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ANTIBODIES IN HUMAN AND MONKEY SERA TO HERPES-TYPE VIRUS FROM A CHICKEN WITH MAREK'S DISEASE AND TO EB VIRUS DETECTED BY THE IMMUNOFLUORESCENCE TEST

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SUMMARY Sera were obtained from 72 subjects whose work was intimately connected with chickens, and from 72 office workers who did not have much contact with chickens. The antibody activities of these sera to herpes-type virus (HTV) (Biken C strain) isolated from a chicken with Marek's disease and to Epstein-Barr (EBV) were examined by the indirect immunofluorescent technique. The frequency distributions of the antibody titers to chick-HTV and to EBV were higher in sera of subjects working with chickens, than in sera of office workers.

Sera obtained from monkeys contained little or no antibody to chick-HTV or EBV.

INTRODUCTION

Antibody titers to chick-HTV from a chicken with Marek's disease, have been examined by the agar gel double diffusion technique (Chubb and Churchill, 1968; Yuasa et al., 1969; Purchase and Burgoyne, 1970) and by the indirect immunofluorescent antibody (IF) technique (Naito et al., 1970; Purchase and Burgoyne, 1970). Naito et al. (1970) demonstrated antibody activity against chick-HTV in human sera by the indirect IF technique, and also antibody activity against EBV in chick sera. This is probably due to a cross reaction due to an antigenicity in common to chick-HTV and EBV antigens found by the agar gel diffusion test, as described by Ono et al. (1970). Naito et al. (1970) also reported higher titers of antibody to chick-HTV antigen in sera from 8 workers on a poultry farm than in those of 53 other individuals whose work was not connected with chickens.

To confirm this, we titrated the antibodies to chick-HTV and to EBV antigens by the indirect IF technique in sera of 72 subjects working with

chickens and of 72 office workers. The antibody activities to chick-HTV and to EBV antigens in the sera of monkeys were also examined.

MATERIALS AND METHODS

1. *Virus antigens*

1) Preparation of chick-HTV antigen

The preparation of duck embryo fibroblasts (DEF) infected with chick-HTV (Biken C strain) was described previously (Kato et al., 1970). Antigen for the indirect IF test was prepared as follows; DEF infected with chick-HTV (Biken C strain) were dispersed in Leighton tubes with coverslips. When typical focal lesions were well developed, the coverslips were removed, rinsed in phosphate buffered saline (PBS), pH 7.4, dried at room temperature, and fixed in cold acetone for 15 min.

2) Preparation of EBV antigen

The P3HR–1 subline of the P3J (Jiyoye) Burkitt's cell line was supplied by Dr. Y. Hinuma (Tohoku University). Cells were grown in Eagle's minimum essential medium containing 20% calf serum at 37 C for 4 days and for virus production the cells were transferred to 33 C. Cultures were incubated at 33 C for 7 days and always yielded 10 to 20% immunofluorescent positive cells. The cells were washed five times with PBS and suspended in PBS at a final concentration of approximately 10^6–10^7 cells/ml. A drop of the suspension was smeared on a coverslip, dried at room temperature and fixed in cold acentone for 15 min.

2. *Human sera*

Sera were obtained from 72 subjects working with chickens in four different poultry farms and control sera were obtained from 72 office workers who had little contact with chickens. The distribution of the ages of subjects in these two groups is shown in Table 1. All sera were stocked at −20 C before examination.

3. *Monkey sera*

Forty eight sera from Cynomolgus monkeys were obtained from the Kanonji Institute of the Research Foundation for Microbial Disease of Osaka University. These sera were stocked at −20 C before use.

TABLE 1. *Age distribution of subjects*

Age	Workers in poultry farms	Controls
10~19	7	10
20~29	20	35
30~39	10	11
40~49	15	14
50~59	17	2
60~	3	0
Total	72	72

4. *Indirect immunofluorescent antibody technique for titrations of the antibody levels to chick-HTV and to EBV.*

The general procedure of the technique was described previously (Naito et al., 1970). Cells on coverslips which had been fixed with aceton were allowed to react with serial four fold dilutions of human or monkey serum in PBS (pH 7.4), usually starting at a dilution of 1 : 10. Then they were allowed to react with fluorescein isothiocyanate-conjugated antibodies. Preparation of fluorescent rabbit anti-human globulin was described previously (Naito et al., 1970). Rabbit anti-monkey globulin for preparation of a conjugate was supplied from the Kanonji Institute of the Research Foundation for Microbial Disease of Osaka University. The conjugate was prepared according to Kawamura's procedure (Kawamura, 1969). Titers are expressed as reciprocals of the highest dilution of serum which gave fluorescence.

RESULTS

The antibody levels in the two groups of human sera against the antigens of chick-HTV and EBV were titrated simultaneously by the indirect IF technique. The frequency distributions of the anti-chick-HTV antibody titers of the sera are shown in Fig. 1. The difference in the frequency distributions in sera of workers in poultry farms and control sera was maximal when the percentage of sera with titers of above and below 1 : 160 were compared. Similar results were obtained on the frequency distributions of the anti-EBV antibody titers. Thus the

frequency distributions of the antibody titers to chick-HTV and to EBV of sera of workers in poultry farms were higher than those of sera of control office workers. The age disitributions of anti-chick-HTV and anti-EBV antibodies in subjects with positive reactions for IF antibodies are shown in Fig. 2 and 3. These figures show

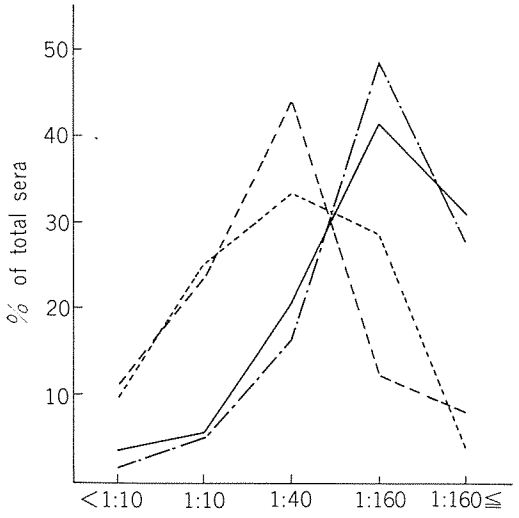

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FIGURE 1. *Distributions of anti-chick-HTV and anti-EBV titers in sera of workers in poultry farms and control subjects.*
———— *Anti-chick-HTV of workers in poultry farms,*
— — — — *Anti-chick-HTV of controls,*
— • — • — *Anti-EBV of workers in poultry farms,*
..... *Anti-EBV of controls.*

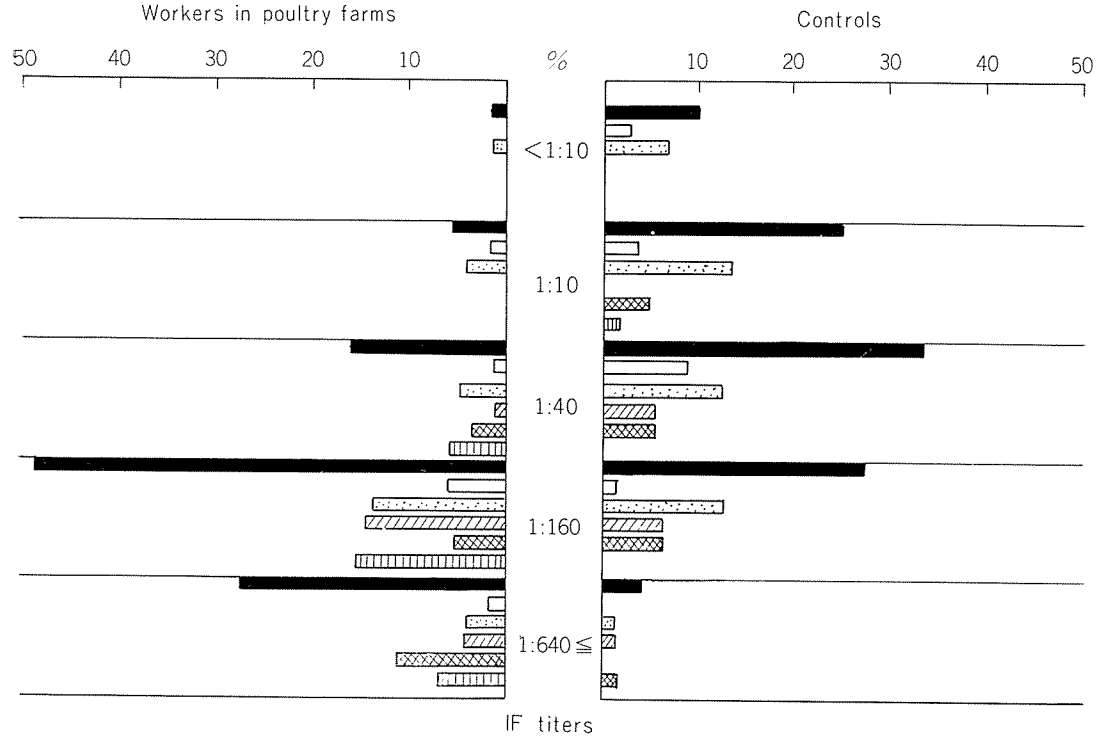

FIGURE 2. *Age distributions of anti-chick-HTV antibodies in subjects with IF antibody.*
Total *10–19 years,* *20–29 years,* *30–39 years,*
40–49 years, *50–years.*

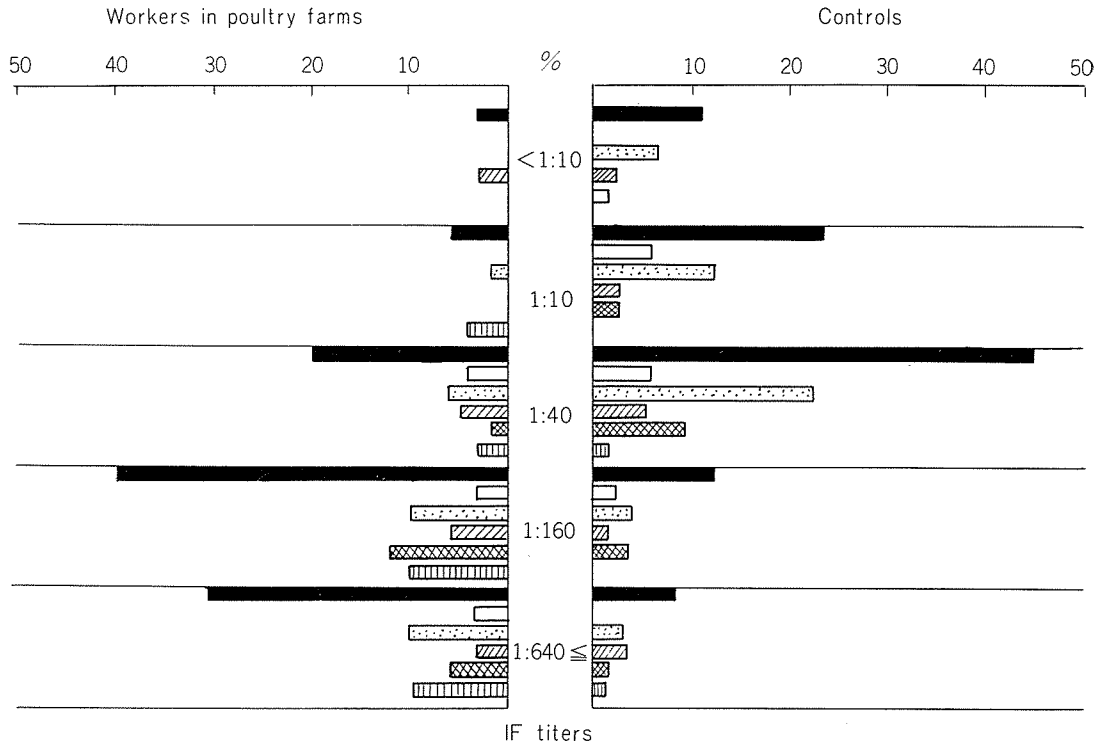

TABLE 2. *Distributions of anti-chick-HTV and EBV titers in monkeys*

Antigen	Anti-chick-HTV and EBV titers					Total
	<1:10	1:10	1:40	1:160	1:640≦	
chick-HTV	36	10	1	1	0	48
EBV	25	18	5	0	0	

that the frequency distributions of anti-chick-HTV and anti-EBV antibodies are similar in each age group. Sera from 70.9% and 76.4% of the workers in poultry farms had anti-chick-HTV and anti-EBV antibody titers of 1 : 160, while only 20.8% and 32.0% of the control sera had (Fig. 4).

The antibody activities of monkey sera against chick-HTV and EBV antigens were also examined. As shown in Table 2, the sera had little or no antibodies against the two antigens.

▲

FIGURE 3. *Age distributions of anti-EBV antibodies in subjects with IF antibody.*
Total, 10–19 years, 20–29 years, 30–39 years, 40–49 years, 50– years.

DISCUSSION

The present data have confirmed the unusual frequency of high levels of antibody to chick-HTV and to EBV antigen of workers in poultry farms, as compared to those of control subjects. Kato et al. (1970) and Konobe et al. (1971), reported that there was no evidence of infection of cultured mammalian cells including human cells, and several species of mammals including monkeys, by chick-HTV. Therefore, it is unlikely that chick-HTV infects man and induces antibody. Calnek et al. (1969) and Purchase (1970) demonstrated many enveloped

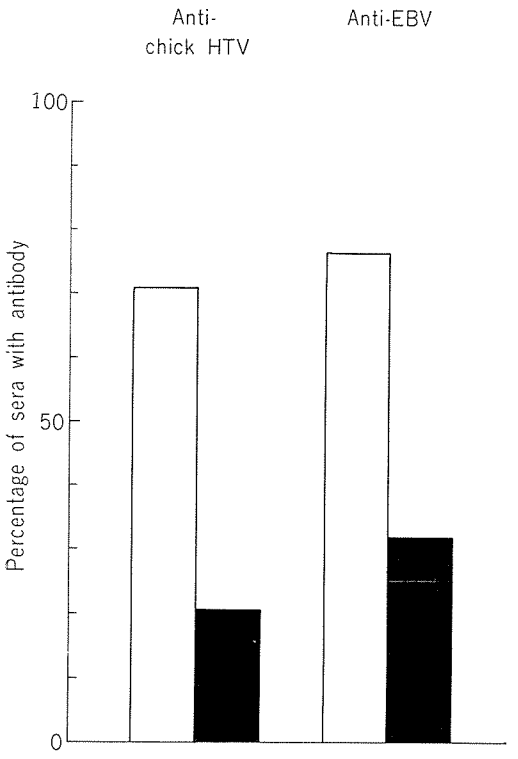

FIGURE 4. *Percentage of sera with antibody titers to chick-HTV and EBV of 1 : 160 or more.*
□ *Workers in poultry farms (72 cases),*
■ *Controls (72 cases).*

herpes virus particles in the epithelium of feather follicles of chickens with Marek's disease. The high levels of antibody to chick-HTV in the sera of workers in poultry farms may be due to repeated antigen stimulation from inhaled dust derived from the epithelium of feather follicles of chickens with Marek's disease, since there were numerous cases of the disease on the poultry farms. Unexpectedly the exact parallelism between the antibody levels to EBV and chick-HTV could not be observed, inspite of the suspect of the common antigenicity between them.

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