THE TRANSMISSION AND TREATMENT OF INFECTIOUS OPHTHALMIA OF CATTLE.

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In a previous publication\textsuperscript{1} an acute inflammation of the eye of dairy cows was described. From a series of cases a characteristic paired bacillus was isolated. It was possible to reproduce the disease with pure cultures of this organism. It was clearly established that in certain cases the bacilli persisted in the ocular secretions after the acute symptoms had subsided.

\textit{History of the Infection.}

Cows from a midwestern state suffering with acute ophthalmia had been introduced into the herd early in September, 1922. A considerable number of cases occurred in cows in two adjoining barns during September and October. With the onset of cold weather the epidemic subsided but sporadic cases occurred during the colder months. The disease again became epidemic during the months of July, August, September, October, and November, 1923. New cases occurred as late as December. On the whole the inflammations were less severe during the second outbreak, but cases of considerable severity were not uncommon. In our previous paper we called attention to the possibility of a recurrence of the disease in epidemic form since five of ten cows reexamined 3 or 4 months after our first observation still carried the organism. At that time attention was also called to the possibility of transmission by flies. Certain facts seemed to incriminate the house-fly with the spread of infection. Flies feed readily on the exudate at the inner canthus of the affected

\textsuperscript{1} Jones, F. S., and Little, R. B., \textit{J. Exp. Med.}, 1923, xxxviii, 139.
eyes. While direct infections doubtless occur, in the main new cases appear irregularly. The fact that the epidemics reached their maximum during the warmer months also suggests fly transmission. The transmission of the Koch-Weeks bacillus of human ophthalmia in Egypt by flies has been reported by Budd, Laveran, Howe, Biskra, Koch, and others. Howe called attention to the prevalence of the disease during the season when flies were most numerous. He further asserted that the same organism which occurred in the conjunctival exudate could be found on the feet of flies. We attempted by a series of experiments to test the hypothesis that flies transmit the disease.

**EXPERIMENTAL.**

At first we attempted to experiment with flies caught feeding upon the exudate about the eyes of spontaneous cases. Too few were obtained in a viable condition to afford sufficient material. Flies were therefore fed artificially on exudate containing large numbers of viable diplobacilli.

*Experiment 1.*—Six house-flies were caught feeding on the eyes of spontaneous cases. Within 1 hour they were placed in a hemispherical cage and applied over the eye of a cow. The flies refused to feed on the lacrimal fluid but some came in contact with the eyeball. The eye remained normal and diplobacilli were not found in the lacrimal fluid.

In the next series of experiments flies were captured in rooms in the laboratory. They were given only water for 10 or 12 hours before feeding. Exudate from a number of spontaneous cases was obtained on sterile cotton swabs. The material was prevented from drying by the addition of a little sterile bouillon. Flies were permitted to feed on this material shortly after it had been obtained.

*Experiment 2.*—Sept. 20, 1923. A number of flies were permitted to feed on the exudate from spontaneous cases. After 1/2 hour a number were introduced into a hemispherical cage applied over the right eye of a cow. Six flies were observed to walk over the lids and feed on the lacrimal fluid. After 10 minutes

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2 Budd, Laveran, and Howe, cited by Graham-Smith, G. S., Flies and disease, Cambridge, 1914, 188.
a number of other flies of the same lot were introduced and kept in the cage 3 or 4 minutes. The eye remained normal, diplobacilli could not be found in the lacrimal fluid 4 days later.

Experiment 3.—Sept. 25, 1923. Forty flies obtained on Sept. 24 were given a little water but no food until next morning when they were permitted to feed on the exudate from ten spontaneous cases. The flies ate ravenously. ¼ hour after they had commenced to feed, twenty-one were removed. Nine were at once applied by means of a cage to the right eye of a cow. Twelve others were used 2 hours later in an attempt to infect the left eye. In no case did the flies feed on the lacrimal fluids, but several came in contact with the cornea and the lids. Four of the flies used in the second exposure were stunned or killed and placed directly on the membrana nictitans. No infection resulted to either eye.

TABLE I.
The Results of the Bacteriological Examinations of the Dejecta and External Surfaces of Flies Fed on the Exudate of Spontaneous Cases.

<table>
<thead>
<tr>
<th>Lot.</th>
<th>No. of flies</th>
<th>Examinations after feeding</th>
<th>No. of deposits of regurgitated or fecal material examined</th>
<th>Result</th>
<th>Result of examination of bottle washings</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>5 min.</td>
<td>7</td>
<td>No diplobacilli.</td>
<td>No diplobacilli.</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>15 “</td>
<td>12</td>
<td>“ “</td>
<td>Diplobacilli in moderate numbers.</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>1 hr.</td>
<td>14</td>
<td>“ “</td>
<td>Diplobacilli in moderate numbers.</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>2 hrs.</td>
<td>12</td>
<td>“ “</td>
<td>No diplobacilli.</td>
</tr>
<tr>
<td>V</td>
<td>4</td>
<td>3 “</td>
<td>9</td>
<td>“ “</td>
<td>“ “</td>
</tr>
</tbody>
</table>

In addition to the direct attempts to transmit the virus, another series of flies was examined bacteriologically, but as a more complete observation is given in Experiment 4 the details will be omitted. It is sufficient to say that viable diplobacilli were obtained from flies 3 hours after they had been removed from the feeding bottles.

Experiment 4.—A number of recently hatched flies were obtained on Sept. 27. On Sept. 28, they were fed on the exudate from five spontaneous cases. As soon as feeding had been completed, they were removed to a series of sterile bottles. Bacteriological examinations were made of the different lots at varying intervals. In all cases isolated spots of regurgitated or fecal material were cultured separately. After this a little sterile broth was added to the bottle and the sides and bottom were rinsed. From the bottle washings plate cultures were prepared. The results are given in the appended table.
A more complete discussion of the results of this experiment will be given later. It clearly demonstrates, however, that the organism is incapable of remaining viable in the gastrointestinal tract of flies for an interval as short as 5 minutes. Furthermore it suggests that the organism may be carried on the external surfaces of flies for only relatively short periods. These facts were utilized in the later experiments.

Experiments 5 and 6.—On two occasions a number of flies were fed on the exudates of spontaneous cases. In one instance they were removed to a separate bottle in 5 minutes, in another in 15 minutes. After a second 5 and 15 minute period had elapsed, 5 cc. of bouillon was introduced into the bottles and the flies and the sides of the bottles were washed vigorously. The bouillon suspensions were divided, portions were centrifuged at high speeds, and the sediment was instilled into the left eyes of two cows. A few drops of the uncentrifuged suspension were instilled beneath the lids of the other eyes. Infection did not take place. Microscopic examination of the centrifuged sediment revealed diplococci in small numbers. A few characteristic colonies also appeared in the plate cultures made from the bouillon suspension.

Résumé of Attempts to Transmit the Disease with House-Flies.

Although our experiments failed to show that the disease could be transmitted by flies, it is probable that the methods are at fault. Flies are phototropic and hence gather as close to the light as possible usually at the center of the screen, the greatest distance from the eye. In no way did we find it possible to approach the natural conditions. In the experiments in which the bottle washings were used in attempts to infect, a certain number of viable bacilli must have come in contact with the eye. It is difficult to attempt to explain why the animals failed to become infected. In later experiments the eyes of both animals were inoculated with culture. One received a large dose and developed ophthalmia. The other received a small quantity of culture sprayed on the eyeball. This animal proved resistant; the organisms were able to maintain themselves in small numbers only and produced only mild symptoms. It is possible in the experiments with flies that too few organisms were introduced into relatively resistant animals, or that the organisms had been injured during the manipulation.
The more conclusive features of the experiments are those in which the excretions of flies known to have fed on material containing the bacilli were examined bacteriologically. The organism is apparently killed when ingested by the fly since it cannot be found in the regurgitated material even as soon as this material is deposited on the sides of a sterile bottle. In no instance was the organism obtained from the feces. The examinations of the bottle washings indicate that the bacilli may be carried on the external surfaces of flies for relatively short periods only. The maximum period determined thus far has been 3 hours. In another more complete experiment the organisms were viable only at the end of 1 hour.

The spontaneous disease usually reaches its highest incidence in the warm months. New cases occur, however, in the colder portions of the year. It is not probable that during cold weather the organism is transmitted by flies. It is also difficult to explain these cases by direct contact. That the virus had other means of dissemination seemed logical. Several facts were known, the bacilli were not resistant to drying, their specificity for bovines and their inability to survive 5 minutes in the digestive tract of the fly argue against an existence as a free form. Furthermore the existence of an organism so specific in character and depending for survival only on passive transmission by flies would be extremely precarious.

Further observations were made to determine whether the virus left the body in another manner. During the first epidemic the nasal exudates of a number of cows suffering from acute ophthalmia were examined. The bacillus could not be found. In November, 1923, the nasal secretions from fourteen cases were examined with negative results. In one acute case of considerable severity in a calf the organisms were encountered in the nasal passages. It was necessary to determine, if possible, whether at any stage of the disease the virus gained access to the nasal passages. With this in view three cows were inoculated in various ways and frequent bacteriological examinations were made of the eyes and nasal secretions. The protocols are given in detail.

Case 1.—On Dec. 3, 1923, a few drops of bouillon containing a portion of the surface growth from a 48 hour blood agar culture of diplobacilli were instilled beneath the lids of the left eye of a cow. ½ hour later the nasal passage in the
region of the opening of the tear duct was brushed gently with a sterile swab. Cultures were prepared from this material at once. They failed to show diplobacilli. The material about the opening of the tear duct was examined 4 and 6 hours after the inoculation with negative results. On Dec. 4, a little material was collected from the inner canthus of the left eye. It showed a moderate number of diplobacilli. Cultures were prepared from the secretion of the left nasal passage. They showed the diplobacilli present in considerable numbers. On Dec. 5, the organism was not cultivated from the nasal passages. On Dec. 6, the eye was appreciably inflamed and diplobacilli were present in the exudate. Cultures from the nostril gave negative results. Cultures were made daily during the ensuing week from the left eye and left nasal passage. The ocular reaction was pronounced and the organisms were always present. The cultures from the nasal passage were negative.

Case 2.—On Dec. 17, 1923, the growth from the slant of a 48 hour blood agar culture was suspended in 10 cc. of bouillon and by means of an atomizer a little was sprayed on the left eyeball of another cow. In addition a considerable amount was sprayed over the septum of the right nostril. 7 hours later a sterile swab was inserted into the nostrils and cultures made from the collected material. They failed to reveal diplobacilli. On Dec. 18, cultures were prepared from both eyes and both nostrils. Those from the left eye and the left nostril contained diplobacilli. The right eye and right nostril were negative. On Dec. 19, the cultures from the left eye were positive. Those from the right eye and both nostrils were negative. On Dec. 20, there occurred a visible inflammation of the left eye, the right eye was normal. The cultures from the left eye were positive. Those from the right eye and both nostrils were negative. On Dec. 21, the inflammation of the left eye was more severe, large numbers of diplobacilli were present in the exudate. Material obtained from the left nostril contained considerable numbers of diplobacilli. The right eye and right nostril failed to reveal the organism. No further observations were made on this animal.

Another cow treated in a similar manner proved resistant. The organisms were able to maintain themselves in the inoculated eye and produce a mild inflammation, but as far as we could determine they failed to reach the nasal passage.

The observations tend to suggest an indirect method of egress for the organism. It is apparent that in certain infections the bacilli gain access to the tear duct at least early in the course of the disease. In two of three experimental inoculations they were isolated from the nostril before symptoms of ophthalmia developed. It seems well established that their appearance in the nasal passage cannot be explained by the immediate washing down of the fluids used in the inoculations, since material from the nostrils obtained shortly after
inoculation always failed to reveal the organism. It has been also definitively established that the diplobacilli are incapable of surviving on the mucosa of the nasal passages. In spontaneous infections the organisms probably gain access to the tear duct shortly after they have come in contact with the eyes. They may even produce considerable inflammation of the duct. The irregular appearance of the bacilli in the nostril is explainable by the hypothesis that early in the disease the lumen is not sufficiently occluded with exudate and thus permits the lacrimal fluid carrying the bacilli to reach the nostril.

**TABLE II.**

*The Effect of Treatment with Argyrol and Zinc Sulfate.*

<table>
<thead>
<tr>
<th>No. of cases treated</th>
<th>Results of bacteriological examination of eyes before treatment</th>
<th>Results of bacteriological examination of eyes after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>7</td>
<td>Diplobacilli in enormous numbers.</td>
<td>2 negative, 5 showed diplobacilli.</td>
</tr>
<tr>
<td>with 20 per cent argyrol.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Diplobacilli in enormous numbers.</td>
<td>None showed diplobacilli.</td>
</tr>
<tr>
<td>with 21 per cent zinc sulfate.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inasmuch as certain bacterial infections of the eye are amenable to treatment and in some earlier experiments we had succeeded in ridding the eye of the organism in certain experimental cases with a solution of zinc sulfate, we decided to test the remedial efficiency of zinc sulfate and argyrol. The cases chosen were typical infections occurring in a dairy herd. They were divided into two groups and treated by instilling the agents beneath the lids twice a day. Table II gives a brief summary of the results of the treatment. It will be noted that both drugs are of value as therapeutic agents. Zinc sulfate in the proportion of 1:40 promptly rids the eye of the bacilli.

**DISCUSSION AND SUMMARY.**

The experiments in which transmission of the disease was attempted by flies hardly parallel the observations within the herd. It is not unusual to observe large numbers of flies feeding on the exudate. Slight disturbances may interrupt feeding and cause the
flies to disperse and within a short period alight about the eyes of other cows.

The experiments, however, bring out the fact that the bacterium will not remain viable for even a few minutes in the digestive tract of the fly. Its life on the external surfaces of the fly is extremely short and in our observations has not exceeded 3 hours. The latter fact strengthens the opinion that in the main the infection is not dust-borne since the bacterium soon dies when not in contact with the eye.

Two other points are of considerable significance. The ability of the organism to maintain itself on the eye for considerable periods after the acute symptoms have subsided may explain the reappearance of cases during the warmer months. The organism can exist in the eye throughout the winter and with the warm weather flies may transmit it to other susceptible individuals and thus a nucleus of an epidemic may be established.

The presence of the organism in the nasal passages in the incubation stage and early in the disease in two of our experimental animals affords an explanation for the appearance of the disease in sporadic cases in the colder months. It is assumed that nasal exudate as a fine spray may be forcibly expelled and directly reach the eyeball of a normal individual. It has been shown that small quantities of culture sprayed on the cornea are capable of giving rise to the characteristic disease. The irregularity of the elimination of the organism through the nostril may be explained by the effect of inflammation on the tear duct. In experimental cases a small quantity of bouillon containing the culture was dropped or sprayed on the cornea. Doubtless the bacilli are deposited on the mucosa of the tear duct. Here they may multiply and set up an inflammation and thus gain access to the nasal passage. To what degree the virus is spread by the forcible expulsion of nasal secretion containing lacrimal fluid cannot be determined. The elimination of the bacilli from the nasal passage in our experimental inoculations leads us to believe that in the main the phenomenon is associated with early infections. The examination of the nasal passages of a large number of well established cases with negative findings tends to corroborate this view.

Of interest to those concerned with the treatment of animal diseases is the readiness with which the inflammation subsides when treated with 1:40 zinc sulfate.