body to SLE in birds from these winter flocks. The immune status of these birds might be of value in determining the expected levels of virus activity the following summer.

A roost estimated to contain 6–8 million blackbirds was sprayed with the detergent Tergitol by the Kentucky Agriculture Department. Many of the birds succumbed to the freezing temperatures. We selected 23 birds, which could easily be captured, and exsanguinated them by cardiac puncture. The serum was extracted and stored at −4°C.

Work in our laboratory (Tesh and McCammon 1978) has shown that the complement fixation inhibition (CFI) test is a rapid, reliable technique for assaying bird sera for antiarbovirus antibodies. The microtiter adaption of the CFI technique was used as previously described (Olsen et al. 1973). Reference antigen was SLE-infected mouse brain (courtesy of CDC); complement fixation (CF) titer 1:32. Control antigen was normal mouse brain. Reference antiserum was a human serum with a CF antibody titer of 1:64 toward SLE. The microtiter test consisted of incubating twofold dilutions of heat-inactivated experimental serum with 2 CF units of reference antigen for 1 hr at 37°C. The above reactants were subsequently mixed with 2 CF units of heat-inactivated (56'C for min) reference antibody plus $5C°H_{50}$ units of guinea pig complement and incubated for an additional 16 hr at 4°C. Optimally sensitized sheep red blood cells (SRBC) (1.25%) were added to the test and incubated for an additional 30 min at 37°C. In the microtiter CFI test, experimental serum titers were reported as the reciprocal of the highest serum dilution that visually reduced the CF reaction between reference antibody and reference antigen by 50%, i.e., from a 4+ reaction to a 2+ reaction. A titer of 1:4 or greater was considered positive. The microtiter CFI test used 25 u liters each of experimental and reference sera, reference antigen, and SRBC, and 50 u liters of complement.

Determination of units of complement, reference antibody, reference antigen, and hemolytic amboceptor were all performed under conditions which simulated those of actual CFI test procedures.

Twenty-three bird sera were titrated for antibody by the CFI test. Results of these test showed that 70% of the sera tested were positive at least at the 1:2 level and that 57% exhibited titers of 1:4 or greater (Table 1). It may be significant that over 50% of the birds tested possessed low levels of antibody to SLE. These findings suggest the value of the winter roots as a source for determination of the susceptibility of these birds to infection. Further studies are warranted, examining larger number of birds and correlating with age. Such a study combined with a banding program could provide new information concerning the epidemiology of SLE.

Table 1. Antibody to St. Louis encephalitis virus in birds as measured by complement fixation inhibition.

Species	No. Tested	CFI titer <2	2	4	8	16
Grackle	15	4	1	4	2	4
Red-wing	4	2	1	1	—	—
Starling	3	—	1	1	1	—
Brewer's blackbird	1	1	—	—	—	—
Total	23	7	3	6	3	4

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COLONIZING *AEDES DORSALIS*

BEULAH M. PARKER[1]

Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

The mosquito *Aedes dorsalis* (Meigen) is an important pest of man and domestic animals. Females are said to be "fierce" biters, and the attack rate may be sufficiently annoying near

[1] Present address: Department of Biological Sciences, Illinois State University, Normal, Illinois 61761.

massive production sites to cause domestic animals to panic. This species warrants detailed work on its bionomics, using techniques to determine effects of stress. This account is submitted as a means to that end.

Large populations of the species have been maintained easily in this laboratory through more than 10 consecutive generations with no diminution in survival, rates of growth, or fecundity. Colonies may be started from eggs or from active stages collected from the field. Populations of any size provided as eggs remain viable for a year or more. Handling of adults and larvae requires minimal space and attention.

Eggs should be incubated on a wet surface in a moist chamber at 24–30°C throughout embryogeny. Eggs 5 or more days old are ready for hatching and remain so as long as they are exposed to a daily photoperiod of 18 hr natural or artificial light. Eggs may be stored at about 4°C for months without injury to the embryo. Cold is not a pre-condition for hatching, however.

Eggs held at room temperature are easily hatched by gradually lowering the amount of dissolved oxygen in the water as has been done for other floodwater species (Borg and Horsfall 1953). The gradual reduction in the amount of dissolved oxygen is most easily accomplished by flooding eggs with an infusion of unsterilized nutrient broth at dilutions of 1:1000 in aerated deionized water. Usual practice is to place 50 eggs in the infusion in a glass hatching tube, 4 mm inside diameter × 20 mm long. The hatching tube containing eggs is then immersed in the same dilution of nutrient broth in an open shell vial in the manner noted by Horsfall et al. 1973. Hatching occurs within an hour or so. Larvae should be removed from the hatching tube with a dropping pipette to a casserole containing deionized water to remove gross microbial contamination before transferring them to rearing chambers.

A standard medium of deionized water containing a small amount of oak-leaf infusion (5 ml: 95 ml of deionized water) is adequate for rearing and requires minimal preparation. The infusion is prepared by soaking dry brown oak leaves in deionized water for about 1 week and then removing coarse particles by straining. The infusion may be stored at room temperature for about a month and used as needed. Approximately 30 larvae may be reared uniformly in a covered pan (25 × 16 × 10 cm) in the standard medium about 7 to 10 mm deep.

A high survival rate for larvae may be obtained consistently when the daily diet is a mixture of 1 part comminuted (not finely powdered) TetraMin®[2] and 4 parts live pelletized dry Fleischmann's® yeast. The TetraMin and yeast mixture should be moistened to a slurry and placed on spots of sand on the bottom of each rearing pan with a dropping pipette. To insure adequate exposure to food, larvae may be concentrated in a small casserole (placed inside the pan) for 24 hrs. At the end of day 1, they can be pipetted into the pan. Fouling of the rearing medium by adverse microbes can be avoided by transferring larvae, about the end of day 4, to clean pans containing fresh rearing medium at the end of the 3rd stadium.

When larvae were fed the TetraMin-yeast mixture and reared in the standard medium of deionized water and oak-leaf infusion at 25°C, 92–99% of them survived to the pupal stage. Mortality during the pupal stage was less than 10% in each experiment (Table 1). In each experiment, 3 lots of 30 larvae constituted a test. About 6 days were required for larvae to develop to pupae.

Pupae may be reared in a small jar placed inside a Lucite® "Illinois emergence cage" measuring 17.5 × 17.5 × 17.5 cm (Horsfall et al. 1973). Males and females should be placed in separate cages. Adequate moisture inside the cage may be provided for emerging adults by evaporation from a wet cellucotton wick over wet paper toweling on the floor of the cage. Humidity in the rearing room is best held at 50% RH or even lower; that in holding cages for females should range from 60–100% RH, that in cages of males should be about 50% RH. Adults may be fed on a dilution of honey to a 10% solution soaked in a wad of cellucotton placed in the feeding slot of the cage.

This species does not mate readily in cages (especially small ones). It can be maintained by induced copulation, using the technique first described by McDaniel and Horsfall (1957) and modified in this laboratory by Novak and Liem (1975). Males mate most readily 5 to 7 days after emergence. Females may be ready to mate 2 days after emergence as reported by Blakeslee et al. (1966) but can be mated easily 4–12 days after emergence. After mating, females are placed in *Illinois oviposition cages* described by Kardatzke (1976).

Ovipositional cages are placed on strips of wet cheesecloth folded to form mats. Each mat

[2] TetraMin is an aquarium food made by TetraWerke, Dr. rer. nat. Baensch Melle, W. Germany (available at pet shops). It may be comminuted in a ball mill or mortar.

Table 1. Survival of aquatic stages of *Aedes dorsalis* reared at constant 25°C in 3 lots of 30 larvae for each experiment.

		Individuals alive at end of each stage				
Experiment	No. of larvae	*Instar 1* %	*Instar 2* %	*Instar 3* %	*Instar 4* %	*Pupa* %
1	90	100	100	100	99	96
2	90	100	98	96	92	90
3	90	100	100	93	92	91
4	90	100	100	100	98	93
Total	360	100	99	97	95	93

rests on a wet bat of cellucotton in an enameled pan (300×220×55 mm) elevated at one end so that the water table at the low end stands well above the bottom of the bat. Capillarity in the cellucotton provides a gradient from saturation at the bottom to barely moist at the top. Females may be maintained in a lighted room where the air temperature is about 25°C and the surface for oviposition is 24–26°C.

A single blood meal is sufficient for each oviposition effort. A plastic box (20×20×20 mm) containing a wad of cellucotton soaked with a solution of honey (10%) when placed on the screened top of the cage provides carbohydrate nutrition. The honey-soaked cellucotton can be maintained in a moist condition with deionized water. About 150–200 eggs may be deposited per female within 4–5 days after a blood meal. However, when temperature and moisture level of the ovipositional mat are not suitable, eggs may be retained for days or weeks after maturation. When deposited, many long-retained eggs may be abberrant.

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OCCURRENCE OF *AEDES HENDERSONI* IN MANITOBA

R. A. BRUST

Department of Entomology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

The adults of *Aedes triseriatus* (Say) reported by Brust and Kalpage (1967) were examined by Dr. D. M. Wood, Biosystematics Research Institute, Ottawa, Canada and were found to be *Aedes hendersoni* Cockerell. Larvae had been collected from tree holes in Winnipeg in 1965, and reared to adults. In the fall of 1971, mosquito eggs were collected from these same tree holes by removing pieces of rotting wood from inside the tree hole. The eggs were cold-conditioned at 5°C for 3 months and then hatched. Larvae were reared to adults while