Bulletin No. 140 - Further Report on the Injection of Cattle with B. Tuberculosis (Avian)

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Further Report on the Injection of Cattle With B. Tuberculosis (Avian)

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*In cooperation with U. S. Department of Agriculture.
Further Report on the Injection of Cattle
With B. Tuberculosis (Avian)

CECIL ELDER AND A. M. LEE

In a preliminary bulletin (1) and paper (2) the above authors reported upon work they had done upon the injection of cattle with B. tuberculosis (avian) with results of subsequent tuberculin tests run upon the same group of experimental cattle. This bulletin is a continuation of the work started some time ago and reported upon in those preliminary papers.

In that work, as was reported previously, cultures of B. tuberculosis (avian) which were proven to be pathogenic for chickens were injected into cattle. The cattle used were ten head of good healthy range steers which were purchased on the range, tested and found to be free from tuberculosis. Several months following injection they were tested with all three tuberculin tests, namely, the ophthalmic, intradermal, and subcutaneous tests. The following conclusions were published:

The work done tends to prove that cattle can be infected with the avian tubercle bacilli when they are injected beneath the skin or into the muscular tissue with comparatively large numbers of organisms. When introduced between the layers of the dermis no infection was produced. The results of the tuberculin tests indicated that the intradermal was the most reliable test for detecting tuberculosis in cattle when infected with avian tubercle bacilli. The ophthalmic test gave no indications of infection. The subcutaneous test did not give a positive reaction, although in steers in which lesions were produced a small rise in temperature was noted, following injection of tuberculin.

Three of the calves had good, well-marked local lesions and they were retained on the experiment. The future work outlined at that time has been carried out with some elaboration and the results are written up and published in this bulletin.
PROCEDURE OF EXPERIMENT

The following questions were raised regarding the three calves which carried the lesions which had been formed by artificial inoculation. (a) Could these lesions be removed intact by a surgical operation? (b) If so, would the animals continue to react afterwards to the tuberculin tests? (c) How closely would the structure of these lesions resemble that observed in the skin form of tuberculosis? (d) Was there any tendency towards metastasis or the formation of internal lesions? (e) Did the local lesions still contain tubercle bacilli and if so were they alive? (f) If alive, might they still be pathogenic for chickens? With these objects in mind the experiment was carried along.

The three calves in which the authors had been able to produce lesions by artificial inoculation were used for the work. Two of them, numbers 84 and 85, had been inoculated subcutaneously and one, number 87, intramuscularly. The latter lesion, however, was mostly in the subcutaneous tissue after it had developed rather than deeply situated within the muscle tissue.

DESCRIPTION OF LESIONS

In July, 1923, calves numbers 84, 85 and 87 were caught, securely controlled and the local lesions which were located in the posterior part of the forearm (point of injection) were completely removed.

The lesion from calf number 84 weighed 9.5 grams, including a small amount of fascia and connective tissue surrounding it. Numerous nodules were found, many of which contained a whitish pus. Most of the nodules were about the size of a pea and some of the pus was somewhat caseated.

The lesion from calf number 85 was situated a little more deeply in the fascia. After removal it weighed 9 grams. There were also numerous nodules here and the largest was about the size of a butter bean. Many smaller nodules were found which contained varying amounts of pus, some being caseated. Each was surrounded by a capsule-like structure.
The lesion from calf number 87 weighed 5 grams and contained four or five nodules of pea size to peanut size. Each was surrounded with a capsule and contained whitish pus and some caseated material.

Smears were made from these lesions and stained by the acid fast method. Unmistakable tubercle bacilli were found in smears from every lesion.

Zinsser and Petroff (3) state, from work they had carried on, that tuberculin skin reactiveness, both to residue antigens and to old tuberculin, can be obtained in guinea pigs by injections of dead tubercle bacilli.

Since the reactions in these three cattle occurred with the intradermal test, which is a skin reactivity, the question was raised if the organisms found in the local lesions might be dead organisms and still be producing this reactivity. Or might they be alive and, if so, were they still pathogenic for chickens?

REPORT OF WORK ON CHICKENS

In an attempt to ascertain answers to the above questions, the following work was carried out with healthy chickens. A small portion of the lesion from calf number 84 was ground up in mortar with sterile water and 2 cc of the suspension was injected intraperitoneally in hen number 5.* A small portion of lesion from calf number 85 was prepared in same manner and injected into hen number 6, likewise a portion of the lesion from calf number 87 was injected into hen number 7 on the same date. At time of injection these hens were in perfect health and fat. They were obtained from the University flock.

Hen number 4, which was a chicken that contained some of the virulent material after it had been passed on down through chickens from the original virulent culture which had been injected into the calves, died February 23, 1924. Hen number 8 was injected with material from liver of hen number 4 in order to keep the material alive and virulent. On March 26, 1924, hens numbers 5, 6, 7, and 8 were injected in the left wattle with avian tuberculin (Jen-Sal); 48th hour and 72nd hour readings

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*Injections in chickens were made day following removal of lesions from calves.
were all negative. On April 22, 1924, hens numbers 5, 6, 7, and 8 were again injected, this time in the right wattle with avian tuberculin. On April 24, 48-hour readings were taken with the following results: Hen number 5 showed a very good positive reaction, the swelling producing a thickness in the injected wattle approximately five times the size of the control wattle. As this hen was very emaciated and weak and it was thought she would die at any time, she was killed for the purpose of photographing the lesions. Smears made from the lesions and stained by the acid fast method showed the presence of tubercle bacilli. It might be well to state here that hen number 5 at time of inoculation with material from the local lesion of the calf, weighed four and one-half pounds. On April 22 (date of last tuberculin test) the same individual weighed two pounds six and three-fourths ounces.

On the 48th hour reading, hens numbers 6, 7, and 8 showed negative and continued to be negative on the 72nd hour reading. Hen number 7 died on the same date that the 72nd hour reading was taken. At the time of inoculation this hen was very healthy and weighed five and one-half pounds. On April 22 (date of tuberculin test) she was very anemic and so weak she could not walk. She was very emaciated, weighing but two pounds three and one-fourth ounces. Her comb and wattles were markedly shrunken. Two days previous to injection she had been very lame when she walked. Post-mortem examination revealed on the serous surface of the intestinal wall a few very hard nodules that ranged in size from a small pin head to a small pea. These nodules contained a very dry yellowish material from which smears were made. When stained with the acid fast method and examined, findings were at first negative, but a careful search through a large number of fields revealed the presence of a few acid fast organisms which appeared to be typical tubercle bacilli.

The fact that chickens which are in a well-advanced stage of tuberculosis will sometimes die shortly after receiving tuberculin has been noted a few times in the work of the authors and the same reason, whatever the connection may be, has been assigned to the case of hen number 7.
The remaining hen of the three, number 6, along with hen number 8, was tested on May 24, and both showed negative reactions at 48 and 72 hours following injection. They were again tested on August 4th with the same results.

Hen number 6 died November 7, 1924, in a very emaciated and weakened condition, but upon post-mortem examination failed to show any macroscopic lesions. At time of inoculation this hen weighed four and one-half pounds, while, on April 22, 1924, some months following, her weight was three pounds fourteen and one-third ounces. Unfortunately, her weight was not taken at time of her death and smears were not made from any of the internal organs. Clinically she was a typical picture of tuberculosis.

Van Es and Schalk (4) fed tuberculous material of mammalian origin to sparrows and found that they would die in a markedly emaciated condition but were almost entirely free from any gross lesions resembling tuberculosis. Several chickens were injected with material from guinea pigs and sparrows. In none of the chickens in their series 1727 could any lesions suggestive of tuberculosis be observed, but all birds dying spontaneously showed a most marked state of emaciation. Many other injections were made and they report that several times they found tubercle bacilli in the liver but no macroscopic lesions.

In another publication Van Es and Schalk (5) state that the mammalian tubercle bacillus, when injected into birds, quite commonly fails to provoke the formation of tuberculous lesions in the latter. They also state that artificial infection succeeds best by direct inoculation into a vein, while intraperitoneal and subcutaneous injections are apt to yield less certain results. This further statement is made, “We do not know, however, how soon an avian organism in a mammal may become adapted to its new surroundings and become capable of producing mammalian disease with regularity.”

Schalk (6) in a somewhat recent paper states that not all tuberculous fowls show macroscopic lesions but bear all symptoms and external signs of the disease. This condition was encountered several times in his laboratory. Microscopic smears
made from pulp of liver and spleen, however, revealed the presence of acid fast organisms which they interpreted to be tubercle bacilli. In some cases they found large numbers of organisms, while in others they were in moderate or limited numbers. He says this type is known as "yersin type" of bacilli. They multiply more or less in the tissues but show no inclination to colonize and produce tubercles.

As was stated earlier, it was very unfortunate that smears were not made from the internal organs of hen number 6. However, from the references cited above, it is indicated that this hen had tuberculosis. Clinical symptoms and outward signs of the disease were very typical. The question is raised, could the avian organisms injected into the calf's body have remained there long enough to adapt themselves to that environment? Then when they were removed and passed again into the chicken's body, were they simulating the mammalian type sufficiently to produce results similar to what other workers have experienced when injecting chickens with a mammalian type? Or if instead of intra-peritoneal injections, intravenous injections had been made macroscopic lesions might have been produced. Or, might the type in this hen have been what Schalk refers to as "Yersin type"? If so, no doubt smears from the internal organs would have revealed the presence of tubercle bacilli upon microscopic examination. If such can be assumed the evidence and data collected would seem to indicate that the organisms present in the local lesions of the calves were alive and the tuberculin reactivity was not being produced by dead organisms.

REPORT OF WORK ON CALVES

As has been reported, the local lesions were removed from the three calves. The operative wounds healed with a rapid, uneventful recovery. The calves were held in the same quarantine lot on practically the same ration, which was little more than a maintenance ration, until December 17, 1923. On this date calves 84, 85, and 87 were injected for the intradermal test and a sensitizing dose of tuberculin* was placed in the left eye for the

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*Intradermal tuberculin was used for the ophthalmic test.
ophthalmic test. December 20th 72-hour readings were taken on the intradermal tests and all were found negative. The diagnostic dose of tuberculin was placed in the left eye at nine o’clock and readings were taken every two hours up to and including five P. M. These readings were negative.

Subcutaneous tests were started December 20, 1923, and here the tests were negative, as is shown in accompanying temperature chart.

**TEMPERATURE CHART FOR SUBCUTANEOUS TESTS**

<table>
<thead>
<tr>
<th>Pre-Injection Temperatures Dec. 20, 1923</th>
<th>Post-Injection Temperatures Dec. 21, 1923</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ear Tag No.</td>
<td>A.M.</td>
</tr>
<tr>
<td>84</td>
<td>98.8</td>
</tr>
<tr>
<td>85</td>
<td>99.4</td>
</tr>
<tr>
<td>87</td>
<td>99.8</td>
</tr>
</tbody>
</table>

NOTE:—United States Bureau of Animal Industry tuberculin used.

As it was learned that the calves gave negative reactions through all the tests after local lesions had been removed and there was no further use for the calves on the experiment, they were butchered as soon as they could be handled by the meats class. At the time of killing, which was January 16, 1924, a very careful examination was made for any local or internal lesion of tuberculosis, but none was found, and the three animals were pronounced as being free from any tuberculous infection.

**DISCUSSION OF WORK**

Work done upon this project brings out several interesting points and although conclusive evidence is not at hand, it raises several questions which will bear further investigation. Possibly, it will not be amiss to briefly discuss some of these at this time. The possible relationship of infection with the avian tubercle bacilli to tuberculosis and diseases resembling tuberculosis in cattle suggests itself.

Paddock (7) in referring to Bovine Lymphangitis states that it is sometimes called skin tuberculosis and thinks there is little
doubt that the two are closely allied, if not manifestations of the same disease. It can be noted in case reports cited by him that cattle with lymphangitis will react to the intradermal test and after surgical removal of the lesions will not react to the intradermal test. The same results have been experienced in cattle artificially inoculated with avian tubercle bacilli.

Schalk (6) states in his paper that there is weighty evidence at hand pointing toward a close relationship between avian and mammalian tuberculosis organisms.

Traum (8) reports on lymphangitis in cattle caused by an acid-alcohol fast organism. It is not the intention of the authors to question the results of this work, but they merely wish to mention that several of their findings when dealing with a known culture of avian tubercle bacilli resemble very greatly the findings of Traum. He refers several times to inoculations in guinea pigs and rabbits which were negative. In his conclusions he states this disease resembles tuberculosis in character of lesions and presence of acid-fast organisms and that a large percentage of cases gave positive reactions to the intradermal test, but autopsy failed to reveal tuberculosis. He states it is not tuberculosis, because large numbers of guinea pigs injected with material from cases failed to develop tuberculosis. In his article no mention was made in conclusions of inoculation of material into chickens.

In Van Es' (9) paper and report at Chicago in 1924, he states that one gets negative results or no lesions in guinea pigs when injected with avian tubercle bacilli. If lymphangitis and skin tuberculosis may be and are confused, what might be the relationship of the avian tubercle bacilli to these infections. From some field work which one of the authors (Lee) did in the western part of Wyoming on avian tuberculosis and from points learned from experimental work, evidence is at hand to show that further investigation is needed regarding the importance of this disease and its relationship to cattle. Other workers are of the opinion that there is some relationship as Connaway (10) states: "There is a possibility we may yet find there is some danger of transmitting avian organisms to the bovines, and the
possibility that these non-lesion reactors may be due to this avian type.”

There are also opinions to the contrary. Hastings (11) in an address before the Society of Bacteriologists reports that he and Beach have examined large numbers of these skin lesions of tuberculosis and were unable to demonstrate the tubercle bacillus in a single instance. They found in some cases acid-fast bacilli in the microscopic examinations, but have been unable to culture them or infect experimental animals with them. They feel that there may be a group of acid-fast organisms that sensitize an animal to tuberculin. In other papers, Beach and Hastings (12) and Hastings, Beach and Weber (13) report upon the sensitization to tuberculin by some members of the acid-fast group other than the tubercle bacilli.

SUMMARY AND CONCLUSIONS

Calves which were injected with virulent avian tubercle bacilli and which developed local lesions gave good positive tuberculin reactions when tested with the intradermal test.

(a) It is possible by a surgical operation to remove completely these local lesions produced by experimental inoculation.

(b) Five months after removal of these local lesions the calves failed in each case to react to the intradermal, ophthalmic, and subcutaneous tuberculin tests.

(c) The local lesions which were removed, resembled greatly the lesions which are observed and described in the skin form of tuberculosis.

(d) Avian tubercle bacilli tend to remain localized when they gain entrance through the skin to the subcutaneous tissues even when introduced in large numbers.

(e) (f) Microscopical examination of the local lesions demonstrated the presence of tubercle bacilli in them eight months after the tubercle bacilli had been introduced. Work done with chickens would tend to indicate that these organisms found in the lesions were still alive at the time the local lesions were removed.
No new lesions developed after surgical removal.

Further work should and will be done on the relationship of avian tubercle bacilli and the skin form of tuberculosis in cattle.

REFERENCES

(5) Van Es, L., and Schalk, A. F. (1914), North Dakota Agricultural Experiment Station Bulletin No. 108.