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CHICKEN ANAEMIA AGENT IN EGYPT: I. A SEROLOGICAL SURVEY OF ANTIBODY AGAINST CHICKEN ANAEMIA AGENT IN SOME COMMERCIAL CHICKEN FLOCKS USING INDIRECT IMMUNOFLUORESCENT TECHNIQUE.

BY

M. M. ZAKI * and A. A. EL-SANOUSI **.

* Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt.
** Department of Virology, Faculty of Veterinary Medicne, Cairo University, Giza 12211, Egypt

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SUMMARY

A serological survey of antibodies against chicken anaemia agent (CAA) in some commercial chicken flocks was conducted by indirect immunofluorescence technique (IIF) using MSBl cells (lymphoblastoid cell line from marek's disease lymphoma) infected with CAA as a source of antigen, CAA antibody could be detected in 103 out of 146 serum samples collected from broiler breeder, layer and day-old broiler flocks with an incidence of 70%. The highest incidence of positive sera was recorded in broiler breeders (78.12-100%) followed by day-old broilers (57.14-83.33%) and layers (54.54-80%). Further detailed investigations are required in order to find out the situation of the disease in different commercial chicken flocks concerning isolation and characterization of CAA in Egypt.

INTRODUCTION

Chicken anaemia agent (CAA) the cause of chicken infectious anaemia (CIA) was first detected in Japan by Yuasa et al. (1979). CAA induces severe haematopoietic and lymphoid damage, which may result in aplastic anaemia in chicks (Taniguchi et al., 1982 and 1983; Yuasa et al., 1979), CAA has been isolated from chickens in Japan (Yuasa et al., 1979), Germany (Bulow et al., 1983), Sweden (Engstrom, 1988), the UK (McNulty et al., 1990), the USA (Lucio et al., 1990) and Australia (Firth and Imai, 1990). Serological surveys have demonstrated the presence of CAA antibodies in commercial chicken flocks in England and Northern Ireland (McNulty et al., 1988), Japan (Yuasa et al., 1985) and the USA (Lucio et al., 1990), as well as in specific-pathogen-free (SPF) flocks in Japan, England and the USA (McNulty et al., 1988; Yuasa et al., 1985). CAA antibodies could be demonstrated by serum neutralization (SN) (Yuasa et al., 1983) or indirect immunofluorescent technique (IIF) (Yuasa et al., 1985). The IIF technique described by Yuasa et al., (1985) using CAA - infected MDCC-MSBl cells, as antigen, offered considerable savings in cost and time and has a similar level of sensitivity to the SN test (McNulty, 1991),. McNulty et al., (1988) found that the optimal serum dilutions to be used in IIF were 1:100 and 1:500 because a non-specific staining was observed at lower dilutions (1:10 to 1:40). In addition, a prozoning effect was observed with some sera which were weakly positive or negative at a 1:100 dilution although they gave strongly positive reaction at 1:500 dilution. Although CAA could not be propagated in standard cell cultures of chicken origin, it grows in some lymphoblastoid cell (MDCC-MSBl) lines derived from Marek's disease lymphoma which can be used for CAA isolation, titration and neutralization test (Yuasa, 1983).

The present paper describs a serological survey of antibodies against chicken anaemia agent (CAA) in sera of some commercial chicken flocks by indirect immunofluorescent technique using MDCC-MSBl cells infected with reference CAA

strain as a source of antigen.

MATERIALS AND METHODS

Cell culture:

MDCC-MSBl cell line were obtained from Dr. M. S. McNulty (Veterinary research Laboratories, Stormont, Belfast BT4 3SD, Northern Ireland). The cells were cultured in RPMI 1640 (Sigma), supplemented with 5% foetal calf serum then incubated at 38°C in 10% CO2 tension. The method for the propagation of CAA in MSBl cells was described by Yuasa (1983).

Chicken anaemia agent:

Cux-1 strain of CAA identified by Bulow et al., (1983) was obtained from Dr. M. S. McNulty (Veterinary Resaerch Laboratories, Stormont, Belfast BT4 3SD, Northern Ireland) to be used to infect MSB1 cells.

Preparation of CAA antigen for IIF:

The preparation of CAA antigen to be used in IIF was described by Lucio et al. (1990). MSBl cells were infected with different dilutions of Cux-1 virus (10^1 to 10^5) by inocculation of 0.5 ml virus dilutions into separate flasks seeded with 25ml of media containing $3x10^3$ MSB1 cells per ml. Infected MSB1 cells were harvested at 48 hr post-inoculation and washed three times with PBS. Ten µl containing 100,000 infected MSB1 were placed in each well of a Teflon-covered glass microscope slide (Flow multiwell slides). Uninfected control smears were prepared with 50,000 uninfected MSB1 cells per well on the same slide. The slides were left at room temperature to be dried then fixed by acetone for 10 minutes at room temperature. Fixed slides were stored at -20 C until used.

Indirect immunofluorescent technique:

Ten µl of field chicken serum, diluted 1:100 to 1:500 in PBS, were placed on CAA-infected MSB1 cells and uninfected cells. CAA-positive ad negative sera (kindly provided by Dr. B. Lucio, Department of Avain and Aquatic Animal Medicine, New York College of veterinary Medicine, Cornell University, Ithaca, NY 14853, USA) were used as positive and negative control for each slide. The sildes were incubated for 30 minutes at room temperature in a humid chamber, then the slides were washed in PBS for 30 minutes at room temperature. Ten µl of 1:10 rabbit anti-chicken IgG conjugated with FITC (fluorescein isolthiocyanate) was applied to the cells and incubated for 30 minutes at room temperature. The slides were then washed in PBS for 30 minutes then mounted with trisglycerol (pH 8.5). Serum samples were considered positive when clear fluorescence granules were detected in the nuclei of CAA-infected MSB1 cells with no fluorescence in uninfected control cells (Lucio et al., 1990).

RESULTS

Detection and percentual distribution of CAA antibodies in chicken sera:

CAA antibodies could be demonstrated in the sera obtained from broiler breeders, layers and day-old broilers which did not show any observable clinical disease. Examined sera exhibited specific reactivities in the IIF test, where table 1 documents the postive reactions given by sera collected from all tested flocks with average incidence of 70%. Broiler breeders showed positivity ranged from 78.12% to 100%, while day-old broiler chickes exhibited a positivity ranged from 57.14% to 83.33%. Tested layers, however, gave positive reactions ranged from 54.54% to 80%.

Mode of immunofluorescence reaction given by positive sera:

MSB1 cells infected with CAA (CUX-1 strain) showed specific reactivities with chicken sera containing specific anti-CAA antibodies. The IIF reactions exhibited itself either in the form of intranuclear granular (Fig. 1) or diffused (Fig. 2) fluorescence as compared with negative control serum (Fig. 3).

Table (1):Distribution and percentage of CAA antibodies in chicken sera.

Farm serial No.	Type of birds	Age	No. positive sera/ Total No.	Percetage of positives
1	Broiler Breeders	34 Week	10/10	100.00%
2	"	40 week	25/32	78.12%
3	"	35 week	10/12	83.33%
4	White Layers	36 week	4/7	57.14%
5	"	19 week	6/11	54.54%
6	Brown Layers	1 week	8/10	80.00%
7	Broiler chicks	Day-old	4/7	57.14%
8	"	"	12/15	80.00%
9	"	"	4/5	80.00%
10	"	"	5/6	83.33%
11	"	"	3/5	60.00%
12	"	"	3/4	75.00%
13	"	"	4/6	66.00%
14	"	"	5/6	83.33%
Total	-	-	103/146	70.00%

Fig. 1: Shows CAA-infected MSB1 cells giving specific intranuclear granular fluorescence with positively reacting chicken serum.

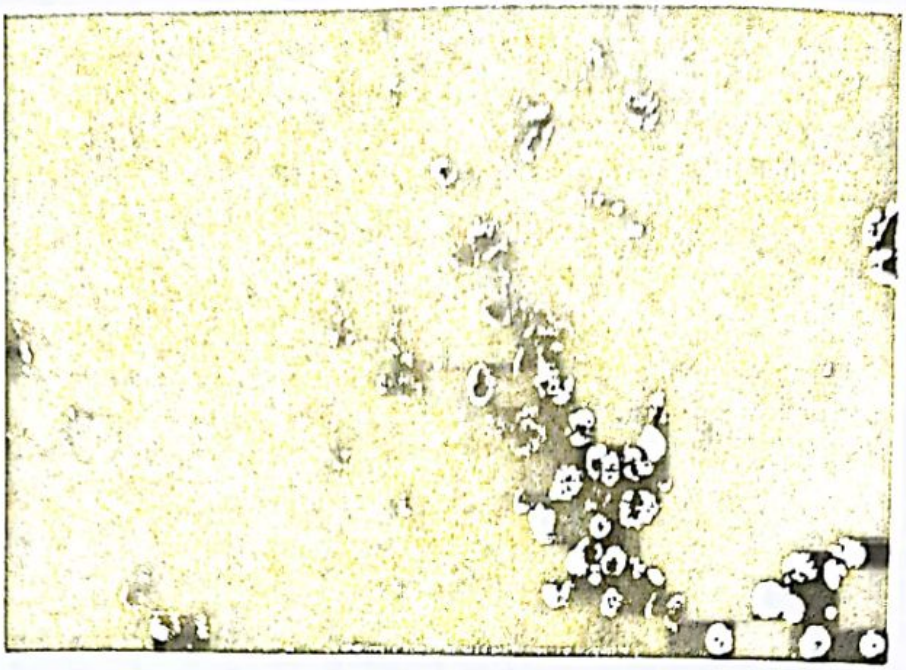

Fig. 2: Shows diffused intranuclear specific fluorescence emitted by CAA-infected MSB1 cells after reaction with positive chicken serum.

Fig. 3: Shows rounded fixed CAA-infected MSB1 cells with no specific fluorescence reaction with negative control chicken serum.

DISCUSSION

Inspite of the wide distribution of CAA throughout the world and the successful detection of CAA antibodies in sera of chickens in different parts of the world (McNulty et al., 1988; Yuasa et al., 1985; Lucio et al., 1990), to our information this is the first documented report about the detection of CAA antibodies in sera collected from some Egyptian commercial chicken flocks without any evidence of clinical disease. The present communication repressents a preliminary serological survey concerning the detection of CAA antibodies in chicken sera. The percentual

distribution of specific CAA antibodies in the tested sera may reflect the state of spread and prevalence of this infectious agent among Egyptian flocks. The Data depicted in table 1 throws a clear spot of light on the wide spread of CAA in the different types of chickens at different ages, with higher percentage of reactivities given by sera collected from broiler breeders (78.12-100%) followed by day-old broilers (57.14-83.33%) then those given by sera of layers.

The existence of CAA antibodies in chicken sera at this high incidence specially in day-old chicks, supports the findings reported by other workers in other countries (McNulty et. al., 1988; Yuasa wt al., 1985). The presence of CAA antibodies in the sera of broiler breeders and day-old broiler chicks might support the suggestion of Yuasa et al., (1979), that chicks with maternal antibody to CAA can be infected and excrete the agent without showing any signs of the disease.

Inspite of the importance of SN test in detecting CAA antibody in chicken sera, however, it requires much more time before final results can be obtained (Yuasa et al., 1980a). In contrast, the IIF test could be conducted more quickly and efficiently and proved its usefulness in the rapid and sensitive detection of CAA antibodies. This finding stand in full agreement with that of Yuasa et al., (1985).

This preliminary study necesitates a detailed survey of CAA antibodies in different localities representing all Egyptian governorates and most of chicken breeder flocks in addition a full investigation concerning the isolation and characterization of this infectious agent is required to find out the real situation of infection with CAA among commercial chicken flocks, specially the implication of the adverse effect of such an agent on the immune system either alone or in combination with other immunosuppresive viruses such as infectious bursal disease virus, reticuloendotheliosis virus as well as the virulent strains of Marek's disease virus (Yuasa et al., 1980b; Bulow et al., 1986).

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