

## COMPARISON OF CHICKENS AND PHEASANTS AS SENTINELS FOR EASTERN EQUINE ENCEPHALITIS AND ST. LOUIS ENCEPHALITIS VIRUSES IN FLORIDA

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**ABSTRACT.** Pheasants and chickens were compared as sentinels for monitoring the transmission of arboviruses of public health significance in Florida during 1991-93. Results suggest that pheasants are better sentinels for eastern equine encephalitis (EEE) virus. They detected virus transmission 3-6 weeks earlier in epizootic years, 2-4 times more frequently during the season, and twice as many birds within a flock seroconverted during a given week. Pheasants detected virus transmission at 2 sites during 2 interepizootic years when chickens failed. Although pheasants detected St. Louis encephalitis (SLE) virus activity somewhat later than did chickens, they had greater seroconversion rates than did chickens. Although both bird species can be used to monitor EEE and SLE viruses, pheasants are more sensitive than chickens as sentinels for EEE virus and as sensitive as chickens as sentinels for SLE virus.

### INTRODUCTION

Chickens have been used as sentinel hosts for arboviruses in Florida since 1978, following the 1977 epidemic (Day 1989). The number of counties that participated each year since then and the number and locations of the flocks have varied greatly over the 16 years. Flocks maintained by county mosquito control programs or public health units were established primarily in areas to detect St. Louis encephalitis (SLE) virus. During the low SLE transmission period that followed the epidemic, some programs relocated flocks to monitor eastern equine encephalitis (EEE) virus.

Because chickens generally are not considered good sentinels for EEE (Kissling 1958, Sudia et al. 1969, Bigler et al. 1976, Crans 1986), we were concerned that Florida mosquito control programs relying on sentinel chickens for EEE surveillance might be misled by negative results. To examine the question, we compared chicken and pheasant flocks located next to each other at several sites in Leon County, FL, during 1991-93. Leon County has a long history of EEE transmission in the southwestern part of the county near the city of Tallahassee (G. Baker, H. Rubin

and L. Stark, unpublished data<sup>5</sup>). Because some counties in Florida are interested in SLE as well as EEE, we also compared chickens and pheasants in Lee County in southwest Florida during 1992-1993. Lee County has an extensive sentinel chicken program and a history of SLE transmission (J. Burgess and L. Stark, unpublished data<sup>5</sup>).

### MATERIALS AND METHODS

Flocks of 6 ring-necked pheasants (*Phasianus colchicus*) were held in cages adjacent to similar cages of 6 chickens (White Leghorns in Leon County and Rhode Island Reds [1992] and New Jersey Giants [1993] in Lee County). To prevent pecking, and potential pheasant-to-pheasant transmission, birds were fitted with blinders or the points of their beaks were cut off. Birds were provided food, water, shade, and protection from predators continuously and were treated in a manner consistent with animal-handling policies of the National Institutes of Health (1985).

In Leon County, paired flocks were maintained at 4 sites in 1991; 3 between June 25 and November 20 and one between July 22 and November 20. Chicken flocks were maintained at all sites between June 3 and November 20, 1991. During 1992 (June 9-November 3) and 1993 (April 7-November 17), pheasants and chickens were maintained only on Sprinel Road and Stoneler Road. Additional chicken flocks were maintained at 5 other sites in 1992 and 1993.

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<sup>5</sup> See weekly and monthly Arbovirus Surveillance Reports, Sentinel Flock Activity, L. M. Stark, Tampa Branch Laboratory, Florida Department of Health and Rehabilitative Services.

Table 1. Seroconversions to eastern equine encephalitis and St. Louis encephalitis viruses in sentinel chickens (C) and pheasants (P) at 4 sites in Leon County, FL, during 1991.

Week	Sprinell Rd.		Jamie Rd.		Sunnyside St.		Stoneler Rd.	
	C	P	C	P	C	P	C	P
Jun. 3	start		start		start		start	
10	0/6 <sup>1</sup>		0/5		0/6		0/5	
17	0/6		0/5		0/6		0/5	
24	1/6	start	—	start	0/6	start	—	
Jul. 1	1/5	1/3 <sup>2</sup>	0/6	0/5	0/6	0/5	1/6	
8	0/4	—	0/6	—	0/6	—	0/5	
15	0/4	2/5	0/6	1/2 <sup>3</sup>	0/6	3/4	0/5	
22	0/6	0/0	0/6	0/1	0/6	0/1	0/5	start
29	0/6	3/4 <sup>4</sup>	0/6	0/3	0/6	0/2	0/6	0/6
Aug. 5	0/6	0/2	1/6	0/3	0/6	0/5	0/6	1/6
12	0/6	0/6	0/5	0/6	0/6	2/5	0/6	0/5
19	0/3	0/3	0/5	0/6	0/6	0/1	0/6	0/5
26	0/4	1/6	0/6	1/3	1/6	0/5	0/6	1/4
Sep. 3	0/6	0/5	0/6	0/5	0/5	1/5 <sup>3</sup>	0/6	0/4
9	0/6	0/5	0/6	1/5	0/4	0/6	0/6	0/4
16	0/6	0/6	0/6	0/4	0/6	0/6	0/6 <sup>5</sup>	0/6
23	0/6	0/6	0/6	0/4	0/6	0/6	0/5	0/6
30	0/6	0/6	0/6	0/6	0/6	0/6	0/5	0/6
Oct. 7	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
14	0/6	0/2	0/6	0/5	0/6 <sup>5</sup>	0/6	0/6	0/6
21	0/6	0/6	0/6	0/6	0/5	0/6	0/6	0/6
28	0/6	0/5	0/6	0/6	0/5	0/6	0/6	0/6
Nov. 4	0/6	0/6	0/6	0/6	0/6	0/6 <sup>5</sup>	0/6	0/6
11	0/6	0/6	—	0/6	0/6	—	0/5	0/6
18	0/6	0/6	0/5	0/6	0/6	0/6	0/5	0/6

<sup>1</sup> Number positive/number of birds tested.

<sup>2</sup> Equivocal positive.

<sup>3</sup> EEE virus isolated from one dead bird.

<sup>4</sup> EEE virus isolated from all 3 dead birds.

<sup>5</sup> One bird had SLE antibodies.

In Lee County, paired flocks were maintained year-round at 3 sites in 1992 and 1993, each with a history of SLE seroconversions in chickens. Sentinel chicken flocks also were maintained at 14 other sites in Lee County.

All sentinels were bled and tested for hemagglutination inhibition (HI) antibodies to EEE and SLE prior to exposure at the sentinel sites. They were bled in the field and kept in the sentinel cages. Birds that were HI-positive on the first bleeding were replaced, and the data were not used. Only birds without antibodies to EEE and SLE were used as sentinels. Birds that seroconverted or died after being HI-negative were replaced with new birds.

Leon County birds were bled (1.5–2.0 cc) weekly (biweekly in 1993) during their exposure periods. Lee County birds were bled (5 cc) biweekly until the first seroconversion, then birds were bled weekly until 3 weeks after the last seroconversion when biweekly bleedings were resumed.

Sera from Leon and Lee County sentinels were tested for HI antibodies to EEE and SLE at the Florida Department of Health and Rehabilitative Services (HRS) Tampa Branch Laboratory (L. Stark). Specimens from Lee County also were tested for HI antibodies at the Lee County Mosquito Control District (J. Burgess). Seroconversions were confirmed by titers at or above 1:10 on 2 consecutive bleedings at least 1 week apart. Birds that seroconverted were removed from the flock after the confirmation.

Because pheasants may die from EEE infection, the brains of recently deceased pheasants found in the cages in Leon County were examined for virus by inoculating approximately 20% of brain tissue in biological field diluent (0.015 cc IC and 0.03 cc IP) into 8 2–4-day-old white laboratory mice at the Tampa Branch Laboratory (L. Stark). Deceased chickens were not examined for viruses. Eastern equine encephalitis virus isolates were confirmed by serum neutralization tests in BGM cell cultures.

## RESULTS AND DISCUSSION

Seroconversions to EEE occurred in all 3 years in Leon County. Seroconversions to SLE occurred in Leon County in 1991 and Lee County in 1993.

*Leon County:* In 1991, during a major epizootic of EEE in Florida, both chickens and pheasants seroconverted to EEE at all 4 study sites (Table 1). When both chickens and pheasants were present, EEE transmission was detected first in pheasants at 3 sites: at Stoneler Road and Sunnyside Street by serology and at the Jamie Road site by virus isolation from the brain of a dead bird.

At the 4th site, Sprinel Road, one of 5 chickens seroconverted on July 1 (although one chicken seroconverted the week earlier, there were no pheasants on site for comparison). One of 3 pheasants at Sprinel Road also had HI antibodies on July 1, but because of laboratory problems with the baseline sample of this pheasant, taken 1 week earlier, we are uncertain of whether or not the bird was negative when first placed at the Sprinel Road site on June 24. Because 5 of the 56 (8.9%) pheasants used during 1991-93 were EEE HI-positive on their initial bleeding, this bird could have obtained its infection from where it was raised. Only 3 of 83 (3.6%) chickens used at the 4 study sites and the 4 other sites in Leon County were EEE HI-positive prior to exposure.

During 1991, 6 chickens and 14 pheasants died in the cages. We recovered EEE virus from 5 of the 9 (55.6%) pheasants examined.

During the period when both bird species were present, chickens indicated EEE transmission was occurring during only 1 week at 3 of the 4 sites and not at all at the 4th site (Stoneler Road). In contrast, pheasants indicated EEE virus transmission was occurring during 3 or 4 weeks at the 3 sites where chickens were positive only once and during 2 weeks at the site where chickens were negative. At Jamie Road and Sunnyside Street, virus transmission was detected by pheasants 3 and 6 weeks before chickens.

In addition, never more than one chicken seroconverted in a flock during a given week, whereas more than one pheasant seroconverted, or was virus positive, in 4 of the 12 (33.3%) site-weeks that were positive. During weeks when seroconversions occurred at a site, 32.7% of the pheasants at the site were positive but only 17.4% of the chickens were positive. Pheasants seroconverted during 12 of the 14 site-weeks (85.7%) when seroconversions were made in either chickens or pheasants. Chickens seroconverted during only 3 (21.4%) of those site-weeks.

Also during 1991, 4 chickens and one pheasant seroconverted to SLE virus at 3 sites in Leon

Table 2. Seroconversions to St. Louis encephalitis virus in sentinel chickens (C) and pheasants (P) at 3 sites in Lee County, FL, during 1993.

Week	Winkler		Williams Rd.		Civic Center		
	C	P	C	P	C	P	
Aug.	2	0/6 <sup>1</sup>	0/5	0/6	0/6	0/6	0/6
	16	0/6	0/5	1/6	0/6	0/6	0/6
	23	0/6	0/5	0/6	1/6	0/6	0/6
	30	1/6	1/5	0/6	0/6	1/6	0/6
Sep.	6	0/6	0/4	0/6	0/6	3/6	0/6
	13	3/6	2/4	0/6	1/6	1/6	0/6
	20	0/6	0/2	3/6	2/6	0/6	0/6
Oct.	27	0/6	2/2	0/6	3/5	0/6	2/6
	4	1/6	—	0/6	1/2	1/6	2/4
	11	2/6	—	1/6	1/1	1/6	0/2
	18	0/6	—	0/6	—	0/6	0/2
Nov.	25	0/6	—	1/6	—	1/6	0/2
	1	0/6	—	1/6	—	0/6	2/2
	8	0/6	—	0/6	—	0/6	—

<sup>1</sup> Number positive/number of birds tested.

County including our Stoneler Road and Sunnyside Street sites. One pheasant seroconverted to SLE 3 weeks after chickens at Sunnyside. There was also a single human case of SLE in an 18-year-old male who lived within 1 mi. of the Stoneler Road site (HRS, unpublished data). The date of onset was September 3, 2 weeks before a sentinel chicken at Stoneler Road seroconverted to SLE. These events were the first and, through 1993, remain the only evidence for SLE transmission in Leon County. Interestingly, there were no human cases of SLE and no chickens seroconverted to SLE in Leon County during a statewide epidemic of SLE in 1990, 1 year earlier.

During 1992, 3 pheasants and no chickens on Sprinel Road seroconverted to EEE: 2 on August 11 and one on October 20. There were no seroconversions at Stoneler Road during 1992. There were, however, seroconversions to EEE in chickens at 2 sites without pheasants in Leon County during 1992: 8 during a 10-week period between August 25 and October 27 and 3 during an 8-week period between August 11 and September 29. The only seroconversion to EEE in Leon County during 1993 was in a pheasant on Stoneler Road on August 11.

*Lee County:* There were seroconversions at sites where both pheasants and chickens were kept only during 1993 (Table 2). A short supply of pheasants made it impossible to replace all seropositive birds with new ones. Therefore, the number of seronegative pheasants at a site decreased as new birds seroconverted.

Pheasants and chickens seroconverted during the same week at Winkler, but chickens seroconverted before pheasants at the other 2 sites, 1 week earlier at Williams and 4 weeks earlier at the Civic Center. During periods when at least one pheasant and one chicken were exposed, pheasants seroconverted during more weeks than did chickens at Winkler and Williams. The rate of seroconversion was higher in pheasants than in chickens.

This study suggests that pheasants are better sentinels for detecting EEE in Florida. The detected virus transmission 3–6 weeks earlier in epizootic years, 2–4 times more frequently during the season, and twice as many birds within a flock seroconverted during a given week. Pheasants detected virus transmission at 2 sites during 2 interepizootic years when chickens failed to show any transmission.

Although pheasants may detect SLE activity somewhat later than do chickens, they are still sensitive to SLE infection and actually had greater seroconversion rates than did chickens. Clearly, pheasants are more sensitive as sentinels for SLE virus than chickens are for EEE virus.

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