but were absent or only present in very scanty numbers in the heart-blood. No growth of these organisms was obtained on agar plates incubated aerobically and as they were Gram-positive they were probably either Welch's bacillus (*B. aerogenes capsulatus*) or the *B. enteritidis sporogenes* of Klein.

Webb and Duncan (1904) found, in a similar investigation, that in some cases the inoculated guinea-pigs died of malignant oedema before anthrax had time to develop and that cultures from the spleen of such animals on more than one occasion revealed the presence of anthrax bacilli. Glynn and Lewis (1912) were also able, in two instances, to isolate the anthrax bacillus by culture from the peritoneal fluid though the guinea-pigs had died of conditions other than anthrax, and these authors are of the opinion that the percentage of deaths from anthrax in their series would have been higher but for the presence of pathogenic anaerobes. In my own investigation cultures were made from the spleen, blood and exudate but no growth of anthrax bacilli could be obtained. In two cases a second guinea-pig was inoculated with emulsion of spleen, but only the original condition was reproduced and cultures gave negative results.

CONCLUSIONS.

1. Infected East India Wool was the cause of the occurrence of the cases.

2. For the detection of the *Bacillus anthracis* in material such as wool animal inoculations are necessary.

3. Guinea-pigs inoculated with wool washings containing anthrax spores tend to survive for a longer period than is usual after inoculation with material direct from cases of anthrax.

REFERENCES.

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Table of Specimens and Results of Examination.

No. and material	Result of cultures	Result of inoculations		Examination of animal tissues	Remarks
1. Wool dust...	*B. anthracis* not found	Negative.	Guinea-pig remained well	—	—
2. Wool dust...	Do.	Negative.	Do.	—	—
3. Unscoured Yarn	Do.	Positive.	Guinea-pig died in 4 days. *Anthrax*	*B. anthracis* found by microscopical and cultural examination in very large numbers in the heart-blood, spleen and exudate	—
4. Dust and Oil	Do.	Negative.	Guinea-pig died in 4 days. Anaerobic infection	*B. anthracis* not found	—
5. BlendedWool	Do.	Negative.	Guinea-pig remained well	—	—
6. Unscoured Yarn	Do.	Negative.	Guinea-pig died in 3 days. Anaerobic infection	*B. anthracis* not found	Second animal inoculated from spleen died in 1 day. *B. anthracis* not found.
7. ScouredYarn	Do.	Negative.	Guinea-pig remained well	—	—
8. Soap Liquor	Do.	Negative.	Guinea-pig died in 2 days. Anaerobic infection	*B. anthracis* not found	Second animal inoculated from spleen died in 2 days. *B. anthracis* not found.
9. Egyptian Wool No. 1	Do.	Negative.	Guinea-pig remained well	—	—
10. East India Wool No. 2	Do.	Negative.	Guinea-pig remained well	—	—
11. French Wool No. 3	Do.	Negative.	Guinea-pig remained well	—	—
12. East India Wool No. 4	Do.	Negative.	Guinea-pig died in 2 days. Anaerobic infection	*B. anthracis* not found in the heart-blood, spleen and exudate	—
13. East India Wool No. 5	Do.	Negative.	Guinea-pig remained well	—	—
14. East India Wool No. 6	Do.	Positive.	Guinea-pig died in 6 days. *Anthrax*	*B. anthracis* found by microscopical and cultural examination in very large numbers in the heart-blood, spleen and exudate	—
15. Cape Wool No. 7	Do.	Negative.	Guinea-pig died in 1 day. Anaerobic infection	*B. anthracis* not found	—
16. East India Wool No. 8	Do.	Negative.	Guinea-pig remained well	—	—
17. East India Wool No. 6	Do.	—		—	—
(*a*) Portion A	Do.	Positive.	Guinea-pig died, 3 days. *Anthrax*	*B. anthracis* found by microscopical and cultural examination in very large numbers in the heart-blood, spleen and exudate	—
(*b*) Portion B	Do.	Negative.	Guinea-pig remained well	—	—
(*c*) Portion C	Do.	Positive.	Guinea-pig died, 4 days. *Anthrax*	*B. anthracis* found by microscopical examination. No cultures made	—