Vet.Med.J.,Giza.Vol.40,No.2.(1992):55-62.

INFECTIOUS BURSAL DISEASE VIRUS INFECTION AMONG EGYPTIAN POULTRY FLOCKS

I - DETECTION AND ISOLATION OF THE VIRUS

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(received:7.2.1992)

INTERDUCTION

Infectious bursal disease (IBD) also called Gumboro disease (Cosgrove, 1962) is a highly contagious disease of young chickens (Hitchner, 1970 a) resulting in severe necrotic lesions in the bursa of Fabricius (BF) (Helmbldt & Garner, 1964 and Peters, 1967). Mortality is commonly low, but morbidity may be up to 100%. The danger of IBD was recognized by the fact that fowls which survive the infection have reduced immune response to subsequent vaccination as well as reduced protection against attacks by other diseases (Okoye, 1984).

In Egypt, Ayoub and Malek (1976) succeeded to isolate two IBD viruses for the first time from two broiler farms. Since that time the disease had been recorded in seversl localities. Therefore, intensive vaccination programs against Gumboro virus in Egypt were applied. Inspite of these intensive vaccination programs, outbreaks of IBD with high mortalities appeared, in 1989, in some farms and spread allover the conntry, where the severe clinical symptoms continued to reappear in susceptible birds of infected farms for a period of two months (Amir El-Batrawi, 1990 and Mousa & saif El-Deen, 1990). These recurrent outbreaks triggered our attention to possible prevalence of variant strains of IBDV that escape the immune-defence mechanisms of vaccinated chickens.

The aim of this present work was directed to the isolation and identification of the IBDV from different flocks in Egypt followed by studying the comparative physico-chemical and biological characers of the local and reference viral isolates. The antigenic characters of the reference and local viral isolates were analysed using a newly accurate serological technique namely the antigen capture ELISA beside other conventional serological techniques.

MATERIAL AND METHODS

Material:

* **Virus:** The reference IBDV "Weybridge strain" was kindly supplied by professor Dr. Silvio pascusci (Instituto Zoo profilattico, Foli, Italy). This virus was primarily propagated in chicken embryo Fibroblast (CEF) cell culture and then further passaged in specific pathogen-free (SPF), embryonated chicken eggs and then transferred to CEF and/or QT_{35}.

* **Cells and Media:** Primary CEF cell cultures were prepared from 9 - 11 days old SPF embryos as described by **Graham (1980)**. The cells were seeded in plastic flasks and allowed to grow in M_{199} tissue culture medium (GIBCO, USA) containing 0.3% Tryptose phosphate broth (TBP) and 6% Fetal calf Serum (FCS). The QT_{35} cell line was obtained from the Instituto Zoo profilattico, Froli, Italy and then propagated In Eagle's Minimum Essential Medium with 0.5% TPB and 5% FCS.

* **Antisera:** Specific anti-Gumboro Virus (Weybridge Strain) serum was prepared according to **McFerran et al., (1980)**.

* **Methods:**

1- Preparation of bursal homogenates:

The typically affected bursae of Fabricius were collected and homogenized in equal volumes of physiological saline, frozen and thawed three times and then clarified by centrifugation at 3000 r.p.m. for 15 m at 4°C. These bursal homogenates were tested by the AGPT and described by **Woernle (1959)** using reference IBDV antigen and antiserum as positive controls.

2- Virus isolation:

The clarified bursal homogenates that were giving precipitation lines by AGPT were then filtered through millipore membrane filter (0.45^{u} pore size) and inoculated on the chorioalantoic membrane of 9 to 11 days old SPE embryonated chicken eggs (ECF). After 3 passages on ECE the chorionallantoic fluid were collected and prepared according to the procedurs adopted by **Cho (1968)**.

3- Inoculation of chicken embryo fibroblast (CEF) and QT-35 cells:

CEF-cells were prepared according to **Graham (1980)**. The infected cells were incubated for 7 days (for CEF) and 10 days (for QT- 35) till a convenient cytopathic effect (CPE) was developed. Infected cells showing characteristic CPE were then exposed to three cycles of freezing and thawing and then clarified by centrifugation at 4°C. Clear supernatents were kept in small aliquots at -20°C till used for further studies.

4- Serum neutralization test (SNT):-

SNT has been performed using alpha procedure as described by Beard (1980) using reference polyclonal antisera produced against the Weybridge strain.

RESULTS

Table (1) shows the percentage of positively reacted bursae by the AGPT and the number of isolates obtained from these bursae. From this table it is clear that samples which were collected from Ismailia and Kaliobia (Banha) Governorates gave a 100% positive reactivity in the AGPT with subsequent isolation of IBDV from all of them. On the other hand, the other governorates showed a varying degrees of positivity and isolation ranging between 80, 75, 66.5 and 40% in Fayoum, Sharkia, Dakahlia and Giza respectively. Based on these

Table (1) : Detection and preliminary isolation trials of IBDV from infected bursae.

Location (Governorate)	Average % of flock mortality	No. of collected bursae	No of positive (AGPT) *	No of isolation **	% Of isolation
Dakahlia	48	12	8	8	66.6
Ismailia	48	10	10	10	100.0
Fayoum	48	10	8	8	80.0
Sharkia	10	4	3	3	75.0
Giza	10	5	2	2	40.0
Kaliobia	10	5	5	5	100.0

* = Bursal homogenates were prepared from the collected suspected bursae and tested aganist specificanti-IBDV serum. The development of clear Iv precipitation was recorded as positive.
** = Homogenate of AGPT - positive bursae were introduced into 9 days old SPF-ECE for isolation trials.

Table (2): Growth characteristics and behaviour of IBDV strains in SPF - ECE.

Virus strain	No of inoculated eggs	No of viral passage	Embryo death (dpi)*										MDT **	Lesions ***
			1	2	3	4	5	6	7	8	9	10		
Weybridge	20	4	0	2	9	3	1	0	0	0	0	0	3.2	The dead embryos and CAM were haemorrhagic and aedematus
Local 1 (Ismailia)	20	4	0	0	0	8	4	1	0	0	0	0	4.4	
Local 2 (Banha)	20	4	0	0	0	0	2	4	0	0	0	0	6.3	

* dpi = Days post inoculation
** MDT = Mean death time
*** Embryos that survived until 7 dpi were stunted with liver necrosis.

Table (3): Cytopathogenisity of IBDV strain in CEF and QT-35.

virus	No. of virus passage	Type of cells	Development of CPE (dpi)				CPE
			1	2	3	4	
Weybridge	3	2ry CEF	++	++++	++++	++++	Cell rounding syncytia and then detachment of cells
Ismalia	3	2ry CEF	++	+++	++++	++++	
Banha	3	2ry CEF	++	++	+++	++++	
Weybridge	4	QT-35	+	++	++	++	Cell rounding syncytia and then detachment of cells
Ismalia	4	QT-35	+	++	++	++	
Banha	4	QT-35	+	+	+	++	

+ = Mark represent the intensity and degree of the developed CPE and cell detachment

Table (4) : Differentiation of Gumboro virus isolates in the Neutralization test *.

Virus strain	Infectivity titers (log 10)		Neutralizing Index (N.I.)
	before Neut	After Neut	
Weybridge	6.5	1.83	4.67
Local 1 (Ismalia)	6.32	1.83	4.49
Local 2 (Benha)	6.17	1.83	4.34

* = Serum neutralization test has been performed using the alpha procedures (varying virus-fixed serum method) described by Beard,(1980) using reference polyclonal anti-sera produced aganist the Weybridge strain.

data we gave our attention for samples that gave 100% positivity in the AGPT and virus isolation (Ismailia and Kaliobia). Isolated IBDV from Ismailia governorate called $Local_1$ and that from Kaliobia (Banha) called $Local_2$. These two isolates were characterized for their behaviour in SPF-ECE, CEF and QT-35 cells, in comparison with the reference Weybridge strain.

Table (2) shows clearly that the behaviour of the three different strains of IBDV, namely $Local_1$, $Local_2$ and Weybridge strains documents the difference in the virulence of these strains based on chicken embryo mortalities. Whereas the Weybridge strain could induce mortalities in 15 out of 20 eggs (75%) with mean death time (MDT) 3.2, the other two local strains could induce mortalities in 13 out of 20 (65%) for $Local_1$ wit MDT 4.4 and 11 out 20 (55%) for $Local_2$ with MDT 6.3. The main predominant lesions observed in infected embryos were deaths with haemorrhagic and oedematous

chorioaltantoric membranes (CAM).

From the data depicted in Table (3), it could be noticed that, CEF cells were more susceptible to IBDV infection, where most of the cell monolayer were showing intense and clear CPE as early as 2 days post inoculation (dpi), while QT-35 cells could show the same changes but some what later, giving only clear and convieniat CPE at 4 dpi. The main cytopathological changes observed in both CEF and QT-35 cells were cell rounding associated with syncytia formation.

Comparing the results obtained from the two local isolates, it has been found that, the $local_1$ strain (Ismailia) could exert clear extensive CPE than those produced by $Local_2$ strain (Banha).

Table (4) shows the neutralizing capability of the polyclonal anti-IBDV "Weybridge strain" serum on the $local_1$, $local_2$ and Weybridge strains. The neutralizing potency of the two local strain by the Weybridge antiserum indicates that these two isolated viruses related to the same type of IBDV. The reference anti-Weybridge polyclonal serum could give a neutral izing index (NI) of 4.67, 4.67, and 4.32 with Weybridge, $Local_1$ and $Local_2$ respectively.

DISCUSSION

Inspite of application of intensive vaccination program against IBDV, a great economical losses have been recorded in Egypt in the last few years among poultry flocks. These losses were due to infection with IBDV. Several trials, therefore, have been done to manipulate such a problem confirming the incrimination of IBDV in causing this disease (**Amer El-Batrawi, 1990, Mousa and Saif El-Dean, 1990**). Because of the importance of IBDV as immunosuppressive agent, and due to its capability of changing its native antigenic configuration as a result of reassortment of its nuclic acid segments, thus giving up an array of different antigenic variants which can escape the immunmechanism of vaccinated chickens; for the aforementioned reasons the present investigation has been undertaken to isolate IBDV from different Egyptian governorates followed by trials to characterize the isolated viral strains. As a firts approach for dealing with this problem, the collected bursae have been tested for the presence of IBD viral antigen (s) using the well known and standardized AGPT. The results obtained in this work revealed that the AGPT could detect the specific IBD viral antigen in the bursae collected from different governorates namely Dakahilia (8 out 10), Ismailia (10 out 10), Fayoum (8 out 10) Sharkia (3 out 4), Giza (2 out 5) and Bnha (5 out 5) as clearly depicted in Table (1). The use of BF of infected chickens as target organs to search fot the specific IBD viral antigen has also

been successfully used by Hiral et al., (1973) and Wyeth and Chattle (1982). The detection of IBD viral antigen (s) as documented in Table (1) throws a clea spot of light on the rate of incidence of IBDV in the Egyptian governorated testes, where 100% of the tested bursae taken from Ismailia and Kaliobia were found to be involved with Gumboro virus infection.

The IBDV strains isolated from Ismailia ($Local_1$) and Kaliobia ($Local_2$) were used for comparing their virulence for ECE and their cytopathogenicity for CEF and QT-25 cells in comparison with the Weybridge strain. Data presented in Table (2) showed that, the Weybridge strain could induce embryo mortalities (75%) with MDT 3.2, whereas the $local_1$ and $local_2$ strains showed embryo mortalities of 65% and 55% with MDT 4.4 and 6.3 respectively. The cytopathogenicity exhibited by the three IBDV strains on CEF and QT-35 cells showed with no doubt that CEF cells are relatively easy to manage more than the QT-35 cells where the cytopathic changes were developing 2-3 dpi in CEF instead of 4 dpi in QT-35 as shown in Table (3).

Inspite of the high susceptibility of both CEF and QT-35 cells to IBDV infection as described by other workers (Cowen and Braune 1988), the CEF cells has been found to be universally preferable for studying the cytopathogenicity of IBDV as reported by Petek et al., (1973), Rosenberger and Gelb (1978) and Jackwood et al. (1989).

For investigating the antigenic relationship and differences between the two local isolates ($Local_1$ and $local_2$) and the reference Weybridge strain, the SNT was applied using the reference hyperimmune serum prepared against the Weybridge strain. The test was previously used by several workers for the antigenic differentiation between several IBDV isolates with the serotype I which was isolated from broiler chicks (Rosales et al., 1989) and those serotype II which was isolated from turkeys (McNutty et al., 1979, McFerran et al., 1980, Lukert, 1986 and McNulty and Saif, 1988). From this work it become clear that the three studied strains (Weybridge, $local_1$ and $local_2$) are related to the same serotype 1 due to the neutralizing effect exhibited against both the $local_1$ and $local_2$ strains by the Weybridge strain antiserum.

Further physicochemical and biological characterization of the two local isolates ($local_1$ and $local_2$) is now in progress to clarify the identity and/or differences between the local Egyptian isolates and other reference foreign strains.

SUMMARY

A total of 46 suspected burse have been collected from different governorates

in Egypt. These samples represented Dakahlia, Ismailia, Fayoum, Sharkia, Giza and Kaliobia (Banha) 12, 10, 10, 4, 5, 5 respectively. These bursae have been firstly tested by the AGPT, using specific chicken anti-IBDV serum, for the presence of specific Gumboro viral antigen (s) befor being subjected to viral isolation. Gumboro virus was isolated from every infected bursae that was giving positive reaction in the AGPT, using SPF-9 days old embryonated chicken eggs (ECE), chicken embryo fibroblast (CEF) and QT-35 cells.

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