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FAILURE OF CHICKENS TO ACT AS SENTINELS DURING AN EPIZOOTIC OF EASTERN EQUINE ENCEPHALITIS IN SOUTHERN NEW JERSEY, USA[1]

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Abstract. A series of 10 chicken flocks placed in areas of southern New Jersey, USA, preceding an epizootic of eastern equine encephalitis (EEE) virus failed to function adequately as sentinels for the outbreak. Epidemiological data including virus isolations from mosquitoes and wild birds, confirmation of equine deaths due to EEE virus, and a confirmed human case showed that most of the chickens placed in areas where virus was known to be present did not develop antibody to EEE. Moreover, those that did develop antibody seroconverted late in the epizootic period. Data suggest that chickens do not function as a warning mechanism for EEE virus but merely act as an added indicator of virus activity.

Domestic chickens are useful for the detection of mosquito-borne viruses in areas where large-scale sampling of mosquito populations is inadvisable for practical or monetary reasons (Sudia et al. 1970). Nichols & Bigler (1969) described the advantages of using privately owned flocks as an added measure of virus activity during epizootics. Rainey et al. (1962) gave plans for a sentinel chicken pen to house domestic chickens as a tool for virus surveillance.

Sentinel flocks have proven to be valuable indicators for the presence of St. Louis encephalitis (SLE) virus (Hammon et al. 1946; Sudia & Chamberlain 1959; LaMotte et al. 1967; Day & Carlson 1985) and western equine encephalitis (WEE) virus (Hammon & Reeves 1946; Smith et al. 1969; Wong et al. 1976). Results with eastern equine encephalitis (EEE) virus, however, have been inconsistent. Kissling (1958) questioned the value of domestic chickens in the detection of EEE virus based on his observation of detectable antibody in only occasional specimens sampled from endemic and epidemic areas. Sudia et al. (1968) collected data from chicken flocks during epizootics in Alabama and found that none of the sentinel birds developed antibody to EEE even though isolations were obtained from mosquitoes collected from the same sites. However, Hayes et al. (1960) placed sentinel flocks at 4 locations in Massachusetts and obtained seroconversions to EEE virus in 35–65% of the birds. Rainey et al. (1962) cited the results of the Massachusetts investigation in suggesting that the sentinel system might be applicable for EEE investigations. King (1983) used chickens as sentinels for EEE virus in Delaware and reported favorable results.

In 1984 a series of chicken flocks was placed in southern New Jersey to function as experimental sentinels for EEE virus. Twenty-two equine cases of EEE were diagnosed in New Jersey that year as either confirmed or presumptive, and 1 human case of EEE was confirmed in a New Jersey resident (Crans & Schulze 1985). During the epizootic, more than 100 virus isolations were obtained from field-collected *Culiseta melanura* (Coquillett). Although some of the birds did seroconvert during the season, results indicated that the chickens functioned poorly as sentinels. This paper describes the methodology and the results obtained from the sentinel birds in comparison to other epidemiological data.

MATERIALS AND METHODS

Beginning in late May 1984, ten flocks, each consisting of five 18-wk-old white leghorn pullets, were placed in areas of southern New Jersey where EEE virus had been reported in the past. Seven of the flocks were housed in pens built to the specifications of Rainey et al. (1962). The remaining 3 flocks were kept at locations where cooperators already had facilities for chickens. From 1 June to 15 October, the young birds were bled from the wing vein every other week using 26-ga, 1.27-cm (½-in.) needles and 3-cm^3 disposable syringes. One cubic centimeter of blood was mixed with an equal amount of the field diluent described by Sudia et al. (1970). The blood specimens were immediately chilled with ice packs and transported to the laboratory for further processing. Each sample was centrifuged at 750 × *g* in a refrigerated centrifuge at 10 °C, and the serum–diluent mixture was stored

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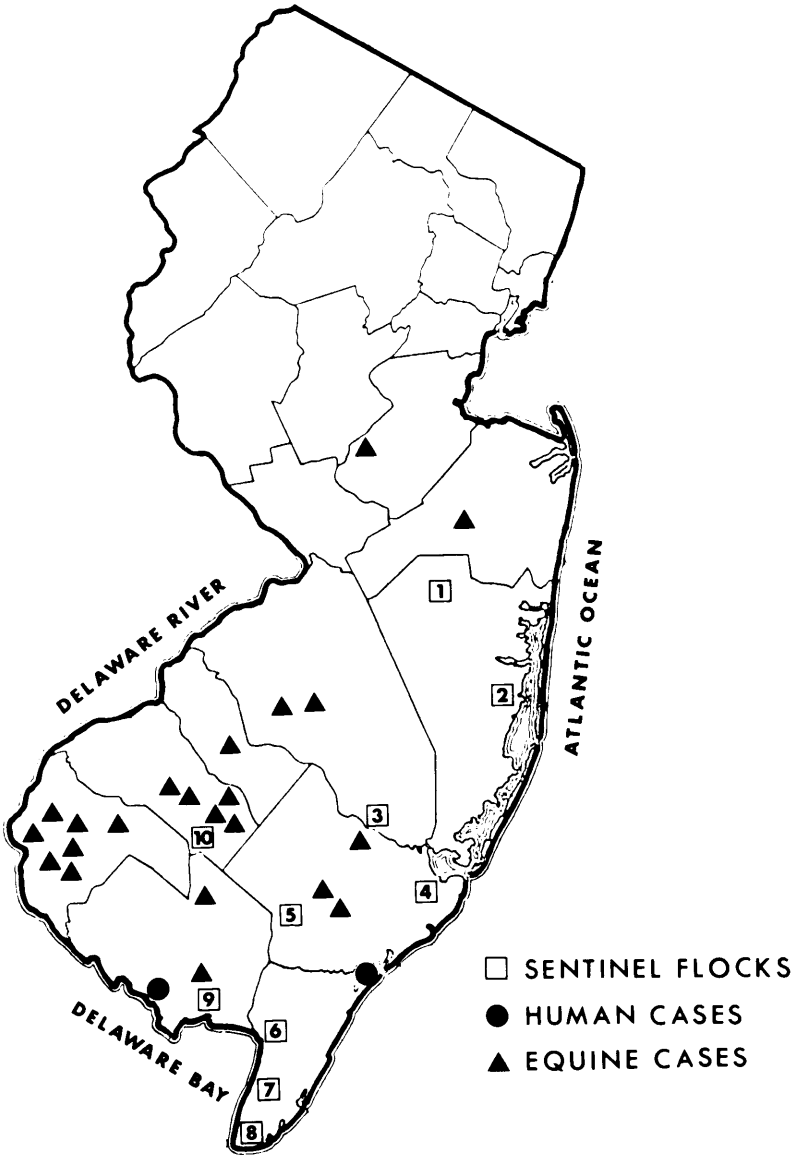

FIG. 1. Location of sentinel chicken flocks in New Jersey in relation to human and equine cases of eastern equine encephalitis in 1984.

at −56 °C. All specimens were tested for hemagglutination inhibition (HI) antibody to EEE virus at the New Jersey State Health Laboratories by the methods of Goldfield et al. (1968). Blood samples that showed a 4-fold rise in HI titer were deemed potential positives and were tested for EEE neutralizing antibody in recently weaned mice. Samples yielding a log neutralization index (LNI) of 1.9 or greater were considered positive.

RESULTS

Figure 1 shows the locations of the 10 flocks tested during the 1984 season and their proximity to the human and equine cases of EEE virus reported during the test period. Table 1 lists the flocks by site and the seroconversions obtained. Four of the flocks showed evidence of contact with EEE virus, but only 5 of the 30 birds in the test sample seroconverted during the period of the experiment. All of the birds that did seroconvert had an LNI greater than 2.1 and tested positive on subsequent bleedings for the remainder of the season.

Six of the flocks were placed in areas where EEE virus was being monitored twice weekly in mosquitoes (Crans & Schulze 1985). As a result, data

TABLE 1. HI antibody to EEE virus in sentinel chicken flocks maintained in New Jersey during 1984.

Flock no.	County	Area	No. with HI antibody	LNI	Date of seroconversion
1	Ocean	Jackson	0 of 5	—	None
2	Ocean	Forked River	2 of 5	2.2, 2.3	12 Sept.
3	Burlington	Green Bank	1 of 5	2.2	26 Sept.
4	Atlantic	Smithville	0 of 5	—	None
5	Altantic	Estelle Manor	0 of 5	—	None
6	Cape May	Dennisville	0 of 5	—	None
7	Cape May	Fishing Creek	1 of 5	2.5	17 Sept.
8	Cape May	Pond Creek	0 of 5	—	None
9	Cumberland	Port Norris	0 of 5	—	None
10	Gloucester	Iona Lake	1 of 5	2.5	18 Sept.

were available to indicate the exact period when EEE virus was being amplified by *Cs. melanura*. Table 2 compares results obtained from virus isolation attempts with those obtained from the sentinel flocks. Data indicate that although EEE virus was common in the enzootic vector, *Cs. melanura*, seroconversions in the sentinel flocks were not detected until late in the season.

The sentinel flock at the Jackson site was on a farm where 2 equine deaths due to EEE virus had been documented in 1983 (Crans & Schulze, in prep.). Although the minimum field infection rate (MFIR) in *Cs. melanura* was 7.10 during August and September, not one of the sentinel birds developed HI antibody to EEE virus. The sentinel flock at Green Bank was immediately adjacent to the resting boxes where *Cs. melanura* were being collected for virus isolation attempts. Fifteen EEE isolations were obtained from the resting box samples, but the single bird that developed HI antibody did not seroconvert until 1½ months after the initial isolation from mosquitoes. The sentinel flock at Dennisville was ca. 1 km from the area where mosquitoes were being sampled. Twenty-five virus isolations were obtained from *Cs. melanura* at this site, but none of the sentinel birds developed HI antibody to EEE. Eastern equine encephalitis virus, however, was isolated from a juvenile Cardinal collected 9 August and from a juvenile Yellowthroat collected 20 September, showing that active transmission to birds was taking place in the immediate vicinity. At the Iona Lake site, 6 equine deaths due to EEE virus occurred within a 16-km radius of the sentinel flock. Collections of *Cs. melanura* from the farms where the equine cases were confirmed yielded 12 EEE isolations and an MFIR of 8.72. The first equine death occurred 31 July, but the single seroconversion in the sentinel birds did not take place until 18 September.

In late October a confirmed case was made known in a boy who lived in Dividing Creek, New Jersey, a coastal community on the salt-marsh ecotone along Delaware Bay. Onset of symptoms was reported as 5 September. The sentinel flock at Port Norris was less than 8 km from the child's home, but none of the birds developed HI antibody to EEE.

DISCUSSION

The results obtained in New Jersey during 1984 support the observations of Kissling (1958), who questioned the potential effectiveness of chickens as sentinels for EEE. The results also agree with the findings of Sudia et al. (1968), who were puzzled by the lack of seroconversions in chickens housed in areas where EEE virus was regularly re-

TABLE 2. Epidemiological data collected from areas in New Jersey where sentinel chickens were monitored for EEE virus, 1 June–15 October 1984.*

	No. *Cs. melanura* tested	No. EEE isolations	EEE minimum field infection rate	Epizootic period	Date of sentinel seroconversion
Jackson	986	7	7.10	24 Aug.–28 Sept.	None
Green Bank	3,341	15	4.49	9 Aug.–8 Oct.	26 Sept.
Dennisville	7,110	25	3.52	2 Aug.–19 Sept.	None
Iona Lake	1,376	12	8.72	31 July–19 Sept.	18 Sept.

* Data for *Cs. melanura* include only the period from the first detection of virus to the end of the season. Minimum field infection rates based on isolates/1,000 specimens tested.

covered from mosquitoes. Although the results appear to contradict the findings of Hayes et al. (1960), a difference in methodologies may have produced the apparent discrepancies. Hayes et al. (1960) did find antibodies in a large percentage of the sentinel chickens monitored during an epizootic in Massachusetts, but the study was not designed to determine if the chickens were functioning as sentinels. In the Massachusetts investigation, the sentinel flocks were exposed from 22 June to 16 October to determine if EEE virus was active outside a large swamp that was producing *Cs. melanura*. Although seroconversions did occur in 35–65% of the birds tested, the results were calculated from bleedings made at the end of the season. As a result, data are not available to determine if the birds seroconverted before, during, or after the epizootic. Likewise, King (1983) used sentinel chickens without any additional epidemiological indicators. Although seroconversions were obtained in the sentinel birds, the positive samples were detected late in the season and may have merely confirmed virus activity that had been present for some time.

Available data suggest that chickens function poorly as sentinels for EEE virus. Even though considerable virus activity was documented in New Jersey during 1984 by confirmation of equine deaths, isolation of virus from *Cs. melanura*, and a confirmed human case, most of the chickens used in these tests did not develop HI antibody to EEE virus. In those cases where seroconversions were noted, the chickens did not seroconvert until late in the epizootic period. By the time the birds indicated that EEE was active, most of the equine deaths had occurred, the human infection had been contracted, and *Cs. melanura* populations were already entering their seasonal decline. The chicken flocks did not function as a warning mechanism for EEE virus; they merely acted as an additional indicator to show that EEE virus was present at some of the locations.

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