STUDIES ON THE TRANSMISSION OF THE POULTRY DISEASE, INFECTIOUS SYNOVITIS, BY USING DIRECT CONTACT AND INSECT VECTOR METHODS

By

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INTRODUCTION AND OBJECTIVES

Infectious synovitis is a disease which effects poultry. It causes crippling and loss of weight, thus hampering the marketability of the birds. Although mortality is relatively low, the number of morbid chickens may be greater than half of the flock. Birds which are effected become run-down and more susceptible to secondary infection. The disease is known to occur in the south-central United States and along the eastern-central seaboard.

Much work, in the past years, has been directed toward an effective control for the disease. Many theraputic compounds have been tested, however as yet there is no known cure for the disease. Some compounds have been shown to alleviate the effects and spread of the disease, but they must be fed continuously to be effective.

There is as yet no information disclosing the method of transmission of infectious synovitis.

The primary objective of this study was to determine if the disease could be transmitted by using certain mosquitoes as vectors, and if direct contact transmission can be obtained. Three approaches were used to this problem. One approach involved the refeeding of mosquitoes on assay birds after they had initially fed on effected birds. Another involved the feeding of mosquitoes on diseased birds, then at various succeeding intervals, these mosquitoes were macerated and

injected into assay chickens. The third procedure consisted simply of inoculating a number of chickens with the causitive agent and then placing these birds into pens with uninoculated assay birds. In some instances fecal material was taken from the collecting pans and placed in the drinking water of each cage respectively.

The effective control of synovitis, and the key to meaningful analysis of the effectiveness of a drug, rest largely with the solution of the method of transmission of this disease. It was with these goals in view that this project was undertaken.

The mosquito, Aedes aegypti (Linn.), was used in these studies, because it is known to be a vector of the virus diseases yellow fever, dengue fever, and encephalitis. Culex fatigans Wied., was used because of its willingness to feed on chickens.

REVIEW OF LITERATURE

Characteristics and Distribution:

An infectious disease of poultry, which is characterized by involvement of the synovial membranes, was described by Olson et al. (1954) and Wills (1954b). It was described as causing ruffled feathers, droopiness, pale or purple combs and enlarged hocks, wing joints and breast blisters. There is a greenish character to the feces as the disease progresses. Olson et al. (1958) found that in laboratory tests inoculated birds showed a slight swelling in the hocks four days after inoculation. Cover et al. (1956) states that internally it causes anemia, enlarged spleen, enlarged and mottled liver and caseous or yellow cheesy exudate in affected joints.

Shelton et al. (1957) said an increase in leucocytes and a reduction of erythrocytes occured as the disease progressed. He also stated that weight gains were reduced till the disease reached a certain stage at which dehydration and emaciation would cause a weight loss. He further reported that in the early stages of the disease splenomegaly occured while later stages caused enlarged and pale colored kidneys and greenish and/or enlarged liver. Sevoian et al. (1958) observed that blood disturbances were present between five and sixteen days post inoculation, and that the one lasting or persistent symptom was the presence of cheesy material in the hocks.

Madsen (1942) reported an arthritis in turkeys which resembles the condition described by Olson et al. (1954) and Wills (1954). Since the condition was different from any previously described he labeled it "synovitis". However, Wills (1954b) named the condition "infectious synovitis", which is the term in general use in the literature.

Sevoian et al. (1958) reports that flock mortality from infectious synovitis runs around 3 per cent but morbidity may be as high as 50 per cent. Olson et al. (1956) states that he observed flock mortality at 5 per cent and morbidity up to 75 per cent due to infectious synovitis. Wills (1954a) reports that he observed the disease to cause low morality with morbidity running around 20 per cent.

The disease has been reported in Texas a d Arkansas by Wills (1954b); in the Delaware, Maryland, West Virginia and Virginia area by Coffin (1955); in Connecticut by Jungherr (1954); and in West Virginia by Olson et al. (1954).

Agent Involved:

Wasserman et al. (1953), while working on the cultivation of some strains of the chronic respiratory disease and turkey sinusitis agent, found that after testing for bacterial sterility the agents still gave typical swollen hock responses. Snoeyenbos and Olesiuk (1955) could not get the agent, which was causing arthritis, to grow in tryptose, blood agar, Bacto-PPLO broth and other agar mediums. Also tests for chronic

respiratory disease antibodies were negative. Cover and Galeta (1955) found that the agent, which was causing arthritis, lived best in the yolk sac. They also discovered the particle size to be from 1 to 2 microns. They observed that the agent would live 5 hours at 37° centigrade but only 1 hour at 56° centigrade. Olson et al. (1957b) found that they had three pathogenic agents which caused infectious synovitis. The viral and the Pleuro-Pneumonia-Like- Organism (PPLO) agent produced typical signs of infectious synovitis, however a third, and separate, agent was pathogenic only to chick embryos. It was learned by Thayer et al. (1958) that the agent he was working with would grow in tissue cells. They were also able to store it at freezing temperatures. Sevoian (1958) also reported variation among strains of infectious synovitis as well as varied sensitivity to drugs. The agent was propogated in a tissue culture, composed of Sinn-Saunders medium containing fragments of chicken embryo tissue, by Wichmann et al. (1960). They found that serial passes of the agent, in tissue culture, resulted in a modification to a nonpathogenic form between the tenth and fourteenth passage. It was also discovered that when birds showing no symptoms to original inoculation were challenged, they were resistant to a virulent culture of the agent. They were able to keep the agent at -72° centigrade for 66 days.

Hale and Purchase (1931) cultured Staphylococcus pyogens

aureus from pheasants affected by an arthritis condition. They felt infection occured by bacteria entering through an open wound. Jungherr (1933) was able to culture Staphylococcus aureus from turkeys affected with arthritis. Jungherr and Plastridge (1941) reported that an avian staphylococcus caused swelling in foot pads and hock joints but seldom in wing joints. They also found swelling usually occured only on one leg. Material in swollen joints would become hardened or solid and would be orange in color. Bowness and Fahey (1954) report a lameness in turkey poults caused by a staphylococcus.

PPLO was found to be the cause of a turkey sinusitis, according to Markahm and Wong (1952). This sinusitis showed symptoms somewhat similar to synovitis. Olson et al. (1956) felt that the agent may be a PPLO. They found the particle size to be between 0.2 and 0.5 micron in diameter. Testing revealed the agent was not filterable and would not grow in artificial media. They were able to keep a yolk culture 271 days at -20° centigrade. A differentiation between PPLO synovitis and infectious synovitis was made by Olson et al. (1957c). The main difference is that in PPLO synovitis there is no caseous exudate, there are more monocytes and preforms in the blood, and the infection is not as severe. They report that sinusitis rather than synovitis occured in birds inoculated intratracheally with PPLO synovitis. Chalquest and Fabricant (1960) grew a PPLO around a culture

of Staphylococcus aureus. They later derived a method of growing it without S. aureus. They were able to isolate PPLO from several laboratory colonies of infectious synovitis as well as from field cases. When the PPLO was inoculated into chickens it gave a typical infectious synovitis response. They also found the agent to remain virulent through seven passages in artificial media.

Lecce et al. (1955) felt that the agent was either a rickettsia or large particle virus. They reported the agent to be unfilterable and range in diameter from 0.2 to 0.5 u. They warned that PPLO is easy to confuse with a large particle virus and state that PPLO is associated with arthritis in other animals. Cover et al. (1956) indicated their findings led them to believe the agent is either a small bacterium or large particle virus.

Control:

Olson et al. (1955b) found aureomycin and terramycin effective in controlling the spread of infectious synovitis but learned that streptomycin and penicillan were not effective. Lecce et al. (1955) reported that the infectious synovitis agents were more sensitive to terramycin and achromycin than to aureomycin. Munro et al. (1956) stated that aureomycin was an effective control for infectious synovitis if fed continuously in effective quantities. Cosgrove and Coffin (1956) found furazolidone to be effective if fed continuously

in effective quantities. Shelton et al. (1956) stated that aureomycin was more effective than furazolidone for controlling the disease. Olson et al. (1957a) stated that chlortetracycline fed continuously at 200 gm/ton of feed would also control infectious synovitis. Shelton and Olson (1957) found dihydrostreptomycin sulfate was effective if inoculated intramuscularly at the rate of 50 mg/lb. before signs of the disease appeared. But they said it was ineffective if delayed for four or more days after initial symptoms appeared before treating the flock. Price and Zolli (1958) discovered that dietary calcium-phosphorus seemed to have some effect in aiding the maintenance of weight in birds affected with infectious synovitis. They found that terephthalic acid appeared to have some effect in controlling the spread of lesions. Shelton et al. (1958) reported that chlortetracycline was effective in controlling infectious synovitis. Shelton and Olson (1959a) found that chlortetracycline was more effective when fed orally than when inoculated intraperitonealy. Shelton and Olson (1959b) discovered that chlortetracycline was more effective when fed continuously at 100 to 300 gm/ton of feed than when treatment was started four days after inoculation, continued for five days and then dropped. The latter procedure was found to be ineffective.

Transmission and Infectivity:

Wills (1954b) reported that he observed no contact

transmission to controls, however, in a later experiment Wills (1954a) observed cage mate transmission to 20 per cent of the uninoculated controls. Two contact birds died 26 days from the time the first affected birds appeared in the flock, and the last one died about 15 weeks from that time. Olson et al. (1956) observed that contact controls contracted the disease from 25 to 80 days from time first affected bird appeared in the flock. Shelton and Olson (1959b) however, reported that none of the uninoculated controls used in their test contracted the disease and all showed normal weight gains. Skamser and Seeger (1960) inoculated birds with synovitis agent number 1829, obtained from N. C. Olson, by using the sinus and foot pads routes. The birds infected via sinus route showed greater tendency toward infection of cage mates than the foot-pad-inoculated birds.

Wills and Delaplane (1955) examined 1,470 eggs taken from a previously infected flock. One chick, 21 days after hatching, was observed to have synovitis. Olson et al. (1956) found a possibility of transovarian transmission in some of their work. Snoeyenbos and Basch (1958) found it difficult to infect mature or egg laying birds. However, the did demonstrate transmission of the disease, through the egg, in two cases. Sevoian (1958) was able to infect laying hens with infectious synovitis and was successful in recovering the agent in the eggs.

Wills (1954b) found he could not transmit the disease

by oral, ocular or respiratory inoculations. He reported, however, that infection occured mostly in birds 2 weeks to 5 months of age. Later, research by Wills (1954a) uncovered transmission by intratracheal and intracrop inoculations of intestinal and cloacal contents, taken from a bird inoculated 7 days previously. The birds receiving intratracheal inoculation came down with the disease while those inoculated via crop were negative. Snoeyenbos and Olesiuk (1955) stated that their material was more infective to chickens than to turkeys. They also noted that 4 to 5 week old birds were the most susceptable. Olson et al. (1955a) were able to obtain transmission by inoculating yolk material, from a previously inoculated embryo, into the foot pad of a check bird. They also found that mucous from the trachea of a chicken was more pathogenic than the joint exudate. Cover and Benton (1957) recovered the agent in the following tissue at one time or another during the course of infection: duodenum, rectum, liver, gall bladder, kidney, testes, ovary, trachea, lung, air sac, cardiac muscle, skeletal muscle, keel bursa, tendon shouth and mesentery. It was found in the liver, spleen and blood from 48 hours to 10 days post inoculation. Although they recovered the agent in the intestinal tissue, they rarely, if ever, found it in intestinal contents, bile or synovial membranes. Snoeyenbos et al. (1958) found no difference in susceptibility, to infectious synovitis, in chickens 23 to 104 days old. They did find that the amount

of agent used modified the incubation period. Benton and Cover (1959) bled inoculated chickens at 4, 8, 24, 28, 32 and 48 hours after inoculation. This blood was then re-inoculated, intravenously, into unaffected birds. The birds inoculated with 4-hour blood did not produce the infection. Two of the three birds inoculated with 8-hour blood showed the infection. All of the birds were inoculated intravenously. A similar test where birds were inoculated intramuscularly proved entirely negative. They found that the agent was present in the blood from the fourth to the thirteenth day at about constant virulence. They also learned that the agent was not present in the liver, blood, spleen, kidney, ovary, testes, or joint exudate after 52 days in chronically affected birds. Olson and Shelton (1958) found that birds at age 3 weeks were more susceptible than those age 5 weeks. They also found the sinus route of inoculation to be less effective than the foot pad.

It was discovered by Cassidy and Grumbles (1959) that there was a slight immunity in chickens which had once been inoculated with a splenic suspension of synovitis. Those birds which did not come down were reinoculated and again failed to exhibit symptoms.

Mosquitoes as Vectors:

Hinshaw and McNeil (1952) noticed a correlation between incidence of infection and presence of mosquitoes, while working

with staphylococcus in turkeys. They reported that one area observed a decrease in incidence of the disease following mosquito control operations. They also hinted at the possibility of transmission through open wounds. However, Fahey (1954) found no mosquito correlation in his work with staphylococcal arthri is in turkey poults.

Benton (1959) discovered that cagemate transmission occured in correlation with mite infestations. In one case nine of the ten check birds contracted the disease. However, in the two tests in which cage mate transmission occured a heavy mite infestation was also noted. He also took fifty mosquitoes and allowed them to feed on chickens which had been inoculated seven to ten days previous. He then macerated and froze ten of these mosquitoes. Later these were inoculated into assay birds to see if they were infective. The others were allowed to refeed on assay birds five days following initial feeding. No transmission by mosquitoes were observed.

EQUIPMENT

Chicken Restrainers

Two chicken restrainers were constructed in order to prevent the birds from moving around and disrupting the mosquitoes while they were feeding.

Basket type.-The restrainer consisted of a piece of plywood 5 in.-12 in. which had a slit one half inch wide running down the middle from about three inches from the top to four inches from the bottom. The plywood was mounted on four strips of lumber, two were 1 in.-1 in.-5 in. and two were 1 in.-1 in.- 12 in. Two hook screws were placed in the plywood, about two inches apart at the bottom right of the board. A wire basket ($\frac{1}{4}$ in.mesh), shaped like a cone, was fashioned and the two ends soldered together. This basket was then fastened to the plywood with the small end to the front, by placing two bolts through the wire mesh and then through the slit in the board. The basket was then held in place by attaching wing nuts to the screws beneath the board. By loosening the wing nuts, the basket could be moved back and forth to fit the bird. Three separate sizes of baskets were used to accomodate the bird as it grew. The chicken was placed head first into the cone and its feet were placed between the two screw hooks. A wire spring was attached to one hook, wrapped around the chicken's legs, and then secured on the other hook. It was desirable to fasten another spring

on the side of the basket, run the spring between the chickens legs, and then fasten it to the opposite side of the basket. This prevented the bird from sliding out of the basket.

Spring type.-The "floor" of this restrainer was a board, ½ in.-8 in.-12 in. This board was mounted on four pieces of lumber, two were 1 in.-1 in.-8 in. and two were 1 in.-1 in.-12 in. Two hook screws were located in the "floor", about two inches apart at the bottom right of the board. Two "eye" screws were placed on the left hand side of the board. One was placed about six inches from the bottom and two inches from the left side. The other was placed about four inches from the bottom and three inches from the left side. Two more hook screws were placed in the board; one two inches from the right and six inches from the bottom, and the other two inches from the right edge of the board and three inches from the bottom. A small link, light weight chain was attached to each "eye" screw. A wire spring was attached, to the middle of each chain, in such a way as to create a slack in the middle of the chain. A chicken was laid on the board and its legs were placed between the hook screws on the bottom of the board. A wire spring was then fastened to one hook screw, wrapped around the birds legs and attached to the other hook screw. The chains were then placed over the bird and attached to the hook screws on the opposite side. The chain was kept taut by the spring incorporated in it. This restrainer proved less satisfactory than the basket type, as birds were able to

"wriggle" out from beneath the chains.

Mosquito Cages

Three types of cages were used to contain the mosquitoes. The culture cage was a large structure where the laboratory culture was maintained. The feeding cage was utilized for holding mosquitoes which were being fed or refed for a particular test. The holding carton was a device used to contain the mosquitoes for use at a later date.

Culture Cages.-This cage was 3 ft.- 3 ft. square. The frame was constructed of twelve 2 in.-2 in.-3 ft. boards. The top, two side and back were covered with clear polyethylene plastic. The plastic was held in place by strips of plyboard, ½ inch wide and three feet long. The bottom was covered by three foot square piece of plywood. The cage front was composed of a six inch strip of plywood which ran around the outer edge. It was fastened to the frame by small finishing nails. Then a separate piece of plywood, two feet eight inches square, was fastened to the six-inch strip by bolts and wing nuts. In the right center of the 2 ft.-3 in. board was cut an 8 in.-12 in. hole. This hole was surrounded by a cheesecloth "sleeve". The "sleeve" was fastened by plywood strips tacked around the hole. A flap of clear plastic, which covered the hole, was tacked on the inside. Another hole, 4 in.- 6 in., was cut in the upper left hand corner. Over this hole was fitted a piece of window glass. The glass was held in place by

strips of plywood.

Inside the cage there was a heating bar and a thermostat to keep the temperature at 80°F. A porcelain pan filled with water was placed beneath the heating bar, and a towel, which acted as a wick, was hung from the cage top into the pan. This kept the humidity between 70 and 90 per cent. Two battery jars were placed in the cage for oviposition and larval development. The larvae were fed finely ground dog biscuits. A petri dish of raisins were placed in the cage for the adult males. Also used was a petri dish in which a cotton pad was soaked with a ten per cent honey solution.

Feeding Cage.-The frame was constructed of four 1 in.-2 in.- 1 ft. boards. The top was covered with clear plastic, the bottom and back with one foot square plywood, the front with a fine mesh wire screen, and either side was provided with a cheesecloth "sleeve". The sleeves and plastic were held down by strips of plywood $\frac{1}{2}$ in.-1$\frac{1}{2}$ in. The plywood was tacked down by small carpet tacks. Small staple nails were used to tack down the screen.

Holding Container.-This apparatus was simply a one-gallon ice cream carton. The top was completely cut away and it was fitted instead with a piece of clear plastic. Two $\frac{1}{2}$-inch holes were then cut in the plastic. One hole was fitted with a cork, the other was covered with a small square of fine mesh nylon cloth. In the bottom of the container

was cut a hole, just large enough to hold a tin pill box snugly. There was a piece of paper towel crumpled up in the pill box. This towel was kept moist with water for mosquitoes to drink. Raisins were also placed in the carton.

Aspirators

Straight tube aspirator.-A piece of glass tubing, six inches long, was fitted, at one end, with a piece of fine mesh nylon cloth. This cloth was held in place by a piece of plastic tubing which fitted over that end of the glass tube. The plastic tube was placed in the mouth and the mosquitoes were sucked up in the glass tube.

Receptacle aspirator.-This aspirator consisted of two pieces of "L" shaped glass, cork stopper, ten inches of rubber hose (¼ in. diameter), plastic vial, square of fine mesh nylon cloth and a rubber band. Two holes were bored through the cork to accomodate the two glass tubes. At the vial end of one glass tube, the nylon cloth was fastened by means of the rubber band. On the other end of this tube the rubber hose was forced on. The cork was then fitted in the vial. The rubber hose was placed in the mouth and the mosquitoes were sucked up through the other glass tube, and into the vial where they were retained. It was not necessary to keep sucking to keep the mosquitoes in the vial.

Cages For Birds

Two separate rooms were set aside for birds used in synovitis work. Room one (1) contained twelve all metal cages each of which was supplied with an individual air supply as well as food and water. These cages had a heavy-wire floor. Birds placed in one of these cages were completely isolated from the birds in the other cages in the room. Room two (2) possessed twelve cages also. The top and back were made of sheet metal while the sides and front were heavy wire. Droppings pans were placed under the cages in both rooms. A two layered battery was also present in this room. Birds placed in cages in this room were not isolated as far as air circulation was concerned.

PROCEDURES AND TECHNIQUES

Aedes aegypti (Linn.) Interrupted Feeding Tests

Test One.-This test was an attempt to obtain mechanical transmission of the disease by using six-week old birds which had been inoculated ten days prior to feeding. Results of the next four tests are outlined in table one.

All birds used were five-weeks old, April 27, 1960. On that date, three birds were inoculated intramuscularly (I.M.) with 0.6 ml. of splenic suspension of synovitis agent in tryptose broth.

The spleens of birds, which had previously been inoculated with synovitis agent, were harvested during autopsy. These birds all showed clinical symptoms of synovitis.

The area to be inoculated was plucked of feathers and disinfected with zinc chloride. A sterilized syringe and needle were used to administer the material. The I.M. inoculations were always made in the leg. This proceedure was followed for all inoculations in all tests described in this paper.

Ten days lapsed from the time of inoculation till the day of initial feeding on May 7th. On this date the inoculated birds showed ruffled feathers, enlarged hocks and an unwillingness to stand and walk.

Fifty mosquitoes, both male and female, were captured from the culture cage, in the receptacle aspirator, and

placed in the feeding cage. An inoculated chicken was fastened in one of the restrainers. Its feet were then inserted through the sleeve on the side of the feeding cage. The sleeve was then tucked around the chickens legs to prevent the mosquitoes from escaping.

A large two-bulb flourescent lamp was placed over the cage to provide adequate light for feeding. The temperature in the room was maintained at about 75°F and the humidity varied from 20 to 50 per cent. These conditions were maintained for all tests involving feeding of Aedes mosquitoes.

Four mosquitoes were all that fed initially. These mosquitoes were removed from the bird at various stages of feeding. A rubber glove was worn on the operators hand and this hand was inserted in the other sleeve. This hand manipulated the straight tube aspirator which was used to remove the mosquitoes. The four mosquitoes which fed were placed in the feeding cage. Immediately two clean 6-week-old birds were inserted in either side of the second feeding cage. These birds were left in the cage overnight. The following morning one of the four mosquitoes was full engorged.

The clean or uninoculated birds were removed and placed in an isolated cage in room 1.

Neither of these birds showed any external symptoms at the end of one month and were destroyed.

Test Two.-This test was also an attempt at mechanical

transmission, however these birds were inoculated 13 days prior to feeding as opposed to a ten day lapse in test one.

Those birds inoculated in test one were used in this test also. Thirteen days passed from time of inoculation till May 10th, the time of the initial feeding. The inoculated birds were well down and apparently very sick. Swollen hocks were quite noticeable. The birds were also listless and reluctant to move.

Fifty mosquitoes of both sexes were again captured, with the receptacle aspirator, and placed in a feeding cage. One of the inoculated chickens was placed on a restrainer and its feet were introduced into the cage, through the sleeve. Twelve partially engorged females were captured, by the method described in test one, and placed in a second feeding cage. Two uninoculated 6-week-old assay birds were immediately placed in restrainers and fed, over night to the twelve captured females. Six of the twelve refed during the night. The assay birds were then placed in a cage in room one (1).

After a period of one month no external symptoms were observed and the birds were destroyed.

Test Three.-This test utilized seven-week old birds which were inoculated seven days prior to feeding. This was the third attempt at mechanical transmission of the disease.

On May 4th, 1960, three six-week old chickens were inoculated intravenously with 0.5 ml. splenic suspension of

synovitis. The preparation of the inoculum was identical with that explained in test one.

Seven days following inoculation a bird which showed external symptoms of synovitis was fastened in a restrainer. Its feet were then placed through the "sleeve" of a feeding cage in which fifty mosquitoes had been previously placed. Nine females were removed from the bird, at various stages of feeding, and were placed in a second feeding cage. Two uninoculated, 6-week old assay chickens were fastened in restrainers and their feet introducted in the cloth sleeve. Following overnight feeding, six of the nine females had refed. The assay birds were then removed and placed in an isolated cage. No external symptoms of synovitis was observed following a month incubation period and the birds were destroyed.

Test Four.-This test employed seven-week old birds which had been inoculated ten days prior to the time of feeding. This was the last test at attempting to obtain mechanical transmission of the disease.

Chickens which had been inoculated for the third test were also used in this test. Ten days lapsed from time of inoculation to day of initial feeding.

On May 14th, fifty mosquitoes, both male and female, were captured and placed in a feeding cage. An inoculated bird, which showed external symptoms of the disease, was

Table One.-Results of *Aedes aegypti* interrupted feeding tests [a]

Test	Age of birds [b]	Days from inoculation until feeding	Mosquitoes beginning feeding [c]	Mosquitoes engorged [d]	Birds indicating transmission
1	5	10	4	1	0
2	7	13	12	6	0
3	6	7	9	6	0
4	7	10	13	9	0

a/ Two check birds were used in each test.

b/ Age of birds at time of inoculation is given in weeks.

c/ Females feeding on inoculated birds.

d/ Females refeeding on uninoculated birds.

placed in a restrainer and its feet inserted in the cloth sleeve. There were thirteen females which partially fed initially. These mosquitoes were removed from the bird and placed in a separate feeding cage. Immediately two seven-week-old uninoculated birds were placed in restrainers and their feet fitted in the sleeves of the second feeding cage. After overnight feeding, nine of the original thirteen had refed. The two assay birds were then placed in a Horsfall unit for observation. Since neither bird showed any external symptoms at the end of one month they were destroyed.

Aedes aegypti (Linn.) Refeeding Tests

Series One.-The birds used in this series were four weeks old when inoculated. There was a time lapse of 48 hours from inoculation till mosquitoes were allowed to feed. A series of refeedings were conducted at 0, 18, 26, 48 and 67 hours following initial feeding. The refeedings were conducted on assay birds in an attempt to obtain transmission. The results of the following six tests are presented in table two.

All the chickens used in these tests were four-weeks old September 7, 1960. On September 10th three chickens were inoculated I.M. with 0.3 ml. splenic suspension of synovitis agent. This inoculum was prepared as described in interuppted feeding test one. The remainder of the material was frozen, at -72°F, for future use. The inoculated birds were placed in a cage in room two (2).

Two hundred and fifty mosquitoes of both sexes were aspirated from the culture cage on September 12th, and placed in a feeding cage. Two of the inoculated birds were placed in restrainers and their feet inserted in the cloth sleeve, one bird at either side of the cage. The lights, temperature and humidity were handled as described in interrupted feeding test one. Neither of the inoculated birds showed external symptoms of synovitis, so 0.5 ml. of blood was taken aseptically from the heart of each and inoculated intravenously into two check birds. These birds were also placed in a cage in room two (2).

After the mosquitoes had fed for three hours all but eighteen females and twenty-two males were removed from the feeding cage by means of the straight tube aspirator. The mosquitoes which were removed were placed in an ice cream carton and stored in the large culture cage. The inoculated birds were removed and placed in their original cage. Two clean birds were then placed in restrainers and fed to the forty remaining mosquitoes, using the proceedure described earlier. These chickens, which remained in the restrainers for twelve hours, were then removed and placed in a cage in room two (2). It was observed that fourteen of the eighteen females had refed. The feeding cage was then sealed off, using a plastic cover, and CO_2 was introduced. The dead mosquitoes were then taken outside and brushed out on the ground. This

procedure was used to destroy mosquitoes following each test in this series.

At eighteen hours past initial feeding thirty mosquitoes, with eleven females, were removed from the holding carton, by means of a straight tube aspirator, and placed in a feeding cage. Two test chickens were strapped in the restrainers and their feet placed in the feeding cage through the cloth sleeve. After five hours the birds were removed and placed in a cage in room two (2).

At twenty-six hours after initial feeding thirty mosquitoes, with fourteen females, were taken from the holding carton and placed in the feeding cage. Two clean birds were again introduced into the feeding cage, as described in the eighteen-hour test. At the end of five hours these birds were removed and placed in a cage in room two (2).

The forty-eight hour test was conducted like the last two. This time however, twenty mosquitoes, with eleven females, were placed in the feeding cage. Feeding time was again five hours. The two clean chickens were removed and placed in a cage in room two (2).

A sixty-seven hour test was the last run in this series. Eighteen mosquitoes, with eleven females, were placed in the feeding cage sixty-seven hours after initial feeding. Two clean birds were fastened in the feeding cage and left there for five hours. The birds were then removed and placed in a cage in room two (2).

During the thirty day observation period no change was noted in any of the test chickens. The net result was no effective transmission and all birds were disposed of on October 20th.

Series Two.-This series allowed a 72-hour lapse from inoculation till time of feeding. Five-week old birds were employed for this purpose. A given number of mosquitoes were then refed, at 0, 24, 48, 72 and 96 hours after initial feeding, on check birds.

On September 16, 1960, three five-week old birds were inoculated intramuscularly with 0.3 ml. splenic suspension of synovitis.

Two hundred and fifty mosquitoes, of mixed sex, were captured and placed in a feeding cage on September 19th. All three inoculated birds were fed to these mosquitoes for one to one and one half hours apiece. The inoculated birds were then returned to a cage in room two (2). Two weeks later, at necropsy, all three of these birds showed clinical symptoms of synovitis. All but thirty mosquitoes were removed from the feeding cage to the holding carton. Of this thirty, fifteen were females. Immediately two clean birds were fed to the remaining thirty mosquitoes. These birds were left in the feeding cage for fourteen hours and then removed to room two (2). All check birds used in this series were five-weeks old September 14, 1960.

Twenty-four hours following initial feeding, fifteen females and some males were captured, from the holding carton, and placed in the feeding cage. Once again two check birds were used for these mosquitoes to refeed on. The refeeding time was nine hours, after which the check birds were removed to room two (2).

Twenty mosquitoes, eight of which were females, were caught from the holding carton and transfered to the feeding cage. Then forty-eight hours post initial feeding, two clean chickens were introduced in the feeding cage. After a feeding time of nineteen hours these birds were removed and transfered to room two (2).

In the seventy-two hour post initial feeding test, fifteen female mosquitoes were present out of a captured number of twenty. These mosquitoes were removed from the holding carton. Two clean birds were placed in the feeding cage, as before, and removed seventeen hours later. They were then relocated in a cage in room two (2).

In the ninety-six hour test, seventeen mosquitoes, with thirteen females, were removed from the holding carton and transfered to the feeding cage. These mosquitoes were allowed to refeed on two clean birds for a period of six hours, after which the birds were removed and placed in a cage in room one (1).

None of the birds used in any of these test showed any

external symptoms of synovitis at the end of the thirty day observation period.

Series Three.-This series, using six-week old birds, allowed 96 hours between inoculation of chickens and initial feeding of mosquitoes on these chickens. Check birds were refed to a given number of mosquitoes at 0, 24, 48, 72 and 96 hours following initial feeding.

Three six-week old chickens were inoculated I.M. with 0.3 ml. splenic suspension of synovitis on September 22, 1960. All these birds developed typical symptoms of synovitis.

Approximately two hundred mosquitoes of both sexes were captured from the culture cage and placed in the feeding cage on the 26th of September. Each inoculated bird was fed to these mosquitoes for a period of eight hours. In this series, immediate, 24-, 48-, 72-and 96-hour refeeding tests were run.

The immediate test employed fifteen females out of a total of thirty mosquitoes retained, after the bulk of the mosquitoes had been removed to the holding carton. Again two clean check birds were used and removed at the end of twelve hours. The check birds were then banded with metal wing bands and placed in the cage in room 2 with those birds used in a similar test in series two. The chickens used in these tests were six weeks old, September 21, 1960.

For the twenty-four hour test thirty-three mosquitoes, including ten females, were utilized. These mosquitoes were removed from the holding carton and placed in the feeding

cage, as previously described. Refeeding time was ten hours, after which the two check birds were removed, banded and relocated in room 2 with two birds used in a similar test in series two.

Forty-eight hours following initial feeding thirty mosquitoes, with fifteen females included, were deposited in the feeding cage and allowed to refeed on two clean birds provided for that purpose. These birds remained in the restrainers for eighteen hours, then removed, banded and placed in a cage with two other birds in room 2.

For the seventy-two hour test seventeen females out of a total of thirty mosquitoes, were allowed to refeed on two chickens for eight and one half hours. These birds were then removed, banded and transfered to room 2.

Nineteen of the twenty-five mosquitoes used in the ninety-six hour test were females. They were allowed to refeed on two check birds for eighteen hours. After feeding, the birds were removed and placed in a cage in room 2.

It was observed, from the general appearance of the mosquitoes that in the initial feeding few females fed to repletion. This may have been due to a drop in room temperature because of furnace trouble. A poor refeeding was observed for the twenty-four and forty-eight hour tests for a similar reason. None of the check birds developed external symptoms during the thirty day observation period.

Series Four.-Inoculated eight-week old birds were allowed a 120-hour time lapse before being fed to captured mosquitoes. Check birds were then used for refeeding purposes at 24, 48, 72 and 96 hours following initial feeding.

The eleven birds used in this test were seven weeks old September 21, 1960. Three of these birds were inoculated I.M. with 0.3 ml. splenic suspension of synovitis on September 29, and placed in a cage in room 2.

On October 4th, 250 mosquitoes, of both sexes, were captured from the culture cage and transfered to the feeding cage. Those birds inoculated on the 29th were fed to the mosquitoes for a period of six hours. The inoculated birds were then removed from the feeding cage. All three of these birds later developed clinical symptoms of synovitis. The entire number of mosquitoes were removed following feeding and introduced into the holding carton for use in this series of tests.

Twenty-four hours following initial feeding forty mosquitoes, including eighteen females, were taken from the holding carton and replaced in the feeding cage. Two clean chickens were introduced into the feeding cage and were fed upon for sixteen hours. The birds were then removed and placed in room 2. The mosquitoes were killed in this series, in the manner described in series one.

Once again forty mosquitoes, with eighteen females, were used for a refeeding test. This test was the 48-hour

test. Two check birds were restrained and placed in the feeding cage. Following a seventeen hour refeeding period, these birds were removed and placed in room 2.

At 72 hours following initial feeding forty mosquitoes, including twenty females, were allowed to refeed on two check birds. After sixteen hours those birds were taken from the restrainers and locked in a cage in room 2.

For the 96-hour test twenty-one females, in a group of thirty mosquitoes, were transfered to the feeding cage and refed to two test chickens. Seventeen hours later the birds were removed and placed in room 2.

None of the check birds showed any clinical symptoms of synovitis and were destroyed after a thirty day observation period.

Series Five.-A six day time lapse was allowed from inoculation till the birds were fed to the captured mosquitoes. Check birds were used for the refeeding of a given number of mosquitoes at 0, 24, 48, 72 and 96 hours following initial feeding.

Three eight-week old birds were inoculated I.M. on October 6, 1960 with 0.3 ml. splenic suspension of the synovitis agent. Ten other birds the same age were set aside for use in this series.

On October 12th approximately 200 mosquitoes, of both sexes, were trapped from the culture cage and transfered to

the feeding cage. Those birds inoculated on the sixth were fed in shifts of three hours to the captured mosquitoes. After feeding was complete the birds were placed in room 2 and all but thirty mosquitoes were transfered from the feeding cage to the holding carton, for use in this series of tests. Immediately two check birds were fed to the fifteen remaining female mosquitoes. Twelve hours after refeeding began the birds were removed, banded and put in a cage in room 2.

The twenty-four-hour test utilized forty mosquitoes of which twenty-four were females. Two clean birds were again used, and after seventeen hours were removed and handled similar to those described above.

Forty-eight hours after initial feeding twenty-four females and sixteen males were transfered to the feeding cage for refeeding. The two birds used were removed after sixteen hours.

Fifteen females and ten males were used for the 72-hour test. Feeding time here was eighteen hours. The assay birds were again caged in room 2.

A period of one month was allotted for observation of the check birds. During that time no bird exhibited any external symptoms of synovitis.

Series Six.-A seven day lapse in time was allowed from inoculation of the three birds till they were fed on the

captured mosquitoes. Assay birds were refed to a given number of mosquitoes at 24, 48, 72 and 96 hours following initial feeding.

Eleven chicks, three-weeks old October 12, 1960 were set aside for use in this test. On October 11th three of these were inoculated with 0.3 ml. splenic suspension of synovitis.

On October 18th approximately 250 mosquitoes were captured in the culture cage and removed to the feeding cage. The three inoculated birds were fed to these mosquitoes in shifts for a total of twenty hours.

For the twenty-four hour test seventeen females and thirteen males were used. The feeding time was seventeen hours. Two clean birds were used and then caged in room 2.

The forty-eight hour test utilized nineteen females and eleven males. This time the two check birds were restrained for eighteen hours. The birds were then placed in room 2.

For both the 72 and the 96 hour tests twenty-five females were present in the feeding cage. Refeeding time for both tests was about sixteen hours. All four check birds were caged in room 2 following feeding.

The inoculated birds used in this series failed to develop clinical symptoms of synovitis. At necropsy no lesions were observed. This may have been due to the prolonged freezing period which the agent endured before use. None of the check birds developed any smyptoms and were destroyed at

Table Two.-Results of *Aedes aegypti* refeeding tests [a]

Series	Age of birds [b]	Inoculation to first meal [c]	Mosquitoes Female	Mosquitoes Male	Hours after first feeding	Hours refeeding	Birds with symptoms
I	4	2	18	22	0	12	0
			11	19	18	5	0
			14	16	26	5	0
			11	9	48	5	0
			11	7	67	5	0
II	5	3	15	15	0	14	0
			15	--	24	9	0
			8	12	48	19	0
			15	5	72	17	0
			13	4	96	6	0
III	6	4	15	15	0	12	0
			10	23	24	10	0
			15	15	48	18	0
			17	13	72	8½	0
			19	6	96	18	0
IV	8	5	18	22	24	16	0
			18	22	48	17	0
			20	20	72	16	0
			21	9	96	17	0
V	8	6	15	15	0	12	0
			24	16	24	17	0
			16	8	48	16	0
			15	10	72	18	0
			16	6	96	16	0
VI	3	7	17	13	24	17	0
			19	11	48	18	0
			25	--	72	16	0
			25	--	96	16	0

[a] Two check birds were used in each test.

[b] Age in weeks.

[c] Time in days.

the end of thirty days.

Aedes aegypti Maceration Tests

Series One.-In this series a ten-day waiting period was allowed from time of inoculation till the birds were fed to captured mosquitoes. A number of mosquitoes were macerated and inoculated into two check birds at 5, 7, 11, 13 and 17 days following initial feeding. The results of the next two series of tests are recorded in table three.

On December 1, 1960 3 three-week old chicks were inoculated I.M. with 0.3 ml. splenic suspension of synovitis.

Ten days later all birds showed clinical symptoms of infectious synovitis. Two of these birds were strapped in restrainers and their feet placed in a feeding cage. Prior to this time 400 mosquitoes, selected at random from the culture cage, had been introduced into the feeding cage. The lights, temperature and humidity were maintained as described in interruped feeding test one. The mosquitoes were allowed to feed for eighteen hours. The birds were then removed and replaced in their cage in room 2. All engorged females mosquitoes were taken from the feeding cage and placed in a holding carton. This carton was then set in the culture cage to maintain optimum temperature and humidity conditions. The rest of the mosquitoes were destroyed by the method described in series one of the Aedes refeeding test.

Five days following feeding eight mosquitoes were aspirated from the holding carton by a receptacle aspirator. The hose leading from the CO_2 tank was placed over the glass lead-in tube of the aspirator. A slow stream of CO_2 was then run through the tube into the receptacle and out through the mouth piece till all the mosquitoes were dead. The CO_2 tank was then disconnected. Next, the receptacle was removed from the aspirator and the mosquitoes were dumped into a sterile glass homogenizer and 1.5 ml. of sterile tryptose broth was added as a carrier. The mosquitoes were macerated and the suspension then poured in a small sterile vial.

Two check birds were immediately inoculated I.M. with 0.3 ml. of this suspension and locked in a cage in room 1.

Seven days from initial feeding eight more mosquitoes were taken from the holding carton, killed, macerated and placed in a suspension as described above. Once again two birds were inoculated with 0.3 ml. of this suspension.

This procedure was repeated at eleven, thirteen and seventeen days following initial feeding. In all cases eight females and 1.5 ml. of tryptose broth were used for making the suspension. The check birds were always inoculated intramuscularly with 0.3 ml. of the mixture.

Ten birds, three-weeks old on November 30, 1960 were set aside for this series of tests.

On February 27, 1961, all the birds used as check birds were destroyed. None had shown any symptoms of synovitis

during the thirty day observation period.

Series two.-In this series a four day waiting period was employed from inoculation till feeding. A given number of mosquitoes were then macerated and inoculated into two clean birds on the 4th, 6th, 9th, 13th and 21st days following feeding.

On February 22, 1961 three, three-week old birds were inoculated with 0.3 ml. splenic suspension of synovitis agent. These were then placed in a side cage in room 2. Fifteen birds, of the same age, were isolated for use in this series of tests.

Four days following inoculation 200 mosquitoes, 150 of which were females, were placed in a feeding cage. These mosquitoes were captured from the culture cage by using the receptacle aspirator. Two of the inoculated birds showed ruffled feathers and listlessness but no swelling. These birds were strapped on restrainers and their feet introduced into the feeding cage. After eighteen hours the birds were removed and 85 engorged females were aspirated and placed in a holding carton.

On March 4th, four days after feeding, eight females were removed from the holding carton. These mosquitoes were killed and macerated by the method described in series one of Aedes maceration tests. These mosquitoes were blended with 1.5 ml. of sterile tryptose broth. Three clean birds were then inoculated I.M. with 0.3 ml. of this suspension,

Table Three.-*Aedes aegypti* maceration tests [a]

Series	Age of birds [b]	Inoculation to blood meal [c]	Days from feeding until maceration	Birds indicating transmission
I	3	10	5	0
			7	0
			11	0
			13	0
			17	0
II	3	4	4	0
			6	0
			9	0
			13	0
			21	0

a/ Eight mosquitoes were macerated in each test and suspended in 1.5 ml. of sterile tryptose broth, 0.3 ml. of the suspension were injected in each of two test birds used in each test.

b/ Age of birds at inoculation, in weeks.

c/ Time in days.

and locked in a side cage in room 2. This same procedure using the same quantities of ingredients, was repeated on the 6th, 9th, 13th, and 21st days following initial feeding.

Three days after initial feeding all three inoculated birds developed typical external symptoms of infectious synovitis and were destroyed. None of the clean check birds developed any external symptoms of synovitis during the thirty-day observation period.

Culex fatigans Maceration Tests

A four-day waiting period was allowed from time of inoculation till the birds were fed to captured mosquitoes. A given number of mosquitoes were macerated and inoculated into two assay birds on the 2nd, 3rd, 7th, 9th and 15th day following inoculation. Results of these tests are outlined in table four.

An intramuscular I.M. inoculation, of 0.3 ml. splenic suspension of synovitis, was given to three five-week old chickens on March 9, 1961. Ten other birds, also five weeks old March 9th, were isolated for use as check birds in these tests.

Four days following inoculation two of the inoculated birds were restrained and fed to 200 mosquitoes, which had been captured from the culture cage. The birds showed no external symptoms of infectious synovitis. After eighteen hours the birds were retracted from the feeding cage and re-

Table Four.-*Culex fatigans* maceration test [a]

Age of birds [b]	Inoculation to blood meal [c]	Days from feeding to maceration	Mosquitoes macerated	Birds with symptoms
5	4	2	4	0
		3	4	0
		7	4	0
		9	4	0
		15	8	0

[a] The mosquitoes were suspended in 1.3 ml. of sterile tryptose broth, except for the 15 day test for which 1.5 ml. was used. The suspension was inoculated into each of two test birds at the rate of 0.5 ml. per bird.

[b] Age in weeks.

[c] Time in days.

moved to room 2. All the engorged females were then aspirated from the feeding cage and placed in a holding carton. On the 2nd, 3rd, 7th, and 9th day following feeding four mosquitoes were killed and macerated in 1.2 ml. of sterile tryptose broth by the method described in Aedes maceration test one. In each instance 0.5 ml. of the suspension was inoculated I.M. in two check birds. These birds were all placed in separate cages by dates in room 2. On March 28th, fifteen days after initial feeding, eight female mosquitoes were suspended in 1.3 ml. of sterile tryptose broth, and then 0.5 ml. of the suspension was inoculated in two check birds. These birds were also placed in room 2.

The three inoculated birds later developed typical external symptoms of synovitis. Autopsy revealed the presence of enlarged spleen and the cheesy exudate in hocks. None of the check birds developed any external symptoms of synovitis during the thirty-day observation period.

Cage Mate Transmission Tests

This test utilized six-week old birds, ten of which were inoculated and 22 which were used as contact assay birds. The object was to place the inoculated and clean birds in very close contact to determine if contact transmission would occur. The results of the next four tests are outlined in table five.

Test One.-All the birds used in this test were six-weeks

old on July 27, 1960. Ten birds were inoculated I.M. with 0.5 ml. splenic suspension of synovitis on July 25th. Five of these were placed in the upper level of the battery, the other five in the lower level. Sixteen uninoculated birds were then inserted into each level of the battery. Six other clean birds were placed in a side cage to act as controls to check the possability of air transmission. Both the battery and side cages were located in room 2, and all tests concerning transmission to cage mates were run in this room. The inoculated birds were marked with metal wing bands. The first of the inoculated birds died on July 31st, one died on August 2nd, two on the 4th, four on the 7th and one on the 10th. These nine all showed external symptoms and internal lesions characteristic of infectious synovitis. One inoculated bird did not develop symptoms. The last infected bird died on August 10th. The observation period was thirty days long, starting on August 10th. One clean bird died on August 7th but showed no symptoms or typical lesions at necropsy. Autopsy report for this bird indicated it had been badly pecked. On August 10, three check birds died. Two of these had no lesions or typical characters at autopsy. One, however, showed a slightly enlarged spleen. Then on the 15th of August a check bird died whose only observable lesions were pecked comb. On the 19th a check bird died which had a slightly enlarged spleen and breast blister. A cheesy exudate

was found in the hocks. The spleen of this bird was removed (aseptically) and frozen for future use in two challenge birds. Two more check birds died on August 26. One bird showed only wounds left by pecking, the other was pecked around the comb and head but also showed slightly swollen hocks. The last birds to die was on August 28, and they showed slightly enlarged spleen and hocks. No cheesy exudate was found in the hocks. The twenty-three other check birds kept in the battery were destroyed on the tenth of September, after exhibiting no external symptoms of synovitis during the 30-day observation period. None of the six birds kept in the side cage developed any external symptoms and were destroyed with those living in the battery.

The results were complicated by overcrowded conditions and pecking. Some birds were very badly pecked and others had enlarged hocks which may have been due to "knocking-around" inside the cage. This crowded condition was maintained in order to assure close contact of the inoculated and check birds. A splenic suspension was prepared from the bird which died on the nineteenth of August and 0.5 ml. inoculated into two check or challenge birds. Neither of these birds developed typical symptoms during the thirty-day observation period.

Test Two.-The birds used in this test were not as crowded as those used in test one. Also younger birds were used to

reduce deaths due to pecking.

All the birds used in this test were three-weeks old November 16, 1960. Twenty of these birds were inoculated I.M. with 0.3 ml. splenic suspension of synovitis. Ten of these birds were placed in each of the two levels of the battery on November 18th. Six check birds were added to each group of inoculated birds.

The last inoculated bird died on December 8th. All inoculated birds showed typical external symptoms of synovitis. The twelve test birds were then observed for sixty days from the time the last inoculated bird died. None of the twelve birds showed any external symptoms of infectious synovitis during this time.

Test Three.-Side cages were used in this test because in the battery water and feed pans were not located to allow the birds to defecate in them. Young birds were used and relatively crowded conditions were employed for this test.

Twenty-eight birds, three-weeks old November 30, 1960, were used in this test. Twelve of these birds were inoculated on the 29th of November and equally distributed in four side cages. Four clean birds were placed in each side cage with the three inoculated ones. All the inoculated birds were marked with metal wing bands.

The last inoculated bird died on December 17th. All the banded birds showed typical external symptoms of infectious

synovitis. A sixty-day observation period was then allowed for the assay birds. At the end of that time, on February 17, 1961, none had died and none showed any external symptoms of synovitis.

Test Four.-The object of this test was to try to obtain morbid chickens which would live for some time, and to see if these older morbid birds would transmit the disease to younger clean birds. Fresh feces were taken daily from the droppings pan of each cage ontaining inoculated birds and placed in the water of that cage,and in the water of a cage containing three check birds.

Eight, 8-week-old chickens were inoculated on the 22nd of February, 1961. Two of these birds were given an I.M. inoculation of 0.1 ml. splenic suspension of the synovitis agent. Two were given 0.2 ml. of the suspension in the same manner. Two received 0.4 ml. and two others received 0.8 ml. of the synovitis suspension. Each bird was placed in a separate side cage in room 2. Ruffled feathers and slight droppiness was observed of one bird on the 27th, five days after inoculation. At this time four birds, three-weeks old, were placed in each of the eight cages with the one inoculated bird. Also, three other three-week old birds were placed in a cage by themselves in room 2. Starting on the 27th, feces was taken from the pans of each cage and handled as described above. After the inoculated bird died

the feces was no longer removed from that cage. One bird receiving 0.1 ml. of the material appeared to be healthy through-out the test. The first external symptom appeared on February 27th, and the last inoculated bird except for the one mentioned above died on March 14th. A forty day observation period was allowed following the death of the last inoculated bird.

At no time during the entire testing and observation periods were any external symptoms noticed on any of the thirty-five check birds.

Test Five.-Results of cage mate tests [a/]

Test	Age of bird [b/]	Birds inoculated	Check birds	Birds indicating transmission
1	6	10	22	0
2	3	20	26	0
3	3	12	16	0
4 [c/]	8	8	35	0

[a/] Birds in the first three tests were inoculated I.M. with 0.5 ml. splenic suspension of synovitis agent.

[b/] Age in weeks.

[c/] Two birds were inoculated with 0.1 ml. splenic suspension of synovitis agent, two with 0.2 ml., two with 0.4 ml. and two with 0.8 ml.. Feces from droppings pans were placed in the water of the respective cages daily.

SUMMARY AND CONCLUSIONS

There were two major objectives with which this study was concerned. The first involved exploring the possibility of transmission of infectious synovitis by mosquitoes.

Four tests using 14 chickens were conducted in an effort to obtain mechanical transmission of the disease through interrupted feeding of Aedes aegypti mosquitoes. Six tests were enacted, utilizing 74 birds, to investigate the possibility of transmission through refeeding of A. aegypti. The time lapse between initial feeding and refeeding varied from zero to 96 hours.

Three series of tests were conducted to determine if the agent was present in the mosquito's body, in infective quantities, after feeding on an inoculated bird. Two of the series involved the use of Aedes aegypti and one employed the use of Culex fatigans. Mosquitoes were allowed to feed on diseased birds and then macerated and inoculated into check birds at intervals between one and twenty-one days. There were 39 birds used in the three series.

Using materials and methods described, no transmission was observed in any of the tests using insects as vectors.

The second objective was to test the possibility of transmission of the disease, by direct contact, to cage mates. Various ages of birds and combinations of variables were used. In cases where any doubt existed as to whether or not

the clean bird had contracted the disease, the spleen was removed and two assay birds were then inoculated with a suspension of the spleen. Four tests were conducted using a total of 132 birds.

In no instance involving uninoculated chickens could a definate diagnosis of infectious synovitis be made. Therefore, under the conditions of those tests, no cage mate transmission occured.

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LITERATURE CITED

Benton, W. J. 1959. Masters of Science Thesis. University of Delaware.

Benton, W. J., M. S. Cover. 1959. The infectivity of blood and tissues from chickens with infectious synovitis. Avian Dis. 3, (4): 361-370.

Bowness, E. R., J. E. Fahey. 1954. Lameness in turkey poults. Can. J. Comp. Med. 18: 335-337.

Cassidy, D. R., L. C. Grumbles. 1959. Immunity studies on Avian infectious synovitis. Avian Dis. 3, (2): 137-142.

Chalquest, R. R., Julius Fabricant. 1960. PPLO associated with synovitis in fowls. Avian Dis. 4, (4): 515-539.

Coffin, J. M. 1955. Arthritis complex. Broiler Growing. 6: 14-18.

Cosgrove, A. S., J. Coffin. 1956. The treatment of infectious synovitis with furazolidone. Proc. First Nat. Symp. on Nitrofurans in Ag., pp. 61-63.

Cover, M. S., W. J. Benton. 1957. The distribution of the infectious synovitis agent in the tissues of artificially infected chickens. Avian Dis. 1: 312-320.

Cover, M. S., J. M. Galeta. 1955. Studies on avian arthritis of chickens. Proc. Ann. Pull. Conf., Univ. New Hamp.

Cover, M. S., J. M. Galeta, E. F. Waller. 1956. The etiology of an arthritis disease of chickens. Am. J. Vet. Res. 17: 12-15.

Fahey, J. E. 1954. An outbreak of staphylococcal arthritis in turkey poults. Poul. Sci. 33: 661-664.

Hale, N., H. S. Purchase. 1931. Arthritis and periostitis in pheasants caused by Staphylococcus pyogenes aureus. J. Comp. Path. Therapeutics. 46: 12.

Hinshaw, W. R., E. McNeil. 1952. Staphylococcus (synovitis) in turkeys. Poul. Sci. 31: 320-327.

Jungherr, E. 1933. Staphylococcal arthritis in turkeys. J. A. V. M. A. 82: 243-249.

Jungherr, Erwin and W. N. Plastridge. 1941. Avian staphylococcosis. J. A. V. M. A. 138, (766): 27-32.

Kelser, R. A. 1933. Mosquitoes as vectors of the virus of equine encephalomyelitis. J. A. V. M. A. 82, NS (35): 767-771.

Lecce, J. G., F. G. Sperling, L. Hayflick, W. Stinebring. 1955. Tendovaginitis with arthritis, a new syndrome of chickens-isolation and characteristic of an infectious agent. J. Exptl. Med. 102: 489-498.

Madsen, D. E. 1942. Synovitis of turkeys. Turkey World. 17: 24-27.

Markham, F. S., S. C. Song. 1952. PPLO in the etiology of turkey sinusitis and chronic respiratory disease (CRD). of chickens. Poul. Sci. 31: 902-904.

Munro, D. A., N. O. Olson, D. C. Shelton, C. E. Weakley, Jr. 1956. Intra-muscular streptomycin and a comparison of continuous and inter-mittent feeding of aureomycin and

furazolidone. Poul. Sci. 35: 1161-1172.

Olson, N. C., J. K. Bletner, D. C. Shelton, D. A. Munro, G. C. Anderson. 1954. Enlarged joint condition in poultry caused by an infectious agent. Poul. Sci. 33: 1075-1081.

Olson, N. C., D. A. Munro, J. K. Bletner, D. C. Shelton. 1955a. The production of synovitis in chickens by agents from different sources isolated in the yolk sac of embryonating chicken eggs. Proc. Ann. Meet. Poul. Sci. Assoc.

Olson, N. C., D. C. Shelton. 1958. Control of infectious synovitis. 6. Chlortetracycline in field experiments. J. A. V. M. A. 132, (11): 477-482.

Olson, N. C., D. C. Shelton, J. K. Bletner, D. A. Munro, G. C. Anderson. 1956. Studies of infectious synovitis. Am. J. Vet. Res. 17: 747-754.

Olson, N. C., D. C. Shelton, J. K. Bletner, C. E. Weakley, Jr. 1957a. Infectious synovitis control. 2. A comparison of levels of antibiotics. Am. J. Vet. Res. 18: 200-203.

Olson, N. C., D. C. Shelton, J. K. Bletner, C. E. Weakley, Jr. 1955b. Synovitis control. 2. A comparison of levels of antibiotics. (Abst.) Poul. Sci. 34: 1214.

Olson, N. C., D. C. Shelton, D. A. Munro. 1957b. Infectious Synovitis controlled by medication. Effect of strain differences and PPLO. Am. J. Vet. Res. 18: 735-739.

Olson, N. O., D. C. Shelton, D. A. Munro, J. K. Bletner. 1957c. Preliminary blood studies in chickens with a synovitis caused by the infectious synovitis agent, PPLO and a combination of the two agents. Avian Dis. 1, (1): 82-91.

Olson, N. O., D. C. Shelton, C. E. Weakley, Jr. 1958. Control of infectious synovitis. 4. Antibiotics and Nitrofurans. Poul. Sci. 37: 610-614.

Price, K. L., Z. Zolli, Jr. 1958. The influence of dietary calcium-phosphorus and Terephthalic acid on antibiotic control of experimental infectious synovitis. Avian Dis. 3, (2): 321-328.

Sevoian, Martin, G. H. Snoeyenbos, H. I. Basch, I. L. Reynolds. 1958. Infectious synovitis. 1. Clinical and Pathological Manifestations. Avian Dis. 2, (4): 357-365.

Shelton, D. C., J. K. Bletner, N. O. Olson, G. C. Anderson, C. E. Weakley, Jr. 1957. Control of Infectious synovitis. 1. Continuous feeding of antibiotics and the influence of diethylstilbestrol and coccidiostats. Poul. Sci. 36: 113-121.

Shelton, D. C., N. O. Olson. 1959a. Control of infectious synovitis. 7. Comparison of tetrocycline antibiotics. Poul. Sci. 38, (6): 1309-1315.

Shelton, D. C., N. O. Olson. 1959b. Control of infectious synovitis. Continuous and intermitten feeding of

chlortetracycline and furazolidone. Poul. Sci. 38, (3): 575-579.

Shelton, D. C., N. O. Olson. 1957-1958. Control of infectious synovitis. 9. The efficency of dihydrostreptomycin sulfate as related to the time of experimental infection. Antibiotics Ann.

Shelton, D. C., N. O. Olson. 1958. Control of infectious synovitis. 11. The potentiating effect of terephthalic acid chlortetracycline. Avian Dis. 2, (4): 425-431.

Shelton, D. C., N. O. Olson, C. E. Weakley, Jr. 1958. Control of infectious synovitis. 4. Antibiotics and nitrofurans. Poul. Sci. 37: 610-614.

Shelton, D. C., N. O. Olson, C. E. Weakley, Jr. 1956. Synovitis control. 4. Antibiotics and furazolidone. Poul. Sci. 35: 1171-1183.

Skamser, L. M., K. C. Seeger. 1960. Aureomycin chlortetracycline as a preventive for experimental synovitis in chickens. Avian Dis. 4, (1): 637-643.

Snoeyenbos, G. H., H. I. Basch. 1958. A further indication of egg transmission of infectious synovitis. Avian Dis. 2: 494-496.

Snoeyenbos, G. H., H. I. Basch, H. Sevoian. 1958. Infectious synovitis. 2. Drug prophylaxis and therapy. Avian Dis. 2: 514-530.

Snoeyenbos, G. H., O. M. Olesiuk. 1955. Studies of an

agent producing arthritis in turkeys. Proc. Ann. Conf. Lab. Workers in Pullorum Dis. Control, Durham, New Hampshire. June 14-15.

Thayer, S. C., R. G. Stout, W. R. Dunlap. 1958. Observation on infectious synovitis. Poul. Sci. 37: 449-454.

Wasserman, B., J. Yates, D. E. Fry. 1953. The cultivation of certain strains of the CRD agent and turkey sinusitis agent in the tibiometatarsal joints of chickens. Proc. Ann. Conf. Lab. Workers in Pullorum Dis. Control. Univ. Mass. June 16-17.

Wichmann, R. W., R. A. Bankowshi, A. J. DaMassa. 1960. The cultivation and modification of the avian infectious synovitis agent in tissue culture. Avian Dis. 4, (2): 152-164.

Wills, F. K. 1954a. Observation on an agent producing arthritis in Chickens. Tex. Ag. Exper. Sta. Prog. Rep. 1723.

Wills, F. K. 1954b. Preliminary report on transmission of an agent producing an arthritis in chickens. Tex. Ag. Exper. Sta. Prog. Rep. 1674.

Wills, F. K., J. P. Delaplane. 1955. Transmission and therapy studies on an agent which produces arthritis in chickens. Proceedings Book, Am. Vet. Med. Assoc. Ninety-Second Ann. Meeting, Minn. Aug. 15-18, pp 350-357.

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ABSTRACT

The possibilities of transmission of infectious synovitis were explored.

Two types of tests, using mosquitoes as vectors, were employed. One type involved the feeding of mosquitoes on previously inoculated birds, then after a period of from zero to ninety-six hours refeeding the mosquitoes on assay birds. The second type of insect test comprised the feeding of mosquitoes on inoculated birds, then macerating and inoculating them into check birds at intervals of one to twenty-one days following feeding.

Other experiments were conducted in which uninoculated birds were brought into close contact with inoculated chickens and their excrement.

The observation of all tests indicated the absence of transmission of an infective titer of the infectious synovitis agent. These conclusions are drawn from tests using Aedes aegypti (Linn.) and Culex fatigans Wied., with methods and materials described.