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## A NOTE ON THE DURATION OF NEUTRALIZING ANTIBODY AGAINST AVIAN ENCEPHALOMYELITIS VIRUS IN CHICKENS

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Nine adult or young chickens of different flocks naturally exposed to avian encephalomyelitis virus were moved to the laboratory at a very early stage of the exposure and kept separately. Then they were observed for the rise and fall of neutralizing antibody against the virus up to 14~34 months after they had been moved. Two birds with the antibody titers of log neutralization indices of 3.0 and 4.5 showed almost the same titers after 21 months. Seven birds with slightly positive or negative antibody titers (0.6~2.0) at initial stage scarcely gave higher titer during the experiment. Two of the birds became negative after the 5th or 11th month of the observation period. Three birds observed for 30~34 months maintained low but significant levels of antibody with or without intermittent appearances of negative titers.

### INTRODUCTION

VAN ROEKEL has stated that neutralizing antibody against avian encephalomyelitis virus (AEV) in chickens may remain at significant levels for at least several months after exposure to the virus. Moreover, it has been described that the antibody may persist for a lifetime despite the absence of actual data based on long term observations. This seems to be due to the relatively short life span of a chicken. Recently, MIYAMOTO et al. revealed that a total of 20 growing or adult chickens of 4 commercial flocks (5 birds per flock) immunized perorally or by contact infection with a live vaccine of AEV (strain 0596) maintained log neutralization indices (NI) ranging from 1.3 to 4.0 for at least 12 months, and 5 birds of a flock naturally infected which were tested at the same time as the vaccinated birds had larger indices (range 2.8~4.5) for at least 12 months. In addition, the naturally infected chickens indicated an increase in the antibody titer at the 10th month of the observation. IKEDA & MATSUDA, in their study on the immunodiffusion test for AE, showed the rise and fall of the antibody in chickens which were injected intracerebrally, subcutaneously or

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perorally with VAN ROEKEL strain of egg-adapted AEV at 40 days of age and kept for 30 months. Several birds were bled arbitrarily for each monthly virus-neutralization test (VNT) from each group of the injected birds. Birds of the intracerebrally injected group yielded log NI of 1.25~3.8 (mean 2.55) for 30 months and those of subcutaneously injected group did log NI of 1.05~3.4 (mean 2.25) for 2 years. On the other hand, birds perorally inoculated showed a delayed rise of the antibody titer (6 weeks post-inoculation) and log NI of 1.03~1.3 (mean 1.07) in some of them and 0~0.9 (mean 0.39) in the remainder. Moreover, all the positive antibody titers had disappeared 16 months after inoculation. Adult chickens of other group administered perorally with the same virus revealed log NI of 0~1.9 (mean 0.92) for at least one year.

This note deals with the rise and fall of the neutralizing antibody in chickens of commercial flocks, which were naturally exposed to AEV, moved to the laboratory in earlier stage of the exposure and kept separately for about 1~3 years.

#### MATERIALS AND METHODS

##### Experimental chickens

The chickens were obtained from flocks A~D. The AE status of these flocks was as follows<sup>6,7</sup>:

Flock A: A flock of about 2,000 meat-type breeder chickens aged about 9 months. Their progeny chicks including flock B described below were affected with AE. The daily egg production rate dropped to the minimum, Jan. 25, 1968. Four breeders (Nos. 1~4 in table) of the flock were moved to the laboratory Mar. 9, 1968.

Flock B: A broiler flock. Hatched Feb. 15, 1968. Brain smears of five 13-day-old chicks showing AE clinical signs were positive by the direct fluorescent antibody technique (FAT). Further 3 chicks which survived AE gave log NI of 2.3, >3.4 and >3.8 respectively at 30 days of age. Fifteen chicks were moved to the laboratory Mar. 8, and kept in wire floor cages in 2 groups. Then 3 (Nos. 5~7) of them were held separately for this study at about 70 days of age.

Flock C: A flock of White Leghorn chickens. Hatched July 3, 1968. Five 7-day-old chicks showing AE clinical signs were checked by the FAT and proved positive. On Aug. 8, 1968, 3 birds of the flock were moved to the laboratory. Finally, one (No. 8) of them was employed for this study.

Flock D: A broiler flock of New Hampshire chickens. Hatched Mar. 11, 1969. Two 19-day-old chicks with AE symptoms were examined by the FAT and proved positive. On April 18, 1969, 16 birds were introduced to the laboratory. Four of them gave log NI of >3.0 with the pooled serum at 74 days of

TABLE *Avian-encephalomyelitis-virus-neutralizing antibody titer (log neutralization index) in chickens infected naturally or inoculated*

FLOCK* <sup>1</sup>	NO. OF BIRD	MONTHS AFTER THE ONSET OF OBSERVATION																																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	19	20	21	22	23	24	25	27	28	29	30	32	33	34			
A 309 (318)	1	1.8	·* <sup>2</sup>	·	·	·	1.7	·	·	·	·	·	·	1.4	Died																				
	2	1.0	1.1	1.8	·	·	·	1.2	1.2	1.4	1.1	1.0	·	·	0.0	·	0.5	Killed																	
	3	1.6	2.0	1.4	1.2	1.0	0.8	0.6	·	0.6	·	0.6	·	·	0.6	·	0.3	Killed																	
	4	0.6	1.4	1.0	·	1.4	·	1.0	·	1.5	·	1.8	·	·	1.2	·	·	1.5	1.0	·	0.5	·	1.5	·	1.5	0.8	·	0.3	1.3	Killed					
B 21 (28)	5	1.0	1.0	0.4	1.0	·	1.0	0.6	0.8	·	0.0	1.2	0.4	·	0.8	0.6	Killed																		
	6	2.0	1.5	0.8	1.8	·	3.0	·	·	1.4	·	·	1.6	·	1.2	·	·	·	·	2.0	·	·	·	·	>1.8	1.5	1.1	1.1	1.0	2.0	0.4	Died			
	7	1.0	1.3	1.8	1.0	·	1.8	·	·	1.3	·	·	1.2	·	·	1.2	·	·	·	1.6	·	·	·	·	>1.3	·	1.5	·	1.5	1.8	·	1.1	Killed		
C 36 (77)	8	3.0	·	·	·	·	·	·	·	·	2.6	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	>2.8	3.5	Died						
D 38 (80)	9	4.5	·	·	·	·	·	·	·	·	·	·	·	>2.8	·	·	·	>2.1	·	·	·	·	·	·	·	·	·	·	·	·	3.7	Died			
Inoculated* <sup>3</sup> (adult)	10	2.5	2.6	·	·	·	·	·	·	·	·	·	·	·	1.6	·	·	·	·	·	·	·	·	·	·	>1.1	·	·	>1.9	·	·	·	>1.8	Killed	
	11	·	·	0.1	0.9	2.0	·	·	·	·	·	·	·	·	1.4	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1.2	Died		

\*<sup>1</sup> Flock from which birds originated

Figures indicate days of age at which birds were moved to the laboratory (days of age at the first test). Nos. 1~4 were moved 43 days after the egg production rate of the flock dropped to the minimum.

\*<sup>2</sup> Bled but not tested

\*<sup>3</sup> No. 10 was intramuscularly injected with a dose of 1 ml (1:5 diluted) of the VAN ROEKEL strain of chick-brain-passed AEV. No. 11 was not injected but kept in an adjoining compartment to No. 10.

Duration of neutralizing antibody against AEV

age. Four of the remainder were held separately for this study, but one (No. 9) of them survived.

Each bird aged about 70 days was moved from wire floor group cage to single compartment of a wire battery. The battery had 4 single compartments with wire floor allowing excreta to fall into a dropping pan and 2 common troughs for feed and water respectively. The birds kept were bled at monthly intervals and tested for the VNT.

#### Virus-neutralization test (VNT)

The test was made according to CALNEK & JEHNICH<sup>1)</sup>, but incubation of the mixtures of serum and AEV was done at 37°C for 60 min. Fifty percent of embryo-infective dose (EID<sub>50</sub>) was calculated by the method of BEHRENS-KÄRBER. A log NI of 1.1 or greater was considered as positive<sup>2)</sup>.

#### RESULTS AND DISCUSSION

Birds Nos. 8 and 9 which had larger log NI (3.0 and 4.5 respectively) at the initial stage gave almost the same antibody titers after 21 months. On the other hand, Nos. 5~7 revealed smaller log NI throughout the experiment. No. 5 gave a positive titer only at the 11th month of observation so far as the test was made. The other 2 chickens yielded persistently positive titers for at least 32 and 34 months respectively, but their antibody titers were low. As described in "MATERIALS AND METHODS", 3 birds which showed AE clinical signs at 13 days of age showed relatively greater log NI at 30 days of age. These birds seemed to have been egg-infected or infected at early days of life<sup>3)</sup>. Therefore, Nos. 5~7 birds might be infected later or slightly.

Adult breeders (Nos. 1~4) which had been kept for 43 days in the flock after the daily egg production rate dropped to the minimum gave positive but only smaller log NI. Two (Nos. 2 & 3) of them revealed only negative neutralizing antibody titers after the 11th and the 5th month of observation respectively as far as the test was carried out. Recently CHOI & MIURA described briefly that the indirect FAT could detect the AE antibody in chickens which had an NI of less than 1.1 at the early or the later stage of infection. They demonstrated the AE antibody by the test in Nos. 2 and 3 birds at the 14th month, but could not at the 16th. No. 4 bird repeated negative titers during the observation period of 30 months.

No. 11 had been held in an adjoining compartment to No. 10 infected heavily with a laboratory strain of AEV. They were separated by a wire partition. However, No. 11 indicated the first significant level of antibody titer 5 months after it came under observation. This appears to show that, under the experi-

mental conditions used, contact infection or reinfection might not take place so easily. As described previously, both adult chickens and their progeny indicated persistently low titers of the neutralizing antibody throughout the experiment. It remains unsolved whether the persistence of low antibody titers or the disappearance of the antibody may be observed only under the laboratory conditions, where reinfection is infrequent, or also in the farm environment, where sub-clinical infection occurs frequently.

The present data appeared to demonstrate that the virus-neutralizing antibody titers obtained initially were almost parallel to those of the lasting antibody. This seems to mean that the intensity of the initial antigenic stimulation may relate to the persisting antibody. Since the observations are based on small numbers of experimental chickens, further experiment is necessary to confirm the findings.

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