ANTIBODIES AGAINST RETICULOENDOTHELIOSIS VIRUS AND CHICKEN ANAEMIA AGENT IN CHICKEN SERA

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SUMMARY: Antibodies against reticuloendotheliosis virus and chicken anaemia agent were examined by agar gel precipitin test and indirect immunofluorescence antibody test in several commercial and government poultry farms in Malaysia. Nine (5.7%) out of 159 sera were found to possess antibodies against REV and ten (20.4%) out of 49 sera were positive for chicken anaemia agent antibodies. The results indicated serological evidence of the two diseases in the country.

Key words: reticuloendotheliosis, chicken anaemia, serological survey

INTRODUCTION

Reticuloendotheliosis virus (REV) was isolated from turkeys by Robinson and Twiehaus (1974). This virus caused marked reticuloendotheliosis following experimental inoculation into young chickens, turkeys and Japanese quails resulting in high mortality and affected birds had markedly enlarged livers and spleens (Sevoian *et al.*, 1964; Theilen *et al.*, 1966). Natural infection with REV is uncommon and there is no known economic significance. Yuasa *et al.* (1976) reported evidence of a disease caused by vaccine contamination with REV in chicken flocks inoculated with Marek's disease vaccine produced by certain manufacturers in various areas in Japan. Retarded growth, anaemia, ruffled feathers and leg paralysis were observed in the affected chickens. In some countries, significant mortality among turkeys with tumorous enlargements had been attributed to REV. However, in other countries many infected flocks with high serological evidence appeared to have no clinical signs of the disease or obvious performance problems (Witter *et al.* 1982). Witter *et al.* (1982) found serological evidence of infection in about 20 percent of layer and broiler breeder flocks in the United States of America. A subsequent report by the same authors showed that REV was isolated from blood and litter samples (Witter *et al.* 1985).

Chicken anaemia agent (CAA) is a transmissible and filterable agent that produces aplastic anaemia in chicks (Taniguchi *et al.*, 1982, 1983; Yuasa *et al.*, 1979). It was first isolated by Yuasa *et al.* (1979). Besides Japan, isolation of CAA has been reported in Germany (Bulow *et al.*, 1983). It is evident that CAA is widespread in commercial chicken flocks in Japan, since it can be isolated readily (Yuasa *et al.*, 1979; 1983) and the antibody is detectable at a high rate (Yuasa *et al.*, 1985). CAA induces severe haemapoietic and lymphoid damage, which may result in aplastic anaemia in chicks (Taniguchi *et al.*,

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1982, 1983; Yuasa *et al.*, 1979). CAA may play a role in the occurrence of anaemic diseases in the field; however, its economic impact on commercial chickens has not been well studied.

This report describes a serological survey of sera obtained from several commercial and government poultry farms in Malaysia in 1987 for the presence of antibodies against REV and CAA. The existence of these two viruses in Malaysia have not been confirmed.

MATERIALS AND METHODS

A total of 159 serum samples from five flocks was examined. The serum samples were collected from commercial and government farms in different parts of the country (Table 1).

TABLE 1
Identification and sources of test samples

Flock number	Source (Farm)	State	Type	No. of sera collected
1	Government	Perak	Broilers	37
2	Government	Sarawak	Breeders	16
3	Government	Perak	Layers	20
4	Private	Perak	Broilers	17
5	Private	Johor	Breeders	67
		Perak	Breeders	2
Total				159

Detection of antibody against REV

Agar gel precipitin (AGP) test was used for primary screening of the serum samples. The antigen for the AGP test was prepared from REV–T strain which was grown in chick embryo fibroblast (CEF) cultures with the absence of calf sera and tryptose phosphate broth in the culture media. The AGP test was carried out using two to four units of the antigen with agar gel, which contained eight percent NaCl and one percent Bacto agar (Difco) in phosphate buffer, on standard glass slides. A centre well and six surrounding wells of four mm diameter and four mm apart were made on the agar. About 30 ul of the antigen or serum was placed into each well, respectively, and observation for precipitin lines was made after three days incubation in a humidified chamber at room temperature.

Antibodies were also detected by an indirect immunofluorescence antibody (IFA) test to confirm the results of the AGP test, using antigen prepared on coverslip cultures infected with REV–T strain as described previously (Yuasa *et al.*, 1976). The coverslip cultures were stained conventionally with primary test serum and then with FITC conjugated anti-chicken IgG rabbit IgG (Miles Lab. Inc., USA) at 37°C for 40 minutes, respectively. The test serum was examined at 1:10 dilution.

Detection of antibody against CAA

The immunofluorescence antibody (IFA) test for detection of antibodies against CAA was according to the procedure described previously (Yuasa *et al.*, 1985).

RESULTS

Detection of antibody against REV

A total of nine (5.7%) sera were positive for antibodies against REV on AGP test and IFA out of 159 serum samples (Table 2). The specificity of the AGP test is shown in Fig. 1. The precipitin line between the antigen and test serum was continuous with that pro-

TABLE 2
Detection of antibody against reticuloendotheliosis virus

Flock number	No. of sera tested	No. of positive sera	
		AGPT	IFA
1	37	0	0
2	16	0	0
3	20	6	6
4	17	0	0
5	69	3	3
Total	159	9 (5.7%)	9 (5.7%)

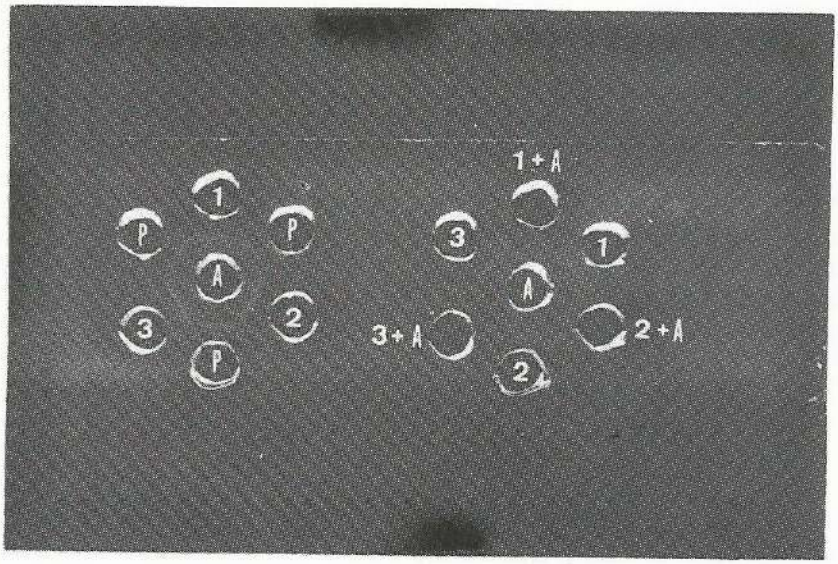

FIG. 1: Left template: Continuous precipitation line observed when test and positive control sera were placed in wells alternately with the antigen in the centre
A; antigen; P: positive control serum; 1,2,3: test sera
Right template: Each of the three test sera showed no precipitation line when neutralised with REV antigen
A; antigen; 1,2,3: test sera
1+A, 2+A, 3+A: test sera neutralised with REV antigen

duced between the antigen and control positive serum when the wells are adjacent to each other. The reaction of the positive serum disappeared when the antigen is added to the serum. These positive sera were from flocks number 3 and 5. The positive sera were later subjected to IFA test and found to be positive for all the samples (Fig. 2).

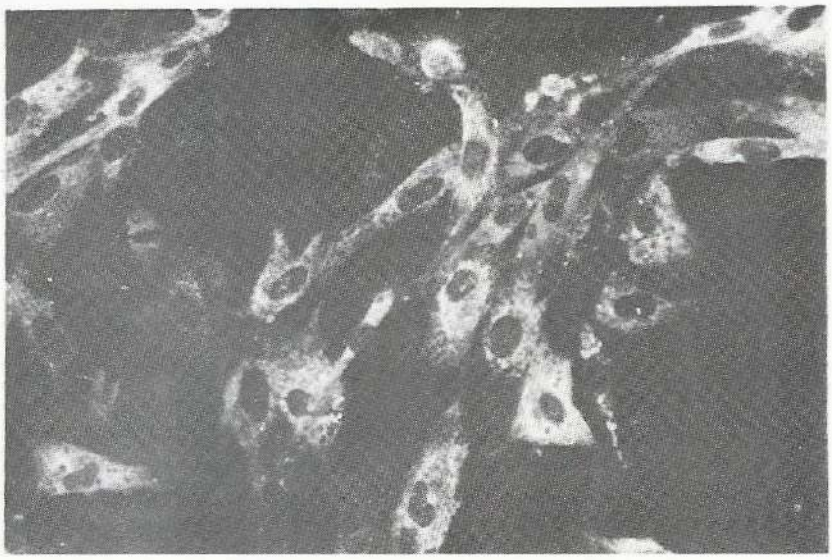

FIG. 2: Specific fluorescence in CEF cultures infected with REV–T strain stained with positive test serum by IFA test (x300)

Detection of antibody against chicken anaemia agent by IFA test

A total of ten (20.4%) sera were positive out of 49 sera (Table 3). These positive sera were from flocks number 1 and 3. Specific fluorescence in MDCC–MSBI cells infected with CAA is shown in Fig. 3.

TABLE 3
Detection of antibody against CAA by IFA test

Flock number	No. of sera tested	No. of positive sera
1	20	3
2	10	0
3	9	7
5	10	0
Total	49	10 (20.4%)

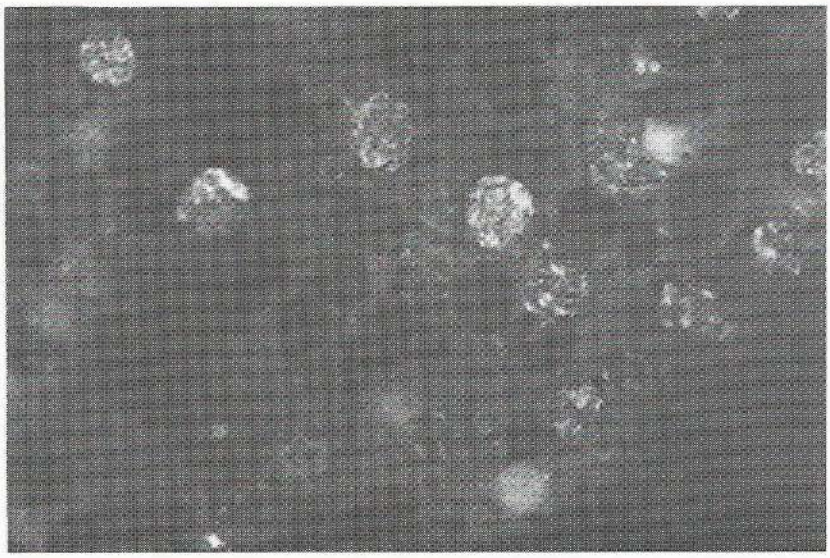

FIG. 3: Specific fluorescence in MDCC–MSBI cells infected with CAA stained with positive test serum (x600)

DISCUSSION

The test results indicated the first serological evidence of REV and CAA infection in several chicken flocks in Malaysia, although only a limited number of sera were tested in this study. The incidence of the serological positive reactors, 5.7 percent for REV and 20.4 percent for CAA, were low as compared to results obtained by researchers in countries like the United States of America and Japan. From a serological survey of REV infection among chickens in Japan, Wakabayashi *et al.* (1977) detected 4.3 percent positive reactors in 1973, 8.9 percent in 1974, 2.4 percent in 1975 and 7.0 percent in 1976. Yuasa *et al.* (1985) showed that 357 (93.7%) out of 381 serum samples were positive for antibody against CAA in breeder flocks in Japan. Witter *et al.* (1982) found serological evidence of REV infection in about 20% of layer and broiler breeder flocks in the United States of America. The presence of serological evidence of these two diseases in Malaysia indicated that further work is needed to be carried out to detect for the presence of clinical cases in the field with positive isolation of the viruses. The epidemiology of these two diseases and their economic importance in commercial poultry farms needs further study.

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RINGKASAN

ANTIBODI TERHADAP VIRUS RETICULOENDOTHELIOS (REV) DAN CHICKEN ANAEMIA AGENT (CAA) DIDALAM SERA AYAM.

Antibodi menentang virus reticuloendotheliosis dan agen anaemia ayam telah dipereksa dengan cara ujian agar gel precipitin (AGPT) dan indirect immunofluorescence antibodi (IFA) di beberapa ladang ternakan ayam swasta dan kerajaan di Malaysia. Sembilan (5.7%) daripaca 159 contoh-contoh sera didapati mengandungi antibodi terhadap REV dan sepuluh (20.4%) daripada 49 contoh sera didapati positif untuk antibodi CAA. Hasil daripada kajian ini menunjukkan bukti-bukti serologi terhadap kedua-dua penyakit tersebut dalam negara ini.