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Authors: WATTS, DOUGLAS M., and WILLIAMS, JAMES E.

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EXPERIMENTAL INFECTION OF BOBWHITE QUAIL (*Colinus virginianus*) WITH WESTERN EQUINE ENCEPHALITIS (WEE) VIRUS[1]

DOUGLAS M. WATTS[2] and JAMES E. WILLIAMS
Department of Virus Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20012

Abstract: Bobwhite quail infected with WEE virus produced viremias 20 and 32 hours post-inoculation with maximum virus titers of $10^{3.0}$ $TCID_{50}$/0.1 ml blood. Hemagglutination-inhibition antibody appeared in about a week, reached maximum titer in 3-6 weeks and disappeared from most birds after 12 weeks. Neutralizing antibody appeared 6 days after inoculation with an average log neutralization index of 2.7. These responses of quail are compared to those of other bird species and confirm the suitability of bobwhite for use as a sentinel animal to detect the transmission of WEE virus.

INTRODUCTION

The usefulness of animals employed for detecting virus transmission is enhanced when patterns of viremia and antibody responses are known, since the times when natural infections were initiated can be deduced more accurately from serial serum-antibody determinations. In addition, the relative risk of infecting mosquitoes in a field study area can be inferred from the levels and durations of viremia characteristic of different species. In field studies utilizing wild birds to study the transmission of eastern and western equine encephalitis,[10] bobwhite quail was the best species investigated in terms of availability and survival, and quail otherwise seemed appropriate as sentinels. This paper describes the response of bobwhite to infection with WEE virus and gives a further evaluation of the species usefulness as a sentinel animal.

MATERIALS AND METHODS

Bobwhite quail, purchased from a commercial source (Georgia Quail Farm, Savannah, Ga.) were 3½ months old when used. Twelve experimental birds were inoculated subcutaneously on the breast with strain m-3249 of WEE virus, which was originally isolated from *Culiseta melanura* mosquitoes collected in the Pocomoke Cypress Swamp, Md., in 1965 and identified by cross neutralization with the Massachusetts strain of WEE. M-3249 had undergone 3 passages in primary hamster kidney cell culture (HK). The dose of virus inoculated was determined by simultaneous titration in HK. Six control birds were inoculated with diluting medium 199-Hank's containing penicillin (100 units/ml) and streptomycin (100 μg/ml). Experimental and control birds were kept in separate constant-temperature rooms to prevent possible transmission of virus to controls by aerosols or feces.[1]

[1] In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care' of the Institute of Laboratory Animal Resources, NAS-NRC.

[2] Present address: Department of Veterinary Science, University of Wisconsin, Madison, Wisc. 53706.

Blood samples were taken by venipuncture with syringes rinsed with a heparin-saline solution (50 USP units/ml) before and at intervals following the inoculations. Plasmas were separated from blood cells by centrifugation at 650 x g for 15 minutes at 4 C. Fecal specimens were taken from the cloaca at times of bleeding prior to and for 6 days following inoculations. Fecal and blood specimens were stored at —60 C.

Isolation of virus was attempted by inoculating 0.1 ml of blood cells on medium-free HK, which were incubated for several hours, rinsed, refed with media and observed 5 days for cytopathic effect (CPE). Cultures exhibiting CPE were confirmed positive for WEE virus by neutralization tests using specific WEE rabbit antisera. Original blood specimens were titrated in HK to determine concentrations of virus.

Isolation of virus also was attempted from fecal samples mixed in 199-Hank's medium with 20% inactivated fetal bovine serum, 1,000 units/ml penicillin, 1,000 μg/ml streptomycin and 10 μg/ml amphotericin B. Samples were centrifuged at 650 x g for 15 minutes at 4 C, and the supernatant fluid was inoculated into HK (0.1 cc/tube), which were treated as above.

The immune response of quail to WEE virus was studied using tube tissue culture neutralization and hemagglutination-inhibition (HI)[2,3] tests. Hemagglutination antigen was prepared with strain m-3249 in primary chick fibroblast cells by the method described by Darwish and Hammon[1] for Japanese B antigen. Plasmas were acetone extracted and adsorbed with goose red cells, diluted 1:25 to 1:1,600 and tested against 4-8 units of antigen in microtiter plates. A tube-neutralization test of heat inactivated (30 minutes at 56 C) plasmas diluted 1:5 in fetal bovine serum was done in HK with approximately 72 $TCID_{50}$ of WEE virus using a method described previously.[10] Log neutralization indices (LNI) were determined on plasma samples taken 6 days, 6 and 12 weeks after inoculations using serial 10-fold dilutions of virus and 1:5 dilution of plasmas. Virus titers and LNI were computed by the method of Reed and Muench.[7]

RESULTS

Viremia.

Experimental birds received 0.1 ml of inoculum containing 400 $TCID_{50}$ of WEE virus. One to 2.5 logs of WEE virus per 0.1 ml were found in blood cells taken 20 hours post-inoculation in 11 of 12 birds, and at 32 hours post-inoculation, virus was found in the bloods of 7 birds (Table 1). Virus was not detected in samples taken thereafter. Levels and duration of viremia were similar in males and females. Blood specimens from control birds were negative for virus throughcut the study, as were pre-inoculation bloods from experimental birds. Virus was not detected in feces.

Bird 227, which had viremia at 20 and 32 hours, died 56 hours after exposure to WEE virus (Table 1). Virus isolation attempts from brain and spleen of this bird were negative.

Immune Response.

Pre-inoculation plasmas from experimental birds were negative for HI and neutralizing antibodies, and plasmas from control birds were negative throughout the study. No evidence was found of hemagglutinin inhibitor in acetone-extracted plasmas[5] or of non-specific neutralization of virus.

Indications of HI antibody were found 6 days after inoculation with WEE virus, when 6 of 11 experimental birds were positive at the 1:25 plasma dilution. All experimental birds were positive 9 days post-inoculation, and maximum HI titers, as high as 1:400 in some birds, occurred at 3-6 weeks (Table 2). Titers then declined, and only 3 of 9 birds gave evidence of HI antibody 12 weeks after inoculations.

Neutralizing antibody also appeared in the plasmas of experimental birds 6 days after inoculation with virus. Whereas sera obtained 3 days post-inoculation did

not neutralize virus in tube tests, sera taken after 6 days did. Neutralization tests of sera from subsequent bleedings confirmed the presence of neutralizing antibody. LNI of plasma samples averaged 2.7 but went as high as 4.5 in one bird. On the average, LNI were slightly higher in females than in males (Table 2).

TABLE 1. Viremia in bobwhite quail following subcutaneous inoculation with 400 $TCID_{50}$ of WEE virus.

		Virus titer as log $TCID_{50}$/0.1 ml of blood				
		Hours post-inoculation:				
Sex	Bird Number	8	20	32	44	56
males	225	0	1.5	2.5	0	0
	228	0	1.5	3.0	0	0
	230	0	0	0	0	0
	232	0	2.5	2.5	0	0
	260	0	2.5	0	0	0
	262	0	2.5	2.5	0	0
females	226	0	1.0	0	0	0
	227	0	2.5	2.0	0	died
	229	0	2.5	0	0	0
	231	0	1.5	0	0	0
	261	0	2.5	3.0	0	0
	263	0	2.0	2.5	0	0

TABLE 2. Hemagglutination-inhibition (HI) titers and log neutralization indices (LNI) of plasmas from bobwhite quail inoculated with WEE virus.

Time after inoculation		HI titers*			LNI**		
		males	females	both	males	females	both
day	3	0	0	0			
	6	5	7	6	2.7	2.6	2.7
	9	71	50	60			
week	2	63	57	60			
	3	79	114	94			
	4	63	76	69			
	5	63	46	54			
	6	71	66	69	2.3	3.1	2.7
	7	36	57	45			
	8	40	35	37			
	9	63	26	42			
	10	17	8	12			
	12	5	2	4	2.5	2.9	2.7

* Values are geometric means for the reciprocal of the highest plasma dilution inhibiting hemagglutination. Zero indicates no inhibition at 1:25 plasma dilution.

** Values are arithmetic means.

DISCUSSION

Bobwhite quail, 3½ months of age, were susceptible to infection with 400 $TCID_{50}$ of WEE virus. Although viremia was not detected in one bird, HI and neutralizing antibody responses indicated that infection had occurred. Viremias in quail lasted less than 2 days with blood titers of WEE virus up to 10^1 $TCID_{50}$/ml (= approx $10^{2.5}$ $TCID_{50}$/.03 ml). Viremia in chickens responding to a low infective dose of WEE virus (0.6 LD_{50}) also lasts about 2 days with blood titers of virus equal to or less than $10^{1.0}$ LD_{50}/.03 ml.[6] Viremia in English sparrows inoculated with 100 LD_{50} of WEE virus lasts up to 4 days with virus titers as high as $10^{5.5}$ LD_{50}/.03 ml, although titers between $10^{2.5}$ and $10^{1.5}$ LD_{50}/.03 ml are typical.[6] Thus, bobwhite generally circulate less virus in the blood than do chickens or English sparrows.

HI antibody in infected quail disappeared in most birds by 12 weeks post-inoculation. In contrast, chickens infected with WEE virus have shown HI titers of 1:320 a year after inoculation.[6] Therefore, unlike chickens, bobwhite quail cannot be utilized for HI antibody surveys conducted late in the year to determine the extent of virus transmission during the previous summer, since the absence of HI antibody may not mean that quail were not infected.

Neutralizing antibody appeared in bobwhite 6 days after inoculation with virus, and LNI averaged 2.7 (max = 4.5). Neutralizing antibody appears in chickens about 2 weeks after infection with WEE virus, and LNI range from 2.3 to 4.0 after 4 weeks.[6] Hybrid doves *(Streptopelia spp.)* may show LNI of 3.3 by 4 weeks post-infection.[8] Thus, LNI for bobwhite quail fall in the range of values observed for other birds, but average titer of neutralizing antibody is achieved quite early.

This study indicates that bobwhite quail are suitable for use as a sentinel animal to detect the transmission of WEE virus. Their rapid neutralizing antibody response will give evidence of virus transmission a week after infection. Both sexes are susceptible and show similar viremias and antibody responses. Contact transmission is improbable since bobwhite are not aggressive, have a low level and transitory viremia, and apparently do not shed virus in feces. In addition, bobwhite quail tolerate repeated bleedings over long periods of time, are of sufficient size to permit relatively large volumes of blood to be taken at bleedings and are easy to handle and maintain.

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