

THE HOUSEFLY (*MUSCA DOMESTICA* LINN.), AS A VECTOR OF *SALMONELLA PULLORUM* (RETTEGER) BERGEY, THE AGENT OF WHITE DIARRHEA OF CHICKENS¹

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For many years flies have been suspected of being one of civilization's worst enemies in the transportation of bacterial organisms. Numerous publications (for historical account refer to Steinhaus, 1946) have appeared furnishing evidence that the adult housefly is a mechanical carrier of bacteria which are not only harmful to man but also to his domestic animals. Fewer studies are found relative to another phase of flies as vectors: that is, the fate of bacteria ingested by the larvae in relation to metamorphosis and the duration of the association. No study, to date has been found concerning the bacteria-fly-chicken association of white diarrhea.

The fact that maggots will grow and develop best in habitats rich with bacteria and yeast is commonly accepted. If the breeding habitats and the feeding habitats of the housefly are studied, one learns that the food and fecal matter of chickens are often utilized by the housefly for breeding and for food. Observations of chicken behavior indicates that they continually capture and devour many flying insects. The possibility, therefore, seems to exist that if the larvae while feeding can be infected with a chicken pathogen and if these bacteria can endure the metamorphosis of the host, there is a direct relationship between the agent of a chicken disease and the flight range of the adult fly.

The organism selected for study was *Salmonella pullorum* (Retteger) Bergey, causative agent of white diarrhea of chickens. The importance of this disease to the poultry industry is summarized in U. S. D. A. Farmers' Bulletin No. 1652, p. 7: "Pullorum disease (bacillary white diarrhea) is quite wide spread, existing in every section of the United States where appreciable numbers of poultry are kept. It causes heavy financial losses, resulting from the deaths of baby chicks, diminishing egg production in hens and pullets, reduces hatchability of eggs, and occasionally the death of hens due to generalized pullorum infection." Young chicks up to three weeks seemed to be most vulnerable. Death rate in infected chick broods may range from 50 to 80 percent.

METHOD

Bacterial-survival Study

To arrive at the subsequent conclusions 15 to 20 newly hatched housefly maggots were placed upon a 48-hour-old pure culture of the test bacterium which was growing on nutrient agar in Petri-dishes. The Petri-dishes were kept at a temperature of 37° C. It was observed that the Petri-dishes would fuse together and seal off the supply of air unless they were opened daily.

After the larvae developed and pupated, the pupae were sterilized in an aqueous solution of 1:1000 mercuric chloride (Glaser, 1923) for five minutes. While in the germicidal solution, the pupae were agitated. This disposed of the air bubbles adhering to the pupae and usually caused them to sink. At the conclusion of the five minute period, each pupa was transferred to a tube of nutrient broth and agitated for five minutes. Then the pupa was washed in sterile-distilled water and placed on a sterile agar plate to await fly emergence. Each pupa received a

¹Part of a dissertation submitted in partial fulfillment for the Ph.D. degree, Department of Zoology and Entomology, Ohio State University, Columbus.

number in the order of its processing and this number remained with the specimen throughout the experiment. Controls were maintained throughout the experiment.

The flies were placed in the following indicative categories: the positive or negative effectiveness of the germicide, the survival of the fly, and the presence of bacterial growth after the fly emerged. If the pupa was free of any evidence of contamination but did not emerge, it was classified under "dead" but, carrying the same identification, was macerated. Those pupae that were free of contamination and that emerged and showed evidence of disseminating bacteria were used in bacterial-survival phase. If any of the experimental flies did not produce evidence of harboring bacterial organisms, they were transferred and macerated along with the dead pupae.

After the flies emerged, the agar plates were observed for bacterial growth. Whenever bacterial growth appeared on any plate, the fly was washed for five minutes in an aqueous solution of 1:1000 mercuric chloride, passed through the broth tube checks, rinsed in sterile, distilled water and transferred to either the bacterial-fly longevity association or the bacteria-fly-chicken association. The plates which held the emerged flies were incubated for 24 hours and at the end of the incubation period a letter of the alphabet was assigned to each bacterial colony and they were examined for the presence of the test organism. The bacteria were identified according to Bergey (1939) and Breed, Murray, and Hitchens (1948). Organisms which did not meet the key characteristics for the test bacterium remained unidentified. Only the experimental bacterium was recorded in the data.

The experimental flies which emerged and did not show any evidence of bacterial growth, and the pupae which did not emerge but were free of contamination, were externally sterilized in an aqueous solution of 1:500 mercuric chloride for ten minutes. Each specimen was passed through the nutrient broth check and placed in a sterile, distilled water blank in which it was macerated with sterile needles. The macerated substances were agitated so as to disperse the material evenly within the water blank and then streaked upon sterile agar plates. The plates were incubated for 48 hours, and if colonies appeared they were identified for *Salmonella pullorum*.

The flies which emerged and showed positive evidence of carrying the test organisms were divided into groups. These groups were arranged and handled to permit study of (1) how long the bacterial organism remained with the housefly and during what part of this period it was disseminated, (2) whether the infected housefly, when devoured by a host, sufficiently transferred the test organism to the chicken.

Existence of Bacteria within the Housefly

A portion of the flies that showed positive evidence of carrying the test species through their metamorphosis, was studied to determine the longevity of the bacteria within the housefly and during what part of this period the bacteria were disseminated. Flasks plugged with cotton and autoclaved for one hour at 250° C. were filled to a depth of two inches with sterile nutrient agar and after solidification of the medium, the flasks were tilted to collect the moisture of condensation. The surplus moisture was poured out and then the flasks were ready for the flies. As soon as the bacterium had been recovered from the housefly in the survival phase, the fly was sterilized in an aqueous solution of 1:1000 mercuric chloride for five minutes, then passed through the nutrient broth check, rinsed in sterile, distilled-water and transferred to a 500 cubic centimeter filter flask which had been previously sterilized and contained two inches of sterile agar. The flies that showed contamination by bacterial growth in the broth tubes were eliminated for the present but were successively sterilized until free of contamination or discarded.

After the fly had been placed in a flask, observations were made frequently for bacterial growth. Whenever growth appeared, the fly was sterilized and

transferred to a second sterile flask and the bacterial colonies of the first flask were identified.

Every second day a sterile piece of cotton which had been soaked with boiling simple syrup (10 parts water and one part sugar) was hung from the cotton stopper for three to six hours. This constituted food for the fly.

As soon as it was apparent that the fly was dying or it was found dead, it was sterilized in an aqueous solution of 1:500 mercuric chloride for ten minutes, passed through the nutrient broth check, rinsed and macerated. The different colonies growing from the streaked macerated material were identified and recorded.

Bacteria-fly-chicken Association

A certain portion of the flies which had produced evidence of carrying a test bacterium were studied to determine the bacteria-fly-chicken association. They were sterilized in an aqueous solution of 1:1000 mercuric chloride for five minutes, passed through the nutrient broth tubes and rinsed in sterile, distilled water. Until the effectiveness of the germicide could be observed each fly was stored in a sterile Florence flask, the bottom of which was covered with one inch of agar. After no contamination was evident, the flies were placed in cubicles with chickens.

Chickens of the most susceptible age to *S. pullorum* were secured from a hatchery which guaranteed that they were free of the organism under consideration. The chickens were placed in separate cubicles and on two consecutive days before the flies were introduced, fresh fecal matter from the chickens was examined to guarantee the absence of the test bacterium. The cubicles were cleaned each day by removing the inner liners which caught the fecal matter and other debris. As soon as the experimental flies, which had been placed with these chickens had been consumed, the chickens were fed commercial feed as recommended by the hatchery. Distilled water was available for the chickens and they were kept at room temperature.

After the flies had been introduced, fecal examinations were made every 24 hours until the chicken excrement showed positive evidence of *S. pullorum* and when it was recovered for three days in succession the chicken was discarded with the assumption that the chicken had obtained the bacterium from the flies placed in the cubicles with it. No attempt was made to study the effects of the test bacterium on the chickens.

SUMMARY OF DATA

Five hundred pupae were treated as described under the method for the study of *S. pullorum* surviving the matamorphosis of the housefly. Of the 500 pupae, 301 emerged free of contamination, 135 were discarded because of contamination and 64, free from contamination, did not emerge and were assumed to be dead.

One hundred and twenty-two of the 301 emerged flies, which were free of contamination, gave evidence of carrying *S. pullorum* through the pupal stage by disseminating the test organism on sterile agar. One hundred and seventy-nine revealed no trace of the experimental bacterium and were macerated.

The pupae which were free of contamination but did not emerge and the flies that did not disseminate test bacterium were macerated in the previously described manner. Thirty-one of the 64 pupae macerated and 28 of the 179 flies macerated harbored *S. pullorum*.

The test bacterium was harbored by 181 out of 365 pupae free of contamination. One hundred and twenty-two of the 301 emerged flies disseminated *S. pullorum*.

Thirty flies which previously had disseminated *S. pullorum* were treated for the study of the longevity of the bacteria-fly-association. One of the 30 flies survived 23 days. The test bacterium was last disseminated by this fly on the fifteenth day. The bacterium was not recovered again from the above mentioned fly, although *S. pullorum* was recovered from another fly on the twenty-second day when it was

macerated. This fly survived 22 days and last disseminated *S. pullorum* on the second day. The third longest period of fly longevity was 20 days. The test bacterium was recovered from this third fly upon maceration on the twentieth day but it had not disseminated *S. pullorum* since the ninth day. The test bacterium was disseminated by 26 flies of which 25 produced no evidence after nine days, but the test bacterium was recovered from the macerated material of one fly after 22 days.

Thirty-seven flies which had previously disseminated *S. pullorum* were treated for the study of the bacteria-fly-chicken association. Thirty-one chickens were allowed to feed on 37 previously infected houseflies. Four chickens, though treated similarly to the others, were separated from all flies and used as controls.

Five chickens produced evidence of *S. pullorum* from their fecal matter two days after the flies had been introduced into the cage but only one of the five produced such evidence for three consecutive days. The greatest recovery of *S. pullorum* was on the fifth day. Variance in production of the test bacterium was from 2 to 10 days. All 31 experimental chickens produced *S. pullorum* three consecutive days in their fecal matter. Evidence from this data indicates that flies do carry the test bacterium and that *S. pullorum* does develop sufficiently within the chickens to be recovered from the chickens' excrement.

CONCLUSIONS

The data reveal that *S. pullorum* was disseminated by 40.53 percent of the experimental flies, 49.58 percent of the pupae harbored the test bacterium. The experimental bacterium was disseminated for fifteen days and harbored for 22 days. Four of the 30 test flies used in the longevity association study produced *S. pullorum* after the ninth day. All of the 30 flies gave evidence prior to the ninth day of containing the test bacterium. *Salmonella pullorum* was recovered from the macerated material of 70 percent of the experimental flies at death.

Thirty-seven infected flies were fed to 31 three week old chickens and in two days the test bacterium was recovered from the chickens' excrement. All 31 chickens produced *S. pullorum* in their fecal matter within ten days. In this study, *S. pullorum* was ingested by the larva of the housefly (maggot), survived the metamorphosis of the housefly and sufficiently established itself in the chicken, by a vector (the housefly), to be recovered from the chickens' feces. The above data establish the housefly, *Musca domestica*, as a vector of *S. pullorum* and thus increases the potential area of bacterial dissemination to that of the range of flight of the housefly.

I am indebted to Dr. C. E. Venard, Department of Zoology and Entomology and Dr. H. H. Weiser, Department of Bacteriology, both of The Ohio State University, for their suggestions and criticisms pertaining to this study.

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