Bulletin No. 136 - Avian Type of Tuberculosis in Cattle: Injections and Testing

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Avian Type of Tuberculosis in Cattle: Injection and Testing

A Preliminary Report

By CECIL ELDER AND A. M. LEE

There has been considerable work done with tuberculosis of cattle with regards to its relationship to human infection. Comparatively little has been done on the transmission of the avian tubercle bacilli to cattle, and it is along these lines that this paper is written.

HISTORY

Day (1), Christiansen (2), Washburn and Mohler (3), Bang (4), Hastings and Halpin (5), Dunne (6), and others, have demonstrated that swine may be infested with the avian tubercle bacillus.

Several years ago Arloing (7) stated that the avian form could infect mammals and that the human and bovine types would affect birds. At that time he thought the avian form was an atypical form of Koch's tubercle bacillus. We shall not attempt to discuss this latter point here, but merely mention it. Koch and Rabinowitsch (8) made the statement about 1908 or 1909 that the avian and mammalian tubercle bacilli are varieties of a single species, while Weber and Bofinger (9) said the organisms were not the same. The latter workers believed at that time that there was little chance for infection of mammals with avian organisms.

In 1911 Giltner (10) reported the feeding of twin calves about two weeks old with the finely chopped organs of a tuberculous hen. This material was given in milk. One calf died almost two months later, but revealed no evidence of tubercular infection on post mortem examination. Two months after having been fed the tubercular material the remaining calf was given a subcutaneous tuberculin test along with a healthy calf, avian tuberculin being used. The experimental calf gave a good reaction, but was not slaughtered at that time. De Jong (11) reported that avian tubercle bacilli can spontaneously infect man, ape, pig, bovine,
rabbit, rat, and white mouse, while Schroeder (12) writes that
the avian type lacks the power to serve as the cause of an epidemic
or an epizootic occurrence of tuberculosis among mammals. In
an article written by Schalk (13) the statement is made that a
few calves have been infected experimentally. Biester (14) in
work done at the University of Illinois reports that calves can be
artificially infected by injecting macerated tubercular organs of
chickens and that local lesions are produced by the injection of a
pure culture of the myobacterium of tuberculosis isolated from
tubercular chickens. Himmelberger and Bang have succeeded in
infecting cattle with avian tubercle bacilli.

OBJECT OF EXPERIMENT

The experiment we are about to report upon was suggested
as the result of some field observations made in Western Wyoming
by state employees who were doing tuberculin testing. Several
cases of the so-called skin form of tuberculosis were detected by
the tuberculin test. From history that could be gathered indica-
tions were that the skin form of the disease was being found on
farms and ranches where there were at the time, or had been,
tuberculosis in the chickens. This immediately raised the ques-
tion: (a) Can avian tubercle bacilli infect cattle? (b) If so,
will those cattle react to injections of ordinary bovine tuberculin?
(c) If they are infected, what is the relationship of this infection
to the form of the disease that we ordinarily call the skin form of
tuberculosis? It was with these objects in mind that the experi-
ment was outlined.

PROCEDURE OF EXPERIMENT

Ten head of range yearling steers were used in the experi-
ment. These animals were good healthy animals, rather typical
of our Western country except for the fact that they were a little
off color from our usual white-faced steer. Figure 1 shows the
steers used and illustrates the type. The photograph was taken
at the close of the experiment.
These steers were all tuberculin tested and found to be free from tuberculosis by all of the three tests used, namely, the ophthal-mic, intradermal and subcutaneous tests.

MATERIAL USED FOR INJECTION

It was found rather difficult to get virulent strains of the avian tubercle bacillus which were in pure culture. On November 18th two cultures which were pure and virulent were received from the North Dakota Agricultural College. Subcultures were made on glycerin agar with a pH value of approximately 7.2. These were incubated and, after being found to be pure cultures by microscopic examination, they were washed off and made up into suspension with sterile water. From this suspension six experimental calves were injected on December 1st, as is shown in Table I. Before injection, the area was clipped, shaved, and disinfected with tincture of iodine. Every precaution was used to avoid outside infection.
TABLE I—SHOWING DETAILS ABOUT THE INJECTION OF CALVES WITH AVIAN TYPE TUBERCULOSIS ON DEC. 1, 1922.

<table>
<thead>
<tr>
<th>Ear tag No.</th>
<th>Seat of injection</th>
<th>Kind of injection</th>
<th>No. cc. injected</th>
<th>Approximate number organisms injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>Post. upper portion left forearm</td>
<td>Intradermal</td>
<td>0.1</td>
<td>28,800,000</td>
</tr>
<tr>
<td>92</td>
<td>Post. upper portion left forearm</td>
<td>Intradermal</td>
<td>0.2</td>
<td>57,600,000</td>
</tr>
<tr>
<td>84</td>
<td>Post. upper portion left forearm</td>
<td>Subcutaneous</td>
<td>1.0</td>
<td>288,000,000</td>
</tr>
<tr>
<td>85</td>
<td>Post. upper portion left forearm</td>
<td>Subcutaneous</td>
<td>2.0</td>
<td>576,000,000</td>
</tr>
<tr>
<td>87</td>
<td>Post. upper portion left forearm</td>
<td>Intramuscular</td>
<td>2.0</td>
<td>576,000,000</td>
</tr>
<tr>
<td>88</td>
<td>Post. upper portion left forearm</td>
<td>Intramuscular</td>
<td>1.0</td>
<td>288,000,000</td>
</tr>
<tr>
<td>91</td>
<td>Left un-injected as controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>93</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>81</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EFFECT OF ORGANISM ON HENS

On the same date that the calves were injected (December 1st), two healthy hens, Nos. I and II, were injected from the same suspension as the calves to test the virulence for chickens of the cultures used in the cattle. Both were injected in the peritoneal cavity; No. I received 1 c. c. and No. II 0.5 c. c. of the suspension, which contained approximately 288,000,000 organisms per cubic centimeter.

On February 19th both hens were given the intradermal test, using avian tuberculin. At this time hen No. I was showing every symptom of acute tuberculosis. She was extremely emaciated, weighing only about one-third of her pre-injection weight. This hen showed no reaction to tuberculin test. Hen No. II gave a good reaction that showed up as a diffuse swelling of the wattle, which was hot and painful. Swelling began on the twenty-fourth
hour following injection and still persisted at the seventy-second hour following injection.

Hen No. 1 died on February 27th. An autopsy revealed tubercular lesions throughout the abdominal cavity. Smears made from lesions in the liver, mesentery, intestinal wall, and skin, and stained by the acid fast method, revealed typical tubercle bacilli. Straight and curved rods, many of which were granular, were found which had taken the acid fast stain.

On the same date an emulsion was made from lesions in the liver of hen No. III, injected in order to retain organisms in a virulent form. At the time of injection, hen No. III was very healthy, weighing 33.4 pounds. She was tested with tuberculin and gave a negative reaction. On May 31st Hens No. II and III were both tested with avian tuberculin. Hen No. II showed a slight swelling of the wattle. Hen No. III showed a swelling of the wattle, a good reaction, at 24 hour period following injection. This reaction persisted for 96 hours.

On June 3rd hen No. II died and upon post mortem examination tubercular lesions were found throughout the abdominal cavity. Figure 2 gives an idea of the extent and location of these lesions.

EFFECT OF THE ORGANISM ON THE CALVES

During the progress of the experiment the calves were kept in a large lot isolated from other livestock. They were fed and watered once daily, as is customary under range conditions in the winter. All the calves wintered very well on a ration consisting of alfalfa and prairie or native hay.

On April 1st, four months after being injected, they showed the following lesions at point of injection:

No. 84. Swelling approximately the size of a black walnut noted at point of injection beneath the skin. On palpation, swelling was found to be hard and movable.

No. 85. Swelling at point of injection very similar to that in calf No. 84, but slightly smaller in size.

No. 87. At point of injection swelling was noticed apparently all in the subcutaneous tissue, about the size of an average pecan.
Fig. 2. Tubercular lesions found in hen No. II.
No. 88. This calf received intramuscular injection, but showed no lesions at point of injection.
No. 83. Showed no lesions at point of injection.
No. 92. Showed no lesions at point of injection.
Except for the local lesions noted above, the animals were to all appearances in healthy condition, as were also the four controls.

RESULTS OF TUBERCULIN TEST

All the calves, including the controls, were given three tuberculin tests, namely, the ophthalmic, intradermal, and subcutaneous. On May 5th they were given the intradermal injection with B. A. I. tuberculin. As it was impossible at this time to obtain ophthalmic discs, intradermal tuberculin was used for the ophthalmic test. The sensitizing dose for this latter test was placed in the left eye at the same time the intradermal injections were made. As calves had been running free in the lot, they were turned loose and kept in their usual environment. Readings were taken at the end of twenty-four hours on the ophthalmic test for evidence of any irritation in the eyes. Both eyes in all calves showed normal. At the end of forty-eight hour period, observations upon the eyes showed nothing. Readings were also taken at this time (at the end of forty-eight hours) on the intradermal test and three of the calves (Nos. 84, 87 and 88, as is shown in the accompanying table) began to show a suspicious reaction.

On this date, May 7th, the calves were tied up during the day to accustom them to being tied. They were turned loose in the evening and caught again the following morning, on which date the diagnostic dose of intradermal tuberculin for the ophthalmic test was given. This diagnostic dose was given exactly seventy-two hours following the sensitizing dose. The results were negative throughout the test for all the calves.

On the intradermal test, commencing at forty-eight hour period, readings were taken every twenty-four hours up to and including the one hundred and twentieth hour. Three of the animals, Nos. 84, 85 and 87, showed a positive reaction while No. 88 could be called nothing more than suspicious. The results of the twenty-four hour readings are shown in Table II.
TABLE II—SHOWING RESULTS OF INTRADERMAL TEST ON CALVES INJECTED
MAY 5, 1922

<table>
<thead>
<tr>
<th>Ear tag No.</th>
<th>May 7th, 48 hour reading</th>
<th>May 8th, 72 hour reading</th>
<th>May 9th, 96 hour reading</th>
<th>May 10th, 120 hour reading</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Negative</td>
</tr>
<tr>
<td>92</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Negative</td>
</tr>
<tr>
<td>84</td>
<td>Suspicious</td>
<td>Thick 2X</td>
<td>Thick 2X</td>
<td>Thick 2X</td>
<td>Positive</td>
</tr>
<tr>
<td>85</td>
<td>N</td>
<td>Thick 2X</td>
<td>Thick 2X</td>
<td>Thick 2X</td>
<td>Positive</td>
</tr>
<tr>
<td>87</td>
<td>Suspicious</td>
<td>P3</td>
<td>P3</td>
<td>P3</td>
<td>Positive</td>
</tr>
<tr>
<td>88</td>
<td>Suspicious</td>
<td>Suspicious</td>
<td>Suspicious</td>
<td>Suspicious</td>
<td>Suspicious</td>
</tr>
<tr>
<td>89</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Negative</td>
</tr>
<tr>
<td>91</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Negative</td>
</tr>
<tr>
<td>93</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Negative</td>
</tr>
<tr>
<td>81</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Negative</td>
</tr>
</tbody>
</table>

NOTE.—The symbols used and code followed are those described by Ernest and Lash (15) in their circular on "Tuberculin Testing of Livestock," and are as follows:

N—Negative.

P3—Circumscribed swelling three times as large as a pea.

Thick 2X—Diffuse swelling in which injected candle or tail fold is twice as thick as normal fold.

THE SUBCUTANEOUS TEST

The calves were also given a subcutaneous test, each being injected with 5 c. c. of Bureau of Animal Industry subcutaneous tuberculin. As calves had been running loose during the five months since injection with avian tubercle bacilli, they were tied up daily for a few days, beginning May 7th, to allow them opportunity to quiet down. Temperatures were taken at various hours during the day for several days, but some of the calves continued to run high temperatures. It was not deemed advisable to inject them for the subcutaneous test until all the animals, including controls, had quieted down so that their temperatures were down to normal. Various methods of feeding and watering were followed and it was finally found best to keep halters on the animals and let them run loose in the small lot. This made it easy to catch them and they soon became quiet enough so that one could walk up to them. It was not until May 22nd with continuous daily handling that all pre-injection temperatures came down to normal. During the period in which pre-injection temperatures
were taken and on the day of taking the post-injection temperatures, the animals had access to moderately cold water from a tank. They were fed in the evening on both days.

Pre-injection temperatures and post-injection temperatures are shown in the tables III and IV.

### TABLE III—TEMPERATURE CHART FOR SUBCUTANEOUS TEST

<table>
<thead>
<tr>
<th>Ear tag No.</th>
<th>Pre-injection temperatures, May 22, 1923</th>
<th>Amount injected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11:30 a.m.</td>
<td>2:15 p.m.</td>
</tr>
<tr>
<td>83</td>
<td>101.1</td>
<td>102.5</td>
</tr>
<tr>
<td>92</td>
<td>101.4</td>
<td>101.8</td>
</tr>
<tr>
<td>84</td>
<td>101.9</td>
<td>102.3</td>
</tr>
<tr>
<td>85</td>
<td>102.1</td>
<td>102.4</td>
</tr>
<tr>
<td>87</td>
<td>101.7</td>
<td>102.6</td>
</tr>
<tr>
<td>88</td>
<td>101.6</td>
<td>101.8</td>
</tr>
<tr>
<td>89</td>
<td>101.4</td>
<td>102.5</td>
</tr>
<tr>
<td>91</td>
<td>101.2</td>
<td>102.1</td>
</tr>
<tr>
<td>93</td>
<td>102.1</td>
<td>102.4</td>
</tr>
<tr>
<td>81</td>
<td>102.1</td>
<td>102.3</td>
</tr>
</tbody>
</table>

### TABLE IV—TEMPERATURE CHART FOR SUBCUTANEOUS TEST

<table>
<thead>
<tr>
<th>Ear tag No.</th>
<th>Post-injection temperatures May 23, 1923</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 a.m.</td>
</tr>
<tr>
<td>83</td>
<td>101.6</td>
</tr>
<tr>
<td>92</td>
<td>101.3</td>
</tr>
<tr>
<td>84</td>
<td>101.9</td>
</tr>
<tr>
<td>85</td>
<td>100.7</td>
</tr>
<tr>
<td>87</td>
<td>101.2</td>
</tr>
<tr>
<td>88</td>
<td>101.9</td>
</tr>
<tr>
<td>89</td>
<td>101.6</td>
</tr>
<tr>
<td>91</td>
<td>101.0</td>
</tr>
<tr>
<td>93</td>
<td>100.9</td>
</tr>
<tr>
<td>81</td>
<td>101.6</td>
</tr>
</tbody>
</table>
PLATE I—TEMPERATURE CURVES OF SUBCUTANEOUS TESTS

Pre-injection — — — — — — — — — — — — Post-injection — — — — — — — — — — — —
In Tables III and IV, and in Plate I, pre-injection and post-injection temperatures can be easily compared. Much to our surprise, the post-injection temperatures did not show the rise which would be expected. However, it will be noted from the chart that animals which showed a positive reaction to the intradermal test (Nos. 84, 85 and 87) showed somewhat elevated temperatures as compared with their pre-injection curves. These temperatures remained elevated for some time, as is shown by the curves.

SUMMARY AND CONCLUSIONS

The cultures of avian tubercle bacilli used were pathogenic for chickens, as was shown by producing death in chickens which were injected with parts of the same suspension that was used in the cattle.

No reaction to the ophthalmic test was found in any calves showing lesions or otherwise.

Three very good intradermal reactions were found as follows: in two calves injected subcutaneously and in one calf injected intramuscularly. Another calf injected intramuscularly but with a smaller amount than the last mentioned animal, gave only a suspicious reaction, but there was not sufficient swelling to be called a P3 reaction.

The subcutaneous test on the calves, which showed lesions and had reacted to intradermal test, did not produce a rise of 2° F. or more above the maximum temperature observed prior to the injection of tuberculin nor a temperature above 103.8° F., and therefore the calves cannot be considered as having reacted to this test. In injecting, 5 c. c. quantities of Bureau of Animal Industry subcutaneous tuberculin were used to insure that calves received a full 0.5 gram O. T.

The lesions produced were identical with the lesions described in the so-called skin form of tuberculosis.

At the close of the experiment, the calves were all strong, healthy and in as good or better flesh than when started on the experiment.

The three calves showing local lesions externally will be retained and the lesions found on palpation removed and studied.
After a few months, the calves will be retested to see if they still react to the tuberculin test. If they do not, we shall assume that they had only a circumscribed lesion. They will then be killed and a careful and complete autopsy made.

Our work 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 tests indicate that the intradermal is the most reliable test for detecting tuberculosis in cattle when infected with avian tubercle bacilli. The ophthalmic test gave no indications of infection. Boerner (16) and Kimball (16) report a bull with lesions of tuberculosis in the subcutaneous tissue which a few months previous to their observation had successfully passed an ophthalmic and subcutaneous tuberculin test. This immediately raises the question, might this animal have been carrying the avian tubercle bacilli? This would seem very possible in the light of the above and in view of the fact that artificial infection with that organism gave typical lesions of the skin form of tuberculosis.

Another point is raised—could these several cases in which only suspicious reactions were obtained by the intradermal test and by retesting with the subcutaneous test give negative reactions have any relationship to the avian infection? We are all aware that this is not unusual, as many workers use the subcutaneous test to check questionable reactions to the intradermal test.

ACKNOWLEDGMENTS

We wish to thank Dr. B. F. Davis, former State Veterinarian, for his helpful suggestions and also Dr. G. W. Bond for helping to make the tuberculin tests on the animals before they were placed on the experiment.
REFERENCES