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SHORT COMMUNICATION

DETECTION OF CHICKEN ANAEMIA VIRUS IN FLOCKS OF COMMERCIAL CHICKEN IN MALAYSIA

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SUMMARY

Studies were carried out to determine the presence of chicken anaemia virus (CAV) in Malaysia. Serum and organ samples collected from local poultry farms showed the presence of CAV antibody and antigen respectively. Antibody against CAV was detected by means of CIA ELISA kit, while the CAV antigen was detected by indirect immunofluorescent and immunoperoxidase staining.

Keywords: Chicken anaemia virus, poultry, antibody and antigen detection.

Chicken anaemia virus (CAV) a single-stranded DNA virus which was first described by Yuasa *et al.* in 1979 is now reported in many countries and believed to be a major causative agent of anaemic and immunosuppressive disease in chicken (Mc Connell *et al.*, 1993). The virus which has now been grouped under *Circoviridae* (Fenner *et al.*, 1993) causes a syndrome in susceptible chickens characterised by increased mortality and anaemia. It has been associated with severe anaemia, aplasia of the bone marrow and atrophy of the lymphoid system (Yuasa *et al.*, 1979; 1983). Often, secondary viral, bacterial or fungal infections complicate the course of the disease.

Chicken anaemia virus infection can cause a mortality of 10% to 30% in disease outbreaks and reduce performance of infected flocks due to subclinical infections. Because of its immuno-suppressive effect, it can be involved in multifactorial disease outbreaks (Mc Nulty *et al.*, 1991) and may be the cause of vaccine failures, e.g., with Marek's disease vaccines (Otaki *et al.*, 1988; 1989).

Many serological surveys done on CAV in other countries showed a high prevalence of antibody to CAV in older birds both in laying and meat type breeds (Mc Nulty *et al.*, 1988). Specific pathogen free (SPF) flock infections have the potential to lead to vaccine contamination since CAV can be vertically transmitted (Yuasa *et al.*, 1983).

In Malaysia the detection and isolation of this virus has not been reported. Therefore, this study was carried out to determine the presence of CAV in Malaysian flocks of chicken. Serum and organ samples were collected for antibody and antigen detection respectively.

A total of 322 serum samples were randomly collected from layers, broilers and parent stock of different ages from thirteen farms in three states of

U.K.) was used to detect the presence of CAV antibody. Hyperimmune antiserum to CAV was generously provided by Professor A.E. Castro from Pennsylvania State University prepared in a chicken.

Organs, such as liver and spleen from SPF chickens were taken as a control group. Immediately after hatching, the chicks were slaughtered and organs were fixed in Bouin's fixative for 18 h and processed by standard paraffin embedding methods. Blood was also collected from these chicks and ELISA test was conducted to ensure that these chicks were free from Newcastle disease (KPL test kit), infectious bursal disease (IDEXX test kit) and chicken anaemia virus (Guildhay/Limited, U.K.).

Liver and spleen from chickens positive for CAV antibody were taken and fixed in Bouin's fixative for 18 h and further processed by standard paraffin embedding methods. Positive and negative control tissues were processed at the same time.

Indirect immunofluorescent staining for tissue section (Mc Neilly *et al.*, 1991) was carried out. Tissue sections were dewaxed in xylene with two changes at 4 min each and then transferred into absolute ethanol. They were then fixed in 1:1 (methanol : acetone) for another 5 min and rehydrated through graded ethanol. This was followed by incubation with 1/10 dilution in phosphate buffered saline (PBS) pH 7.2 of the CAV hyperimmune serum in a humidified chamber for 1 h at 37°C. After two 5 min washes in PBS, they were further incubated for 1 h at 37°C with FITC-labelled goat anti-chicken (Kirkegaard & Perry Laboratories Inc.) diluted 1/60 in PBS, and following a further 10 min wash, mounted in buffered glycerol-saline. They were then examined under incident ultraviolet illumination.

To process the tissue sections for indirect immunoperoxidase, a combination of the methods

paraffin embedded sections of infected and control samples were subjected to dewaxing in xylene for two changes of 4 min each. This was followed by transferring the sections to absolute ethanol before their indigenous peroxidase activity was inhibited by 0.3% H_2O_2 in methanol. The sections were then rehydrated in graded ethanol and incubated in blocking buffer (3% BSA in 0.05M Tris buffer pH 7.6) for 15 min. They were then incubated with 1/10 dilution of CAV hyperimmune serum in 1% BSA in Tris buffer. After washing 3 times with Tris buffer, the sections were further incubated with horse-radish peroxidase conjugated IgG for another 1 h. Then 4-chloro-1-naphtol substrate was added after washing, onto the section and incubated at room temperature for 10-40 min or until blue-black reaction developed. Sections were then mounted by buffered glycerol-saline and examined under light microscope.

In this study, CAV antibody could be detected in 218 sera out of 355 sera tested (61.41%). These positive sera for CAV antibody were detected in nine different farms out of thirteen farms chosen (69.23%). The antibodies were detected in all age groups as young as day old chicks to as old as 70 weeks old. CAV antibody was detected in layers, broiler and parent stock flocks in Malaysia. The highest antibody titre found in the positive flocks based on ELISA test was 7112 and the lowest antibody titre was 943. The individual titre of a farm were shown in Table 1.

Table 1. Chicken anaemia virus antibody titre of 13 farms

Farms	Location of birds	Types	Breed	No. of samples	Age	High titre	Low titre	Types of mgt.	No +ve
1	Lenggeng, N. Sembilan	Broiler	Ayam kampung	30	44 days	-ve	-ve	slatted floor	0
2	Linggi, N. Sembilan	Broiler	Arbor Acre	10	10 days	-ve	-ve	slatted floor	0
3	Ulu Langat, Selangor	Broiler	Ayam kampung	30	young (15) adult (15)	4869 4373	1509 943	free range	10 15
4	Semenyih, Selangor	Layer	Hisex Brown	60	448-476 days	6517	1201	battery cages	40
5	Sg. Merab, Selangor	Mix	Unknown	7	60-90 days	4079	1358	free range	7
6	Serdang, Selangor	Layer	Hisex Brown	30	420 days	6998	2217	battery cages	15
7	Serdang, Selangor	Broiler	Unknown	2	40 days	2528	2353	deep litter	2
8	Muar, Johor	Parent stock	Arbor Acre	84	Adult	7112	952	deep litter	79
9	Muar, Johor	Broiler	Arbor Acre	14	Day-old	5922	1079	deep litter	10
10	Ulu Langat, Selangor	Broiler	Avian	5	28 days	-ve	-ve	slatted floor	0
11	Kuala Langat, Selangor	Broiler	Avian	4	21-43 days	-ve	-ve	slatted floor	0
12	Kuala Selangor, Selangor	Broiler	Avian	40	25 days	6465	943	slatted floor	18
13	Kuala Selangor, Selangor	Broiler	Arbor Acre	40	30 days	6056	1004	slatted floor	22

Antigen of chicken anaemia virus was detected in the nuclei and cytoplasm of the hepatocytes from two positive chickens by both the immunofluorescent and immunoperoxidase staining methods. The nuclei of the hepatocytes were stained intensely while the cytoplasm was lightly stained. In the negative control group no positive reaction was detected in any tissue using immunofluorescent or immunoperoxidase test. All tissue sections from negative control birds were free of any specific staining. The spleen section from the positive case group did not show any positive reaction or specific staining.

From these findings, the results confirmed that Malaysian chicken flocks are not free from chicken anaemia virus and the prevalence of chicken anaemia virus infection is quite high.

Epidemiological studies and isolation of CAV are in progress.

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RINGKASAN

PENGESANAN VIRUS ANEMIA AYAM DALAM KELOMPOK AYAM KOMERSIAL DI MALAYSIA

Beberapa kajian telah dijalankan untuk menentu kewujudan virus anemia ayam (CAV) di Malaysia. Sampel serum dan organ dikumpul daripada ladang ayam-itik dan telah menunjukkan wujudnya masing-masing antibodi dan antigen CAV. Antibodi terhadap CAV telah dikesan melalui CIA ELISA kit, sambil antigen CAV pula dikesan melalui pewarnaan imunopendarfluoran tak langsung dan imunoperoksidase.