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EXPERIMENTAL INFECTIONS OF BIRDS WITH TURLOCK VIRUS

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ABSTRACT: Two experiments were conducted with five gallinaceous and one passerine bird species to determine their responses to Turlock (TUR) virus inoculations. Inoculation of TUR strain 847-32 into bobwhites, chukars, ring-necked pheasants, chickens, and Japanese quail did not produce detectable viremias. The first four species, however, did respond with neutralizing antibody. Inoculation of chickens with strain 69V-1095 resulted in a viremia which lasted 5 days and had a peak mean titer of 2.0 $\log_{10}$PFU per 0.2 ml of blood on the third day after infection. The observation that viremic birds exhibited no noticeable virus-associated morbidity or mortality suggested that TUR virus does not have a detrimental effect on free-ranging populations of the avian hosts studied during this investigation.

INTRODUCTION

Turlock (TUR) virus, a single-stranded RNA virus in the family Bunyaviridae (Klimas et al., 1981), was first isolated in 1954 from a pool of *Culex tarsalis* mosquitoes collected in Turlock, California, by Lennette et al. (1957a) during an investigation of western equine encephalitis. Since 1954, TUR virus has been recovered repeatedly from mosquitoes and birds in North and South America. Scott (1981) reviewed reports of TUR virus isolations from field-collected specimens which showed that 305 of 328 mosquito isolates came from *C. tarsalis* and that 20 of 21 isolates from free-ranging vertebrates were from birds. This field information suggests that TUR is a mosquito-borne avian virus.

Avian isolates have been recovered most often from house sparrows (*Passer domesticus*) sampled in Texas. From 1965 through 1970, 16 of 3,964 sparrow sera collected in Hale County, Texas contained TUR virus (Hayes et al., 1967; Holden et al., 1973; D. B. Francy, unpubl. data). Other birds from which fewer TUR virus isolates have been recovered include three house finches (*Carpodacus mexicanus*) from California (Berge, 1975), one plain-throated antwren (*Myrmotherula hauxwelli*) and one amazonia antshrike (*Thamnophilus amazonicus*) (Shope et al., 1966); the last two species were captured near Belem, Brazil. The non-avian isolate came from one of 249 blacktailed jackrabbits (*Lepus californicus*) sampled in Hale County, Texas during 1965 (Hayes et al., 1967).

Because TUR virus infections occur in mosquito and avian populations in the western United States, information concerning the viremia and antibody responses and disease potential in avian species would be useful to individuals in that region who manage gamebird and songbird populations as well as arbovirologists who annually isolate TUR virus. Only limited information of this sort is currently available. TUR virus inoculations were lethal to 1-day-old chickens (Lennette et al., 1957b), and five passerine species responded to inoculation with a viremia range of 3.5–5.9 $\log_{10}$ per ml as well as antibody production (Hardy, unpubl. data cited by Berge, 1975).

This paper, therefore, reports on the response of five gallinaceous and one passerine species to TUR virus inoculations.

MATERIALS AND METHODS

Six bird species (bobwhite [*Colinus virginianus*], Japanese quail [*Coturnix coturnix*], chukar [*Alectoris gracea*], ring-necked pheasant [*Phasianus colchicus*], chicken [*Gallus gallus*], and house sparrow) were experimentally inoculated with TUR virus for the following reasons. House sparrows were studied because TUR virus has been isolated from them during field investigations in Texas (Hayes et al., 1967; Holden et al., 1973) and thus they represent a potential enzootic host. Bobwhites, chukars, and ring-necked pheasants were inoculated because they are important gamebird species in the western United States where TUR virus is enzootic and we wanted to determine the disease potential of TUR virus in these avian species. Chickens and Japanese quail were

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studied because they were convenient to obtain and maintain in captivity and might therefore serve as animal models for the study of TUR virus pathology, if the virus appeared to have a detrimental effect on them and other species inoculated during these initial studies.

The first five species listed above were used in experiment I, which was conducted at The Pennsylvania State University. Those birds were obtained as eggs. Japanese quail, chickens, and house sparrows were used in experiment II which was conducted at the Vector-borne Diseases Division, Centers for Disease Control (CDC), Fort Collins, Colorado. Chickens were obtained when 2 to 3 days of age and quail were obtained at 2 to 5 days of age. House sparrows were captured in Japanese mist nets in or near Fort Collins, Colorado.

Two virus strains were used. Strain 847-32 is the prototype virus, which was recovered by Lennette and co-workers (Berge, 1975). Before its use in this study, it was passed in suckling mice 13 times. Strain 69V-1095 was recovered from a pool of *C. tarsalis* collected in Hale County, Texas, on 9 July 1969 by CDC field workers and was subsequently passed once in suckling mice.

Birds were bled from the jugular vein with a 1-cc tuberculin syringe and 27-gauge needle. Blood (0.05–0.2 ml) was mixed with 0.9 ml of diluent (M199 cell culture medium with 20% heat-inactivated fetal bovine serum and antibiotics). Blood-diluent mixtures were allowed to clot on wet ice, then centrifuged at 1,000 rpm for 10 min, after which serum was removed and dispensed into paired samples, then stored at −70 C.

Specimens were assayed for virus by inoculation of 0.1 ml of the supernatant fluid onto monolayers of cell cultures (VERO, a continuous cell line of African green monkey kidney cells; or DECC, primary Pekin duck embryo cells) grown in six-well plastic plates and 0.02 ml into the brains of 2- to 4-day-old suckling mice (Scott, 1981). Viremia titers were determined by inoculation of serial 10-fold dilutions onto cell cultures and were expressed as plaque forming units (PFU) per 0.2 ml of blood. Comparative titrations of the prototype strain (847-32) were $10^{6.0}$ PFU/0.1 ml in DECC and $10^{6.5}$ PFU/0.1 ml in VERO cells. Antibody determination was carried out by using a constant-virus serum-dilution plaque reduction neutralization test (PRNT) in VERO cells contained in six-well plates (Scott, 1981). The prototype strain (847-32) of TUR virus was used for all PRNT, and ≥80% plaque reduction over controls was considered PRNT positive at a 1:10 serum dilution.

Experiment I: Five bird species (bobwhite, Japanese quail, chukar, ring-necked pheasant, and chicken) were studied. Experimental birds were inoculated subcutaneously (SC) with 60,000 VERO cell PFU of TUR (847-32) virus and controls were inoculated with a diluent placebo. Birds were housed in groups by species and treatment. For the first four species, 15 experimental and 15 control birds were inoculated at 1 day of age. All birds were bled prior to virus inoculation for antibody determination and on days 1, 3, and 5 post-inoculation (PI) for viremia determination and on the 28th day PI for antibody assay. Sera were tested for virus in VERO cultures, DECC cultures, and suckling mice.

Experiment II: Ten Japanese quail aged 7 days, 10 chickens aged 1 day, and nine house sparrows aged ≥30 days were inoculated s.c. with 600 DECC PFU of TUR (69V-1095) virus. Birds were housed in groups by species. Japanese quail and chickens were housed in single cages while house sparrows were housed in two cages, one containing five birds and the other four birds. Five birds from each species were bled on even-numbered days and five different birds (4 in the case of house sparrows) on odd days at 24-hr intervals for 8 days PI; this was done to reduce the effect that stress from handling and bleeding could have on viremia responses (McLean, 1982). Birds were also bled prior to virus inoculation. At 26 days PI, birds were bled for antibody determination. Sera were tested for virus in VERO cultures.

RESULTS

Experiment I: None of the control birds or those inoculated with TUR (847-32) virus were viremic on days 1, 3, and 5 PI nor did any of the birds show noticeable morbidity or mortality that could be associated with virus infections. None of the pre-inoculation sera neutralized virus.

Antibody was detected in the blood of individuals in four of the five species tested (Table 1). Chukars, pheasants, and chickens produced the strongest responses (highest dilution causing ≥80% plaque reduction), while bobwhites responded poorly and Japanese quail did not produce detectable antibody. None of the sera collected from controls neutralized virus.

Experiment II: Of the three species inoculated, only chickens circulated detectable amounts of TUR (69V-1095) virus (Fig. 1). The highest mean titer was 2.0 $\log_{10}$PFU per 0.2 ml of blood on the third day after inoculation. Virus was detected in chicken blood for 5 consecutive days, from the second through the sixth day PI. The greatest variation in viremic responses by chickens occurred on the second day PI. None of the experimental birds showed noticeable virus associated morbidity or mortality.

One of 10 Japanese quail, 10 of 10 chickens, and seven of nine house sparrows developed antibody titers ≥1:10 26 days after inoculation.

DISCUSSION

Experiment I showed that TUR (847-32) virus did not cause viremia, morbidity, or mortality when inoculated into five different galli-

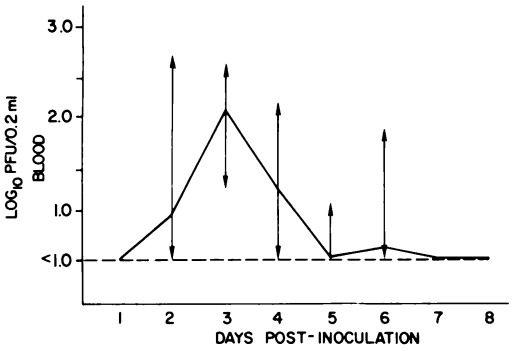

FIGURE 1. Viremic profile of 10 chickens inoculated with 600 DECC PFU of Turlock (69V-1095) virus. Vertical lines represent ranges. The intersection of vertical and diagonal lines represents means for five chickens.

TABLE 1. Antibody responses of five bird species inoculated subcutaneously with 60,000 VERO cell PFU of Turlock (847-32) virus 28 days earlier.

Species	Age when inoc. (days)	No. sampled (% positive)	Birds with antibodies	
			Titer[a]	No. birds
Bobwhites	7	13 (15)	100	1
			1,000	1
Japanese quail	7	15 (0)		0
Chukars	7	14 (100)	1,000	4
			10,000	10
Ring-necked pheasants	7	15 (80)	100	6
			1,000	4
			10,000	2
Chickens	1	7 (100)	100	7[b]

[a] Reciprocal of serum dilution causing ≥80% plaque reduction.
[b] Bled 15 days post-inoculation.

naceous bird species three of which (bobwhites, chukars, and ring-necked pheasants) are important gamebird species in TUR virus enzootic areas. Perhaps birds in this experiment did not become viremic because the virus had been altered by 13 passages in mice (Rosen, 1980). On the other hand, the observation that strain 847-32 did not cause viremia in the avian species inoculated may be representative of what occurs among free-ranging bird populations.

Three of the five species inoculated did respond with high prevalences of neutralizing antibody (Table 1). Thus, serology could be used to detect previous exposure of those three species to TUR virus.

Experiment II showed that the probable duration of potential host viremias, when infected with strain 69V-1095, was less than or equal to 7 days and that viremias reached peak amounts at 2 to 4 days post-inoculation (Fig. 1). Because mosquito thresholds of infection are not known, we cannot state during which portion of the viremia period chicks are infective to mosquitoes. The peak mean viremia, 2.0 $\log_{10}$ VERO PFU per 0.2 ml of blood, occurred on the third day post-inoculation. Three days after initial exposure, therefore, is probably the time when the majority of chicks inoculated with strain 69V-1095 would be most infective to vector mosquitoes.

The actual titer in chicks was probably higher than the titers shown in Figure 1. Chick bloods in experiment II were only tested in VERO cultures, and VERO cultures are inferior to DECC as an assay system for detecting TUR virus (69V-1095) in chick and sparrow blood (Scott, 1981).

The observation during experiment II that house sparrows did not circulate virus was difficult to explain. TUR virus has been isolated from free-ranging house sparrows in Hale County, Texas (Hayes et al., 1967; Holden et al., 1973; D. B. Francy, unpubl. data, 1981) and Fort Collins, Colorado (R. G. McLean, unpubl. data, 1981), as well as from house sparrows inoculated during a mosquito infectivity experiment which compared the susceptibility of *C. tarsalis*, *Culex pipiens pipiens*, and *Culex pipiens quinquefasciatus* to TUR virus (Scott, 1981). During that mosquito infectivity study, six of six house sparrows became viremic after inoculation with 600 DECC PFU of TUR virus strain 69V-1095. Sparrow mean titers were 1.9–2.2 $\log_{10}$ DECC PFU/0.2 ml of blood and 0.8–1.7 VERO PFU/0.2 ml of blood before and after mosquitoes fed on them during the evening of the third day PI. None of the six viremic house sparrows showed noticeable morbidity or mortality that could be associated with TUR virus inoculation.

Sparrows inoculated during this study may not have become viremic because they were pre-exposed to TUR virus. All birds were captured in an area from which TUR virus mosquito and house sparrow isolates have been recovered (D. B. Francy and R. G. McLean, unpubl. data, 1981). Although all birds were bled prior to inoculation and a single antibody positive bird was excluded from subsequent

analysis, immunity could exist without detectable antibody in pre-inoculation sera (McIntoshi et al., 1969; McLean, unpubl. data, 1982). House sparrows previously exposed to virus can lose detectable antibody titers but resist viremia when challenged with the virus a second time. Additional studies with house sparrows and strain 69V-1095, including antibody response curves to determine if responses are primary or secondary, need to be conducted before the results of experiment II can be thoroughly explained.

The absence of a viremic response by Japanese quail to TUR strain 69V-1095 agreed with results from experiment I when strain 847-32 was inoculated. Both experiments showed that Japanese quail are poorly suited as laboratory models for the study of TUR virus.

Although one of the birds species studied herein, the house sparrow, is involved in the natural vector–host transmission system of TUR virus, none of the inoculated birds exhibited morbidity or mortality that could be associated with viral infection. This observation, combined with the lack of reported TUR virus infections from sick or dead birds, suggests that TUR virus does not have a detrimental effect in the western United States on free-ranging populations of the gamebird species or enzootic host species studied during this investigation. It is possible, however, that birds in age classes that were not studied, for example nestling sparrows, are detrimentally affected by TUR virus.

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