

A SENTINEL CHICKEN SHED AND MOSQUITO TRAP FOR USE IN ENCEPHALITIS FIELD STUDIES¹

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INTRODUCTION. For some years, domestic birds have been used as "sentinels" to provide indices on seasonal transmission of the arthropod-borne viral encephalitides (Lockburn *et al.*, 1957; Kaplan *et al.*, 1955). There are several advantages to using them rather than wild birds for this purpose. Wild birds are more difficult to capture and bleed; and because their home range is not constant, fall bleedings may include migrant as well as resident specimens, thus injecting an element of variability in test results. On the other hand, domestic birds more accurately reflect the levels of virus activity in a circumscribed area.

Among the domestic birds, chickens are particularly suitable as sentinels. Because they are more widely distributed than other domestic species, they provide a standard index over a larger area. They have proved to be quite susceptible to infection by the viruses of St. Louis encephalitis (SLE) and western encephalomyelitis (WE) and show good antibody response to both (Hammon and Reeves, 1946; Hammon *et al.*, 1946; Kissling, 1958; Chamberlain, 1959; Sudia and Chamberlain, 1959). There was some uncertainty as to their suitability for studies of transmission of eastern encephalomyelitis (EE), but during recent studies in Massachusetts sentinel chickens showed a good antibody response to the EE virus under field conditions (Hayes *et al.*, 1960). A factor in the practicability of the use of these species as sentinels was the development of *in vitro* hemagglutination-inhibition techniques for determining antibodies in chicken sera (Casals and Brown, 1954), which greatly reduces the time and expense involved in testing.

It is often possible to obtain a reliable index of virus activity in an area simply by making an antibody survey of the farm chicken flocks. In some situations, however, it is desirable to eliminate variability in types of shelter and size of flocks, or to obtain transmission indices where there are no farm flocks. This is particularly important in ecological studies where transmission indices in enzootic habitats are desired, or for site-to-site comparisons of levels of virus activity.

To meet such needs, a sentinel chicken shed was developed in the spring of 1959 by the CDC Encephalitis Section at Greeley, Colorado, for use in field investigations on the natural history and control of encephalitis. After trials that season, the unit was modified to overcome its apparent deficiencies and in trials during the 1960 season the unit proved highly satisfactory.

The unit consists of a demountable and portable shed and pen for housing and holding a flock of 25 to 30 sentinel chickens. It is equipped with removable mosquito traps so that the investigator can obtain mosquito indices, mosquito infection rates, and transmission rates in sentinel chickens simultaneously. This paper describes the sentinel shed, the pen, and the mosquito traps and discusses the improvements made in the unit since it was originally developed in 1959.

SENTINEL SHED AND PEN. An assembled sentinel chicken shed is shown in the foreground of Figure 1. The general layout, the dimensions, and details of the construction features of shed, pen, and trap are shown in Figures 2, 3 and 4. Both the shed and the pen are prefabricated to facilitate transportation, but are easily and quickly assembled or disassembled in the field.

The wall panels of the shed are of 1/4" exterior plywood glued and nailed to 2"

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x 2" wood framing members. The height of the front panel is 4', of the back panel is 3', and of the side panels is 4' at the front and 3' at the back (Figures 2 and 3). A space to accommodate two mosquito traps is left across the top of the front panel, divided by a vertical piece of framing. To keep predators out and the chickens in when the mosquito traps are not in use, two small wood-framed wire panels slip into place over this opening.

The roof is made of two 4' x 8' pieces of $\frac{3}{8}$ " plywood with the front section overlapping the back section by about 6 inches. The finished size allows an overhang of about a foot at both front and back and about 6 inches at each side. At the two back corners of the roof a section is removed from the overhangs (Figure 2) to allow the pen to fit flush against the back of the shed.

To assemble the shed, the four panels are fastened together with wood screws, 3 at each corner, placed in predrilled holes in the overlapping edges of the plywood. A 2" x 4" center cross support is fastened to the upper frame of the two side panels. The roof is then fastened with screws to the upper framing of the wall panels and the 2" x 4" center cross support.

A roost is made of 2" x 2" wooden members—four horizontals spaced about 8" apart and fastened to verticals at each end and in the middle. The upper ends of the side supports are fastened with 4" strap hinges to the rear framing members of the side wall panels, and the lower ends normally rest against blocks fastened to the bottom framing members of the side panels. For ease in cleaning the shed or removing chickens from it, the roost may be raised and held close up under the roof by a wire hook attached to the 2" x 4" center support.

A doorway 20" wide and 33" high is centered in the front wall of the shed. The door for this opening is made of $\frac{3}{8}$ " plywood and is hinged at the side. Another door, this one 10" high, extends the entire width of the back wall. It is hinged at the top and lifts to open. Normally it is kept open, held by a turn but-

ton attached to the upper wall, thus allowing the chickens free movement between the shed and the pen. But when the birds are to be bled, the flock is confined in the shed. Individual chickens are removed through the front door and after being bled are returned to the pen. This procedure serves to keep the bled and unbled birds separated.

The pen is 14' long, 7' wide, and 7' high. The front end is open except for $\frac{3}{4}$ " board 7' long and approximately 1 foot wide nailed across the pen at the top. This open end of the pen fits flush against the back of the shed, and the board fills the space above the back slope of the shed roof. Five 4' x 7' panels make up the sides and the closed end (two for each of the sides). These panels have 2" x 2" wood frames put together with 3 $\frac{1}{2}$ " x 3" metal angles and are covered with mesh poultry wire. In one end of one panel is a 23" x 43" gate that gives access to the assembled pen.

The panels are easily assembled by fastening them in position with loops of 11 or 8 soft wire near top and bottom. Wire loops also are used to secure the pen against the back of the shed. Additional support is provided at the center of the pen by a 2" x 2" spreader member between the upper frames of the side panels and fastened to them with 3 $\frac{1}{2}$ " x 3" metal angles. Poultry wire for the top of the pen is nailed to the upper framing members and to the $\frac{3}{4}$ " board across the front of the pen. If the chickens may be exposed to predators, poultry wire is placed underneath both shed and pen.

Two men can assemble or disassemble a shed and pen in about an hour. The cost of materials to construct 70 sheds and pens in the Encephalitis Section's shop at Greeley was approximately \$50 per unit and the time per unit was about 5 hours each for a carpenter and a helper.

MOSQUITO TRAP. General construction features of the mosquito trap are shown in Figure 4. A frame of wooden members forms the front, and the ends are of $\frac{3}{4}$ " board rounded at the back. The screen-wire covering is tacked to the

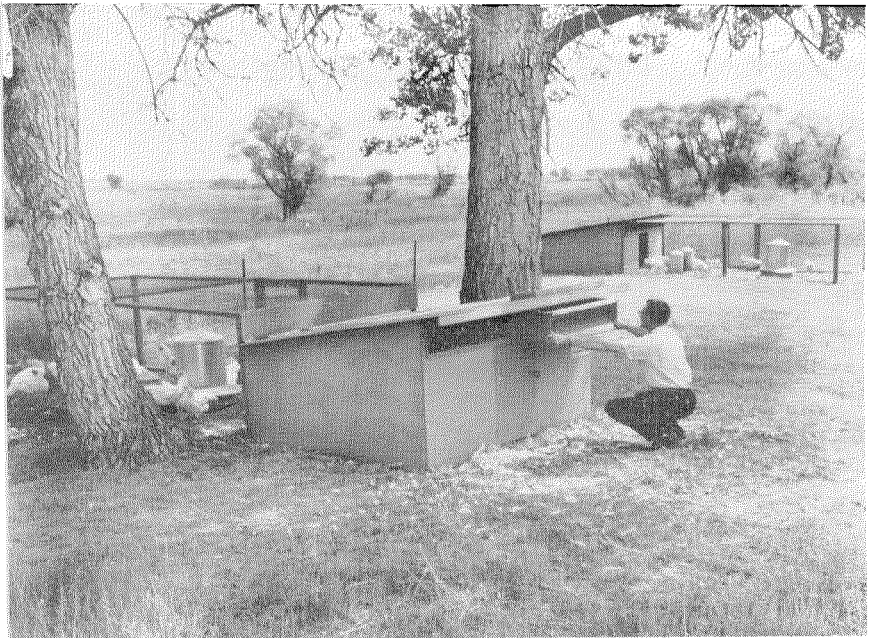


FIG. 1.—Mosquito trap being installed in a modified sentinel chicken shed. Original unmodified unit shown in the background.

inning at the top of the frame, the screen is wrapped around the back and edges tacked securely to the end boards, then brought forward underneath and tacked to the bottom of the front frame. The entrance to the trap was adapted from the horizontal baffle of a double trap developed in Egypt by Bates (1944). Screen wire is tacked to wooden framing to form the lower and the upper parts of the entrance (see figure for details). Mosquitoes attracted to sentinel chickens in the shed enter the trap through the $\frac{1}{2}$ " slot at the rear of the entrance.

Three $\frac{7}{8}$ " holes are provided in the screen at the back of the trap through which mosquitoes can be removed with a vacuum suction tube. One hole is midway between the ends of the trap and each of the others is 10 inches from the first. All

three are spaced well above vertical center so that mosquitoes both above and below the entrance screening can be reached easily. These holes, which are reinforced with metal rings soldered to the screen wire, are kept plugged with corks when not in use.

The only escape route for trapped mosquitoes is through the $\frac{1}{2}$ " entrance slot. Observations have shown that very few of a night's catch of *Culex tarsalis* find this way out of the trap, even when collecting is deferred until well into the following day.

IMPROVED SENTINEL SHED. The general layout of the original sentinel-chicken unit developed in 1959 can be seen in the background of Figure 1. Originally the pen was placed in front of the shed, rather than at the back, and there was no open-

ing in the back wall and no closure for the front doorway. Upon trials of the unit a number of disadvantages were apparent. With the pen in place the mosquito traps were difficult to install and remove; access to the chickens for bleeding was not easy; and keeping the bled birds separated from the unbled was a problem. In humid areas there was need for additional ventilation. Very important was the fact that relatively few *C. tarsalis* were collected in the shed traps.

Moving the pen from the front of the shed to the back solved the first three problems. The last two were corrected by adding the opening in the back wall of the shed. Field observations showed that the cross ventilation keeps the shed floor much drier, and, on the basis of the number of *C. tarsalis* collected, increased the shed's attraction for mosquitoes.

This possibility was tested in 1960. A single shed was modified so that a section could be removed from the middle of the back wall panel, leaving a screened opening 47" wide and 24½" high just above the lower 2" x 2" framing member. From July to September, mosquitoes were collected on 32 nights from traps in the experimental shed—two nights each week with the back panel in place and two nights with it removed. The mean number of *C. tarsalis* collected with the panel intact was 6.3 and with it removed was 31.6. This difference had a high statistical significance. As a result of this test, all of the sheds used in 1961 were provided with the opening in the back wall panel (see Figures 2 and 3).

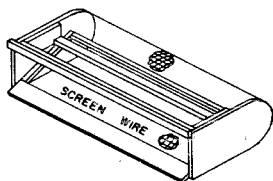
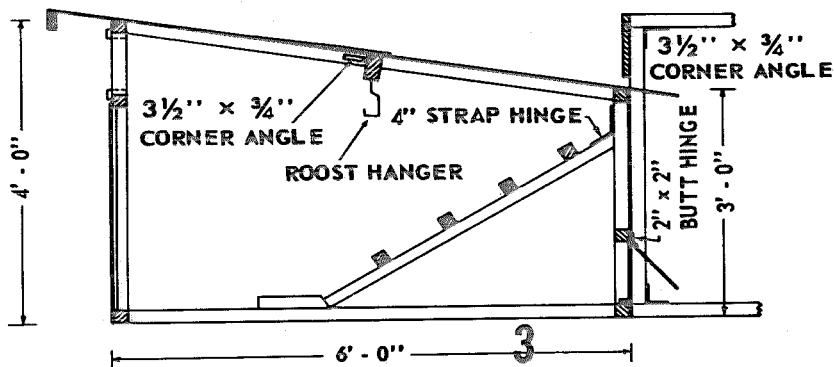
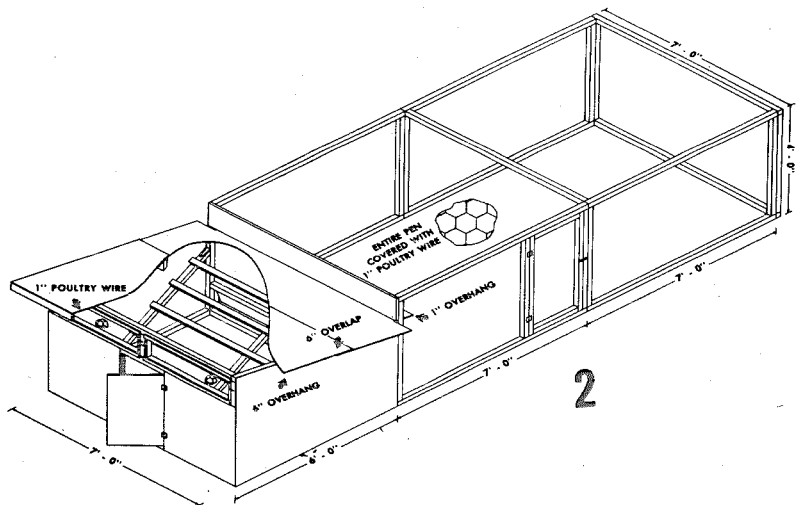
The effectiveness of this change in increasing *C. tarsalis* collections was apparent in every area where the sheds were used during the 1961 season. For example, in the Quincy Valley of Washington, light-trap collections varied little from 1960 to 1961. The average number of *C. tarsalis* per night, collected from 10 light-trap sites, increased from 17.2 in 1960 to 19.8 in 1961, a difference not significant ($t = .78$). In shed-trap collections for the two years, the average number of *C. tarsalis* per trap-night increased from 27.9

to 147.6, a difference highly significant ($t = 3.10$). At Plainview, Texas, light-trap collections of *C. tarsalis* from six sites dropped from an average of 536.9 per night in 1960 to 202.6 in 1961, while the average number taken per night in shed traps increased from 16.4 in 1960 to 19.8 in 1961. Similar increases in *C. tarsalis* collections in shed traps were observed at Greeley, Colorado, during the 1961 season.

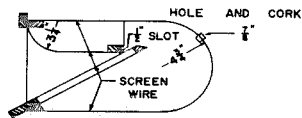
In Massachusetts, where *C. tarsalis* was not known to occur, mosquito collections from shed traps were compared by the numbers of each of four genera. However, the collecting stations were not entirely comparable to those in western areas; in Massachusetts both the sheds and pens had wire floors and were elevated about 6 feet above the ground, which allowed free circulation of air underneath them. In this situation, the opening in the rear wall panel was not expected to affect the flow of air through the shed appreciably. At each of four sites the relative numbers of *Culex*, *Culiseta*, and *Mansonia* collected in shed traps were greater in 1961 than in 1960, but the relative increase for each genus varied considerably from site to site. None of the differences was statistically significant. *Aedes* spp. increased at two sites but decreased at two others.

The overall results in 1961 indicate that the additional airflow through the sheds produced by the opening in the rear wall was responsible, at least in large part, for increases as great as fivefold in the number of *Culex tarsalis* collected as well as general increases in the numbers of other *Culex* and of *Mansonia* and *Culiseta*.

SUMMARY. A sentinel chicken shed developed by the CDC Encephalitis Section at Greeley, Colorado, has been used for several years in encephalitis field investigations to obtain transmission indices in enzootic habitats and for site-to-site comparison of levels of virus activity. The unit consists of a demountable and portable chicken shed and pen and a removable mosquito trap. It can accommodate a sentinel flock of 25 to 30 chickens. Design improvements have increased its effectiveness. With the unit and a single flock



4



CROSS SECTION OF TRAP

FIG. 2.—Sentinel chicken shed and pen. FIG. 3.—Cross section of shed. FIG. 4.—Mosquito trap for tincl chicken shed.

mosquito indices, mosquito infection rates, and transmission rates can be obtained concurrently.

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RESISTANCE TO DESICCATION IN SIX STRAINS OF THE *CULEX PIPIENS* COMPLEX

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INTRODUCTION. While studying the feeding preferences in six strains of the *Culex pipiens* complex (Chamberlain, Sudia and Gillett, 1959) it was noticed that mosquitoes in some of the containers survived the conditions imposed on them better than those in others. It appeared that there were differences between strains in their capacity to withstand desiccation, and two experiments were carried out to test this; the results are described below.

MATERIALS AND METHODS. Two strains of *Culex pipiens* L., and four of *C. quinquefasciatus* Say (= *C. fatigans* Wied.) were used; their places of origin and history are shown in Table 1. The mosquitoes were kept in standard 0.6 litre ice-cream cartons, both ends of which had been replaced with netting (Chamberlain *et al.*, 1959). For the survival tests the

cartons were placed inside desiccators containing 87.5 gm KOH/100 ml. H₂O. These were kept at 26.5° C. and provided a relative humidity of around 32 percent (Peterson, 1959). Records of the number of mosquitoes that had died in each carton were made at two-hourly intervals for 22 hours (in the first experiment three more readings were made at 25, 27 and 32 hours respectively).

Experiment 1 was carried out in Dr. J. W. Chamberlain's laboratory at the Communicable Disease Center, Montgomery, Alabama (31 December 1955-1 January 1956); Experiment 2 was carried out in Professor L. E. Rozeboom's department of Medical Entomology, Johns Hopkins University, Baltimore, Maryland (19-20 January, 1956).

RESULTS. In Experiment 1 (Table 2)