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INFECTIOUS BURSAL DISEASE VIRUS INFECTION AMONG EGYPTIAN POULTRY FLOCKS

II.Physico-chemical characterization of isolated viruses

By

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INTRODUCTION

In Egypt, the IBDV was isolated for the first time by Ayoub and **Malec (1976)**. Since that time, the disease had been recorded in severall iocalities. To control such problem, an intensive vaccination program was applied and after that time Gumboro outbreaks were recorded. Inspite of these, unexpected outbreaks of IBD among vaccinated flocks with high mortalities were bursting out in 1989-1990 in some farms and spread all over the country. The severe clinical symptoms continued to reappear in susceptible birds of infected farms for a period of 2 months **(Amir El-Batrawi, 1990; Mousa and Saif El-Deen, 1990)**.

The recurrent appearance of several outbreaks triggered our attention to nominate other variant strains of IBDV. Therefore, the aim of the present work directed to study the comparative physicochemical charactes of the local **(Madbouly et al., 1992** submitted for publication) and the reference "Weybridge" viral isolates. Besides the electron microscopy investigations, the sensitivity of the isolated virus was tested against treatment with either and choroform, $pH_{3.0}$ buffer and heating at 56°C.

MATERIAL AND METHODS

MATERIAL:

* **Virus:** The reference IBDV "*Weybridge strain* " was kindly supplied by professor, Dr. Silvio Pascusci (Instituti Zoo Profilattico, Forli, Italy). This virus was primarily propagated in chicken embryo Fibroblast (CEF) cell culture and then further passaged in specific pathogen Free (SPE), embryonated chicken eggs and then transferred to CEF and OT-35 cell cultures.

* **Cells and Media:**

Primary CEF cell cultures were prepared from 9-11 days old SPE embryos as described by **Graham (1980)**. The cells were seeded in plastic flasks and allowed to grow in M_{199} tissue culture medium (G IBCO, USA) containing 0.3% Tryptose phosphate broth, (TBP) and 6% Fetal calf serum (FCS). The OT-35 cell line was obtained from the Institute Zoo profilattico,

Froli, Italy, and then propagated in Eagle's Minimal Essential Medium with 0.3% IBP and 5% FCS.

* Buffer (PH_3):

Sorenson's (m/15) mixture was used for preparing pH_3 buffer and described by **Hemperion et al.**, (1983).

METHODS:

* Elctron Microscopy of IBDV:

On observing the IBDV by the electron microscope, infected CEF cells were first subjected to three cycles of freezing and thawing 48 hours post infection (p.i). The tissue culture harvest was centrifuged at 4000 xg for 20 minutes at 4°C in 2 mls. The clarified supernatants were further clarified by a second cycle of centrifugation at 10,000 X g for 10 min. at 4°C. The IBDV particles were then pelleted down in the Airfuge Beckman ultla centrifuge at 104,000 X g for 10 min at 4°C using cellulose nitrate tubes (175 ml capacity) containing carbon-formvar coated copper grid (150-300 mesh) fixed inside the tube by a specific adapter. The IBDV particles on the copper gride were negatively stained with 2% sodium phosphotungestate (NaPt) (pH 7.0) for 1.5 min. The stained copper gride was introuced into the TEM phillips cm 10 operating at 80 kv and 28500 X magnification.

* Treatment of IBD viruses with ether & Chloroform:

The different strains IBDV (Reference, $local_1$ and $local_2$) were subjected to treatment with ether (20%) and chloroform (10%) for different time intervals followed by titration of viral infectivity. The treatment process has been done according to the procedures described by **Hiari and Shimakure (1972) and Elchaman and Wang (1961).**

* Heating of IBD viruses at 56°C:

The stability of IBD viral strains was tested against heating at 56°C for 0.5, 1,2,4, and 8 hrs Following the method described by **Benton et al., (1967).**

* U.V. Irradiation of IBD viruses (Petek et al., 1973):

The local and reference IBD viral isolates were irradiated by U.V light in sterile plastic petridish (60 mm in diameter) under U.V. 60 watt lamp at a distance of 10 cms for a period of 5, 10 and 15 minutes.

RESULTS

* Electron microscopy:

The EM examination showed cleary the presence of icosahedral-like naked particles measuring about 54 nm in diameter for the

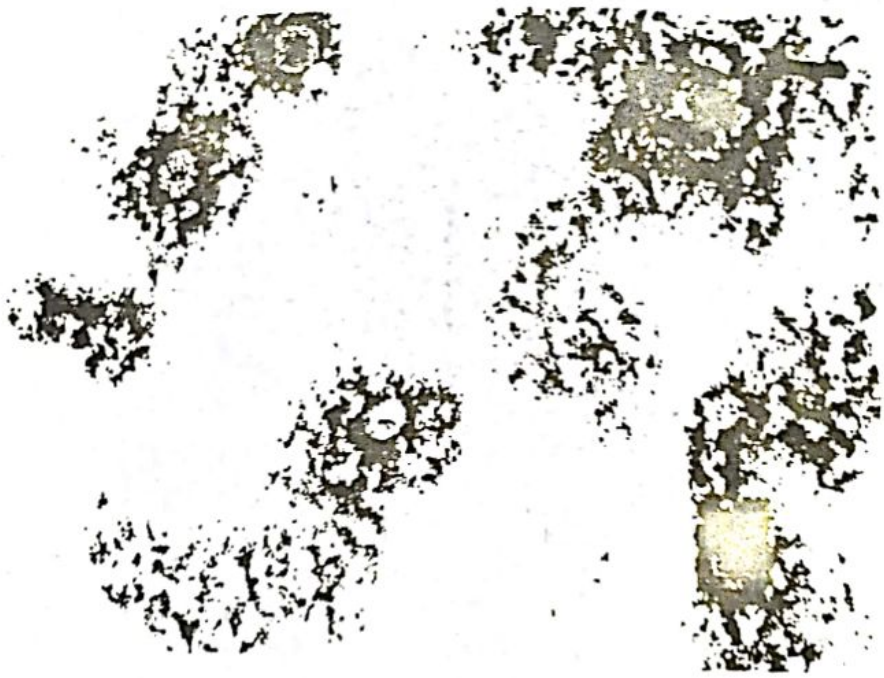

Fig.(1): Electron microscopic detection of Weybridge strain particles in infected CEF cells = 54nm.

Fig.(2): Electron microscopic detection of Ismalia strain particles in infected CEF = 54 nm.

Weybridge strain and local $_1$ isolate (Ismailia) and 53.9 nm for local $_2$ (Banha) isolate (Fig. 1, 2 and 3). The isolated local $_1$ and local $_2$ particles represent typical particles related to the birnaviridae virus group.

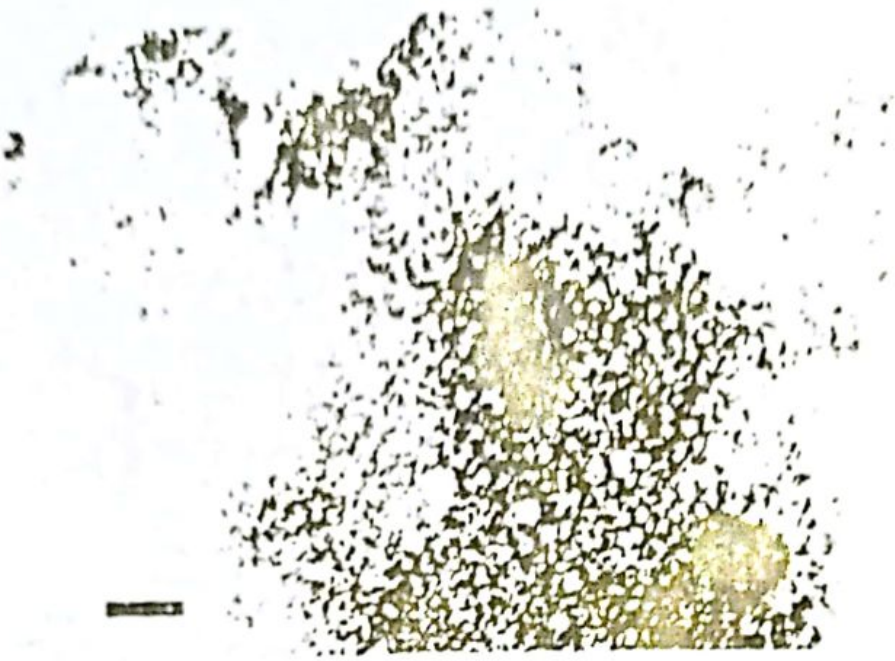

Fig.(3):Electron microscopic detection of Banha strain particles in infected CEF cells = 53.9 nm.

* Stability of Gumboro virus particles to heating at 56°C.

The periodical exposure of the 3 isolates to heating at 56°C for 0.0, 0.5, 1.0, 2.0, 4.0 and 8.0 hours followed by titration of virus infectivity, could reveal that slight decline in the titre of the Webridge strain by 1.20, 1.50 1.87 1.82 and 2.33 (log 10) at the respective periods of time as clearly shown in Table (1).

Local $_1$ and local $_2$ viral isolates exhibited a relative lower sensitivity to heating at 56°C with decline of titer by 0.69, 1.0 1.64, 2.0 and 3.32 for local $_1$ strain and 0.54, 0.67, 0.67, 0.95, 1.0 and 2.0 for the local $_2$ strain at the respective periods of time as depicted in Table (1) and illustrated in Fig. (4).

Table (1): Sequental stability of IBD viruses to heat at 56°C

Virus isolate	Time post exposure (hours)					
	0.00	0.50	1.00	2.00	4.00	8.00
Weybridge	6.5*	5.38	5.00	4.83	4.68	4.17
Local(Ismalia)	6.32	5.63	5.32	4.68	4.32	4.00
Local(Banha)	6.17	5.63	5.50	5.22	5.17	4.17

* The figures depicted inside the table represent the different forces of viral infectivity titers (log 10) in primary CEF cells after periodical exposure of viral isolates to heat at 56°C.

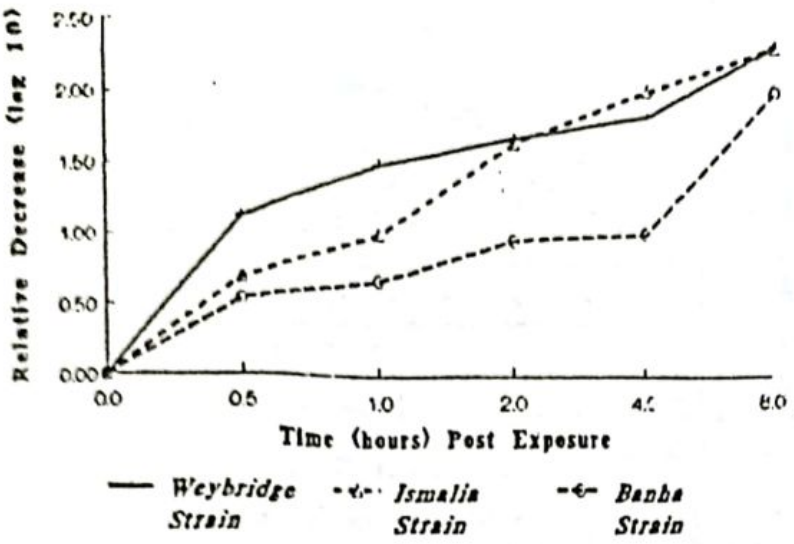

Fig.(4):The relative decrease of infectivity of IBVD isolate exposed to 56 °C

* The influence of exposure to U.V. rays:

CEF viral harvests of the three IBDV isolates obtained 7 days post infection were exposed to U.V. irradiation at a distance of 10 cm for different time intervals (0, 5, 10 and 15 min, followed by subsequent titration of the residual viral infectivity after each time of exposure. All the 3 viral isolates showed a 50% decline in virus titers after 15 min of exposure to U.V. rays as shown in Table (2) and Fig. (5). The relative decline and difference in virus titers before and after U.V. exposure was found to be 2.0, 2.82 and 3.0 for the Weybridge strain, 1.0, 1.94 and 2.64 for the local $_1$ strain and 1.49, 2,79 and 3.0 for the local $_2$ strain at 5, 10 and 15 minutes exposure respectively.

Table(2): Periodical sensitivity of IBD viral infectivity to U.V. irradiation

Virus isolate	Time post exposure (minutes)			
	0	5	10	15
Weybridge	6.5*	4.5	3.68	3.50
Local(Ismalia)	6.32	5.32	4.38	3.68
Local(Banha)	6.17	4.68	3.38	3.17

* The figures depicted inside the table represent the different forces of viral infectivity titers (log 10) in primary CEF cells after periodical exposure of viral isolates to heat at 56°C.

* The influence of chloroform and ether on the infectivity of IBDV isolates:

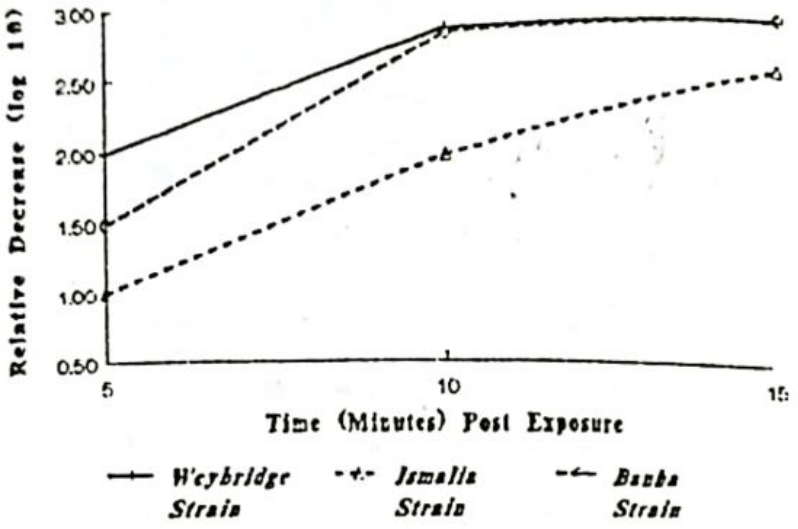

Fig.(5):The relative decrease in infectivity titers of IBD viral isolates exposed to U.V. irradiation.

Table(3): The effect of chloroform and ether on the infectivity of IBDV isolates.

Virus	Pretreated titer (log 10)	Titer after treatment with	
		Chloroform	Ether
Weybridge	6.50**	5.83	5.63
Local(Ismalia)	6.32	6.17	5.68
Local(Banha)	6.17	5.50	5.50

* IBD viral isolates(reference & local strains)have been pretreated with chloroform and eather at an end concentration of 10% & 20%. Therealter the residual viral infectivity was titrated in primary CEF.
** Figures depicted inside the table represent the forces of titers (10g 10) expressed as $TCID_{50}$/ml.

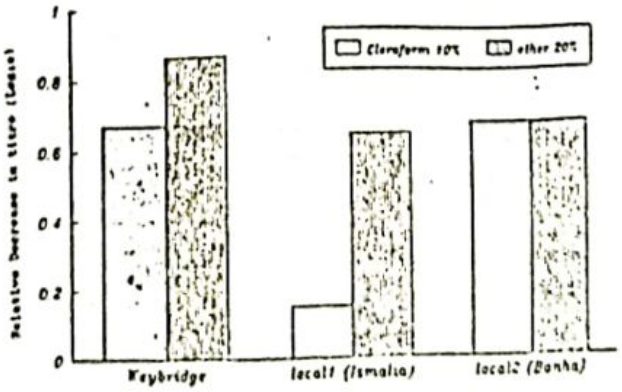

Fig.(6):The relative reduction in infectivity titers of IBDV isolates after treatment with cloroform and ether

Table(4): Sensetivity of IBD isolates to treatment with phosphate buffer pH 3.0.

Virus	Titer of virus infectivity (log 10)		Reduction value (log 10)
	at pH. 7.2	at pH. 3.0	
Weybridge	6.50	4.83	1.67
Local(Ismalia)	6.32	5.50	0.82
Local(Banha)	6.17	5.17	1.00

* IBD viral isolates(Weybridge,Ismalia & Banha strains) were subjected to phosphate buffer at pH 3 and tested for stability under such condition after 18 hours at 4 °C.Reduction in virus titer was properly determined in CEF cells.

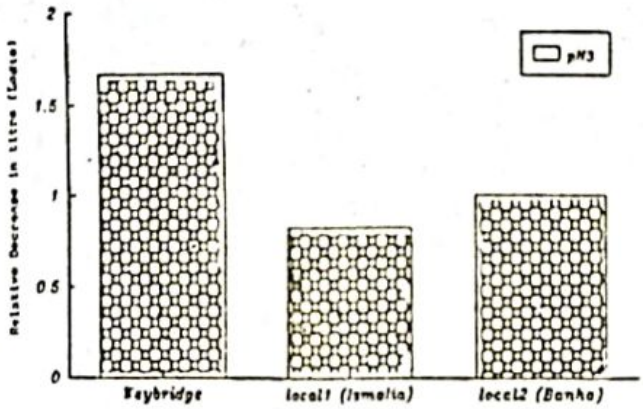

Fig.(7):The relative reduction in infectivity titers of IBDV isolates after 18 hours exposure to pH.3.

After treatment of the IBDV isolates (Weybridge, local $_1$ and local $_2$) with ether (20%) and chloroform (10%) a low decline of virus titers has been noticed. The decline in

virus titers (log 10 expressed as $TCID_{50}$/ml) was recorded to be 0.67, 0.15 and 0.67 after treatment with chloroform and 0.87, 0.64 and 0.67 after treatment with ether for Weybridge, $local_1$, and $local_2$ strains respectively as shown in Table (3) and Fig. (6).

*** The stabllity of IBDV isolates under pH. 3.0:**

For studying the effect of pH 3.0 on the infectivity of IBDV isolates, they were suspended in Sorenson's phosphate buffer solution for 18 hours at 4°C after which the

infectivity titers has been determined. Data depicted in Table (4) shows clearly a reduction in virus titers ranged between 0.82 and 1.67 (log 10) exhibited by the three viral isolates (Fig. 7) with titers of $10^{4.83}$, $10^{5.50}$ and $10^{5.17}$ under pH. 3.0 if compared with titers of $10^{6.5}$, $10^{6.32}$ and $10^{6.17}$ before treatment in case of Weybride, $local_1$, and $local_2$ strains rospectively.

DISCUSSION

The physical characterication of the reference and local isolates ($local_1$ and $local_2$) through their examination with the EM showed icoshedral like naked particles measuring about 54 nm in diameter for Weybridge and $local_1$ and 53.9 nm for the $local_2$ strain (Fig. 1, 2 and 3). This finding oriented us to the consion that the isolated particles besides their identity to the reference Weybridge strain should be related to the Birnaviridae virus gorup. On the other hand, our findings of EM are found to the in consistency with other works published by Hiral et al. (1973); Hiria et al. (1974); Pattrson et al. (1975) and McFerran et al. (1980), who examined purified preparations of IBDV and could detect icosahedral particles measuring about 55 nm in diamater.

The relative stability of the reference and local strains to heating at 56°C was sequentially investigated. The results obtained in this work revealed with no doubt that heating at 56°C does not greatly influence the infectivity of all tested strains of IBDV, where loss in infectivity (estimated approximately 1-2 logs) has been secured within 4 hrs exposure to 56°C (Table 1 and Fig. 4). From these findings and from the reuslts reported by other investigators it can be concluded that the examined IBD viruses express its relative resistance to heating at 56°C, where Benton et al. (1967) recorded virus resistance at 56°C for 5 hrs and Petek et al. (1973) recorded virus resistance for 3 hrs. On the other hand Lukert, and Davis (1974) recorded virus resistance for 90 minutes. In addition, Landgraf et al. (1976) have been able to record resistance of IBDV to heating at 56°C for 30 minutes only.

IBDV could exhibit in this work its relative resistance and slower inactivation after it had been irridiated by U.V. rays, where only a loss of infectivity to a rate between 1 and 2 1.5 log has been given by the $local_1$ and $local_2$ strains, respectivley after 5 min. exposure to U.V. rays (Table 2 and Fig. 5) when compared with a loss of only 2 logs in case of the reference Weybridge strain after exposure to U.V. for the same time. These findings support the previous data reported by Petek et al. (1973) who suggested that, the slower inactivation rate of IBDV by U.V. irridation as com-

pared with the Reo virus (Crawley strain) might be attributed to the smaller target size of the IBDV RNA.

The stability of IBDV reference and local strains to treatment with chioroform 10% and ether 20% has been carried out. Only a very slight decline in virus infectivity has been obtained with approximate loss of about 0.67 and 0.87 logs for the Weybridge strain after treatment with chloroform and ether with a loss of infectivity at rates of 0.15 and 0.64 for $local_1$ strain and 0.67 and 0.67 for $local_2$ strain respectively (Table 3 and Fig. 6).

These findings beside those reported by Benton et al. (1967); Cho and Edger (1969); Lucio et al. (1972); Petek et al. (1973); Ayoub and Malik (1976) and Casnocha (1980) made us to conclude that, the reference Weybridge and local strains of IBDV are generally resistant to the solvent action of chloroform and ether.

Incontrast to the high sensitivity of Reovirus to acidic pH (3.0), the obtained results in this work document the resistance and stability of the studied IBD viruses, as members of the family Birnaviridae, the treatment with pH 3.0 buffer. Table 4 and Fig. 7 show clearly a slight decrease between 0.82 and 1.67 for the three reference and local strains, thus supporting the important finding reported by Petek et al. (1973) who recorded a fast decrease of the titer of Crawley Reovirus 3 logs lower than that of the original titer if compared with IBDV strains (Review by Okaye, 1984).

From Fig. 7, it can also be noticed that there is a noticeable difference in the relative stability of the two strains ($local_1$ and $local_2$) if compared with that of the reference Weybridge strain. This observable difference might give us a base for the differentiation between our local strains and reference one.

SUMMARY

Two isolates of infectious Bursal Disease Virus (IBDV) were isolated from Ismailia ($local_1$) and Kaliobia ($local_2$) governorates during an outbreaks of Gumboro disease from 1989 to 1990. The isolated strains were identified by the agar gel precepitation test (AGPT) and serum neutralization test. Physiochemical studies of the isolated viruses revealed that these icosahedral naked particles measuring 54 nm indiameter, identical to those viruses belonging to the family Birnaviridae. They have been found to be resistatant to heating at 56°C for 8 hrs. beside their relative resistance to U.V. irradiation with slow inactivation rate. The isolates exhibited also a relative resistance to treatment with clolorform (10%) and Ether (20%). $pH_{3.0}$ had no any significant adverse effect on the infectivity of the isolated viruses.

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