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VERY VIRULENT INFECTIOUS BURSAL DISEASE

BY

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INTRODUCTION

Since the first outbreak of IBD in USA (Cosgrove, 1962), the disease had been reported in almost all parts of the world as reviewed by Faragher, (1971) and Okoye et al. (1984), and including Egypt (Ayoub and Malek, 1976). There are two recognized serotypes of IBDV, designated serotypes 1 and 11 (Jackwood, et al., 1982). Only serotype 1 IBD viruses have been known to cause naturally occurring disease in chickens. Recently, workers in USA have been isolated IBD viruses from "problem" broiler farms, that seem to be different from serotype, 1. These viruses are being classified as sub-type of serotype 1 or "variant" viruses. Subtypes are between 10 and 70 percent relatedness (Rosenberger and Cloud, 1985 and Giambrone, 1989). IBD has re-emerged as a major disease problem in the last three years characterized by severe clinical signs with higher than normal level of mortality, recorded in England, Danemark, Holland, Australia , Ireland and Middle East area including Jurdan, Syria and Israel (Box, 1989; Lutticken and Van der Marel, 1989 and Zahid, 1990). The characters of the

re-emerged IBD, designated very virulent infectious bursal disease(VVIBD) was reviewed (Box, 1989). The objectives of this research were to record, isolate, identify and study the characters of 9-VVIBDV isolates obtained from 9-IBD affected farms selected from 9 governorates during an epidemic that had been occurred since June 1989 and could cover the country.

MATERIALS AND METHODS

Specimens:

The bursae of acutely affected chicks were used for virus detection and isolation.

Virus detection:

IBD virus antigen in the bursa was directly detected using the agar gel diffusion (AGD) test as described by Ide (1975).

Virus isolation and titration:

The chorioallantoic membrane (CAM) route of 9-11 day old chicken embryos was used for IBDV isolation and titration as described by Hitchner (1970). Also , chick embryo fibroblast (CEF) was used for IBDV isolation and titration of the IBDV cell culture adapted (Lukert) strain of IBDV as described by Lukert and Davis (1974). The fifty percent end points were computed after Reed and Muench (1938).

Bacterial isolation:

The bursa of acutely survived affected chicks were removed aseptically and the standard method of diagnostic bacteriology described by Blair (1970) was used to identify the isolated organisms.

Pathogenicity tests:

a. Pathogenicity test in chicks with residual of maternally derived antibody (MDA): Twenty-one-day old White Lohman Cockerels were procured from a layer breeder flock, possessing antibodiesdagainst IBD, acquired from their parents according to specific vaccination program including live and inactivated IBD vaccines. Cockerels were reared in small colony type housing units in cages.Blood samples were obtained from all cockerels weekly up to 6 weeks of age to follow the fading of maternally derived IBD antibodies using the AGP test (Woernle, 1966), VN test (Nagi et al., 1980) and indirect ELISA assay (Khafagy et al., 1990). At 42 days age, Five experimental groups were divided, each included 4 chickens. Bursal homogenates of four selected isolates (No. 1, 2, 3 and 5) were inistillated intraoccularly with 100 ul per bird at 1:10 diltution in PBS, uninoculated control group was included. Experimental groups were observed 10 days for signs, deaths and postmortem findings of both dead and survived chicken, bursa-to-body weight rate was calculated according to Dohms et al., (1988), specificity of infection and death was determined by IBD virus antigen detection in the bursa according to Faragher (1971). Further, the bursa, spleen and kidney of live birds were subjected for histopathology by the method described by Culling (1963).

b. **Pathogenicity in SPF chicks:**

9- bursal isolates homogenates were firstly passaged (bursal passage) by intraoccular inistillation of 100 ul of the original bursal homogenate (at 1:10 dilution in PBS) of each isolate into 3-21 days age SPF chicks, and the bursae were collected for each group separately, ground and diluted 1:10 to be used for pathogenicity.

Very Virulent Infectious Bursal Disease.

For pathogenicity, equal pool of the nine bursal passaged homogenate were inoculated intraoccularly into 5- SPF chicks-52 days old and 10 SPF chicks-28 days old,. Also, bursal passaged homogenate of isolates No. 3 and 6 were inistillated into 10 SPF chicks-52 days old and 20 SPF chicks-28 days old. All experimental groups were observed for 7 days post-infection and mortalities were recorded.

Protection conferred by live and inactivated oil emulsion vaccines: Commercial TAD live Gumboro vaccine, strain cu-Im was administered at the recommended level of field dose (10^3 EID 50 per bird) for 3 groups of SPF chicks 24 and 12 days old. 2 and 4 weeks post vaccinations, immune response was measured using ELISA, AGP and SN tests, and challenged with the bursal passaged isolated (pooled and isolate No. 3) using 0.2 ml/bird oculonasal + 0.2 ml/ bird intracloacal, mortalities were recorded within 10 days observation.

Commercial TAD inactivated oil based vaccine was administered at the recommended field dose (0.5 ml per bird) intramuscularly into 20-12 day old SPF chicks, 16 days postvaccination, serological response was measured using ELISA test and challenge was performed at 1:10 dilutions of pooled bursal passaged homogenates isolates using 0.2 ml intraconjunctival + 0.2 ml intracloacal/bird and observed for 14 days postchallenge.

Antigenicity of IBDV isolates:

The method followed was a double immunodiffusion test, in which the standard Faragher strain and variant strain of IBD and their homologous antisera were compared with the 9 bursal homogenated isolates as described by Wyeth and Chettle, (1988).

RESULTS

The nature of the disease:

The disease within affected farms appeared suddenly with high morbidity, usually close to 100%. The clinical disease runs its course in approximately 10-12 days. The mortality usually began at the 2nd day, with peak on the 4th - 5th day and recede in a period of 5-7 days. Actual mortality recorded in **Table, 1.** The clinical signs included whitish-yellowish - greenish and sometimes bloody watery diarrhoea which soiled the vent feather, followed by anorexia, depression, trembling, severe prostration and death.Psot mortem examination of dead chickens showed well developed carcasses and in good bodily condition, with dehydration of the subcutaneous tissue and muscles, with empty crop. Punctate haemorrhages and petechiae were common on the muscles of the leg, breast and wings. Sometimes haemorrhages on the proventriculus, gizzard junction were seen. Kidneys were sometimes enlarged with prominent tubules and varied in colour from grey to dark brown with pale areas. Tubules and ureters, sometimes filled with urates. The liver appeared congested dark red. Thymus appeared hyperaemic pinkish colour. The bursa of Fabricius was enlarged up to twice the normal size, oedematous with yellowish gelatinous peribursal exudate, and yellowish-pinkish or reddish colour and the lumen sometimes, filled with creamy exudate or casious material, while plica showed petechiation **(Table, 2).**

DISCUSSION

Field reports on the recent outbreaks of VVIBDV in vaccinated chicken flocks which had been observed in England (East Anglian strain) and subsequently

Table 1: History of IBDV chicken isolates

Isolate code	Date of isolation	Governorate	Type	Age/days	Breed	System	Capacity	Mortality	MOrtality%	Previous IBD vaccination and type of vaccination
1	Oct.89	Domiat	Layer	42	Lohman(W)[1]	Cage	22000	132000	60	18d,28 d/[2]/DW[3] (TAD),29/sp[4] (TAD)
2	Oct.89	Sharkia	Layer	38,52	Hisex(W+B)[5]	Cage	47000	12000	25	14 d/DW (Intervet)
3	Oct.89	Cairo	Layer	42	Leghorn(W)	Cage	25000	15000	60	14 d/DW (TAD)
4	Nov.89	Kaliobia	Layer	50	Lohman (W)	Cage	25000	2750	11	12 d,34 d (TAD)
5	Aug 89.	Dakahlia	Layer	34	Lohman(W)	Cage	40800	21864	54	14/DW+sp (intervet),36d*/DW(intervet)
6	Sept.89	Giza	Mixed	21	Baladi	DL[6]	800	550	70	14 d/DW (TAD)
7	Oct.89	Fayoum	Broiler	21	Haubbard	DL	5000	2000	40	12 d/ DW (TAD)
8	Oct.89	Behira	Breeder	30	Baladi	DL	68000	30600	45	14 d/DW (TAD)
9	Oct.89.	Kaferelshikh	Layer	45	Lohman(W)	Cage	27000	14000	52	14,35 d/DW (intervet)
						Total	260600	111964	43	

1 w = white
2 d= days
3 DW =drinking water
4 sp= spray
5 B = brown
6 DL = deep liter
* = adminstrated as emergency vaccination

Table (2):Diagnosis of IBDV chicken isolates .

Isolate code	Signs	Post mortem				AGPT bursa	Virus isolation					
		B	K	M	PV		Egg embryo			Tissue culture		
							death	EID_{50}	AGPT CAM	P^1	P^2	P^3
1	+	+	+	+	±	+	+	5.5	+	-	-	-
2	+	+	+	+	±	+	+	4.2	+	-	-	-
3	+	+	+	+	±	+	+	5.5	+	-	-	-
4	+	+	+	+	±	+	+	4.0	+	-	-	-
5	+	+	+	+	±	+	+	4.5	+	-	-	-
6	+	+	+	+	±	+	+	6.0	+	-	-	-
7	+	+	+	+	±	+	+	4.5	+	-	-	-
8	+	+	+	+	±	+	+	4.5	+	-	-	-
9	+	+	+	+	±	+	+	5.2	+	-	-	-

B: Burse K : Kidney M: Muscles PV:Proventriculus

P : Passage No. + : Positive - : Negative

Table (3):Serological follow of IBD maternally derived antibody in white Lohman cockerels .

Age/week	Examined No.	AGP Pos/Ex	VN (Mean)	ELISA absorbancy (Mean)
1	-	ND	ND	ND
2	20	20/20	4096	0.681
3	20	16/20	2048	0.595
4	20	8/20	1024	0.492
5	20	2/20	512	0.375
6	20	0/20	256	0.280

ND: Not done Pos/Ex: Positive /Examined.

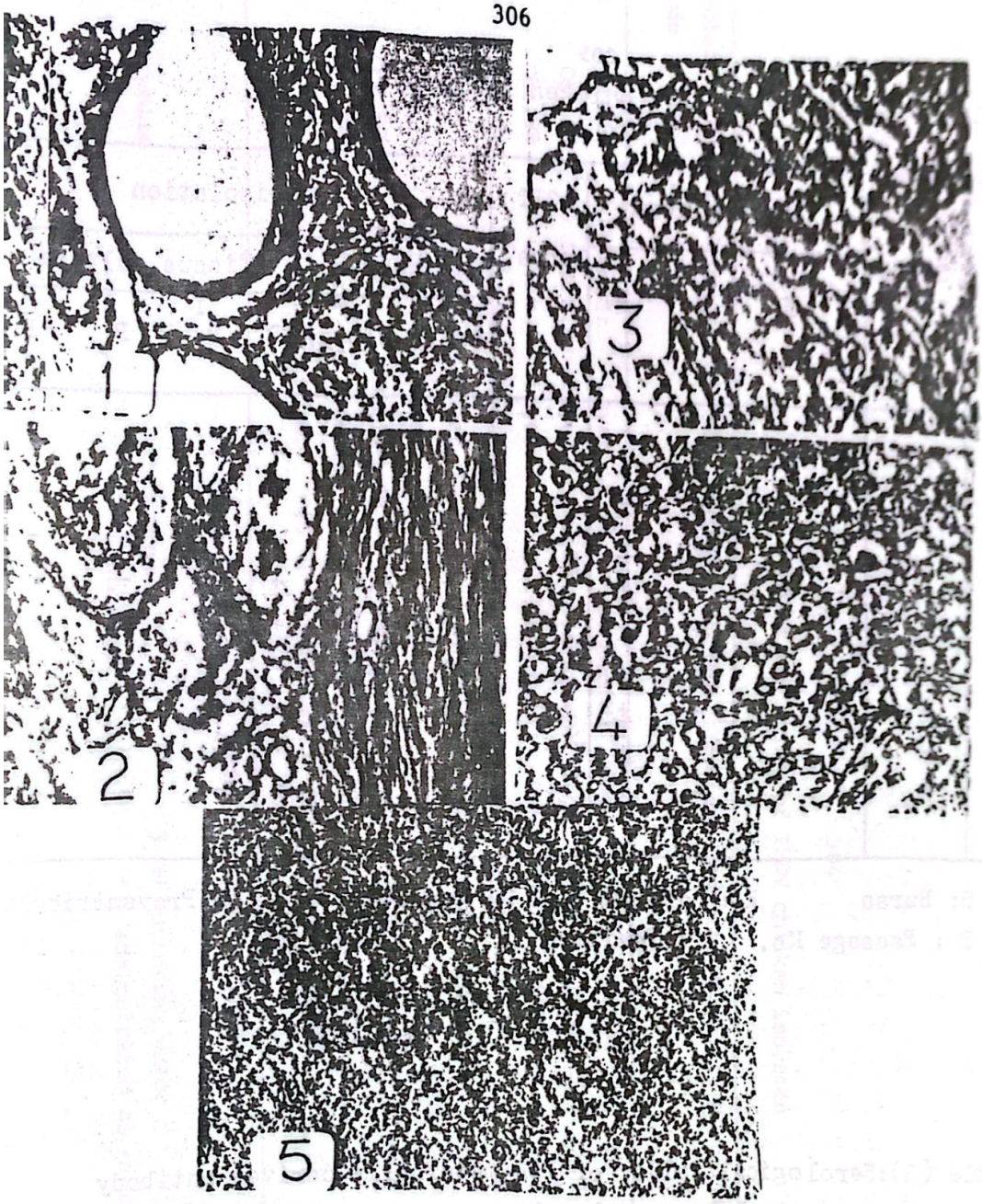

Fig. 1 : Bursa of infected chickens with VVIBDF showing cystic cavities formation. (H & E X 100).

Fig. 2 : Bursa of infected chickens with VVIBDV showing fibroplastic. Changes (H & E X 100).

Fig. 3: Kidney of infected chickens with VVIBDF showing necrobiotic changes (H & E X 100).

Fig. 4: Kidney of infected chickens with VVIBDV showing aggregation of mononuclear inflammatory cells. (H & E X 100).

Fig. 5: Spleen of infected chickens with VVIBDV showing depletion of lymphocytes. (H & E X 100).

other countries (Box, 1989; Lutticken and Van der Marel, 1989 and Zahid, 1990), also, had been reported in Egyptian chicken flocks, characterised by high morbidity, usually closed to 100% and high mortality up to 70%, associated with prominent gross pathological changes as initially reported by Cosgrove (1962) within 10-12 days course. The previous convensional vaccination program using mild and/or intermediate live IBD vaccines once or twice times at 14-35 days age approximately, as recommended by producers for MDA chicks as showed in **(Table, 1)** failed to control the disease. This could be attributable to improper timming of vaccination in presence of MDA chicks, type of live vaccines used and frequency of vaccination (Solano et al., 1985 and Box, 1989).

Isolation of VVIBDV, obtained from clinically affected flocks associated with high morbidity and mortality at 21-52 days age succeded on CAM of embryonated eggs, while failled on CEF on 3 subsequent passages **(Table, 2)**, indicating that isolates belonged to the virulent standard strains of IBDV and not variant types as previously reported (Ismail, 1989).

Results obtained for conurrent bacterial -IBD infected flocks revealed isolation of E. coli, Staph aureus, anthracoid and proteus types, which may be explained as a results of impairment of the defence status of infected chicks consequent to lysis and damage of bursal lymphocytes **(Table, 10)** responsible for development of lymphoid system, which may be further affect the immune status of the recovered chicks (Rosenberger et al., 1975).

The obtained results of pathogenicity for four selective VVIBDV isolates in 42 days age MDA cockerels **(Table, 4)**, revealed that it could penetrate these titer levels of MDA **(Table, 3)** and produced 100%

Table (4): Pathogenicity of selective IBDV isolates for 24 days old white Lohman cockerels inoculated with 100 ul bursal homogenate intraocular .

Isolate code	No. of inoculated chicks	Observation 10-days postinoculation							AGP (bursa)
		Signs.	Deaths		Gross pathological lesions				
			No.	%	B	K	M	PV	
1	4	1	1	25	4/4*	4/4	4/4	1/4	4/4
2	4	0	0	0	4/4	3/4	4/4	0/4	4/4
3	4	2	3	75	4/4	2/4	4/4	1/4	4/4
5	4	0	0	0	4/4	3/4	4/4	0/4	4/4
Control uninoculated	4	0	0	0	0/4	0/4	0/4	0/4	0/4

B : Bursa. K: Kidney M: Muscles PV: Proventriculus

* : Positive No. / Examined No.

Table (5): Pathogenicity of single and pooled* 9-IBDV isolates in various ages of SPF chicks administrated 0.2 ml/bird oculonasal +0.2 ml bird intracloacal .

Isolates code	Age/days	Bursal homogenate dilution	No. birds inoculated	Mortality No.	%
Pool	52	1:10	5	5/5	100
Pool	26	1:10	10	10/10	100
3	52	1:5	10	4/10	40
6	28	1:10	20	16/20	80

* : After 1-chicken SPF passage.

xx : Within 7 days post infection.

Table (6): Protection of SPF chicks immunized with TAD live Gumboro vaccine, strain CU-DM administered via drinking water at various ages and challenged with IBDV isolates 2 and 4 weeks post vaccination using bursal homogenates (0.2 ml/bird oculonasal + 0.2 ml /bird intracloacal).

Age (days) at time of vaccination	No. of vaccinated birds	IBD antibody status before challenge			Challenge			Mortality after challenge	
		ELISA mean	AGP	SN log_2	Isolate code	time	bursal homogenate dilution	Vaccinates	Control
24	5	3754	5/5	ND	pool	4 WPV	1:10	0/5	5/5
12	10	929	8/10	ND	pool	2 WPV	1:10	0/10	10/10
24	10	4716	10/10	11	3	2 WPV	1:5	0/10	4/10

ND : Not done *: 10 days observation period. WPV: weeks post vaccination.

pathological changes as evidenced by histopathological findings of examined bursa, spleen and kidney with variable mortalities ranging from 0.0 to 75% **(Table, 4)**, as previously reported (Box, 1989). On the other hand, the results of pathogenicity in SPF chicks **(Table, 5)** resulted in elevation of mortality percentage to 40%-100% in infected groups, thus emphasized the severity of this current epidemic.

The effect of the VVIBDV isolates on the bursa **(Table, 8)** revealed the same findings reported by Dohms, et al. (1988) where the bursa to body weight ratio was sharply decreased at 10 day post infection, thus indicating the adverse severe effect of the virus resulted in destruction of the bursal cells and consequently reduction of its size to less than four times its normal size. Supporting to these results, were the histopathological findings of the examined bursa, spleen and kidney **(Table, 10 11, & 12)**, where the normal follicular structure of the bursa was altered by degeneration and ncrosis of lymphocytes in medullary area, and replaced by hetrophils and hyperplastic reticuloendothelial cells, inconsistent haemorrhages, oedema and hyperaemia as a result of the inflammatory reaction. , cystic cavities of the medullary areas of the follicles, necrosis and phagocytosis of hetrophils and plasma cells accompanied by fibroplasia in the interfollicular connective tissues. Splenic lesions revealed prominent hyperplasia of the reticuloendothelial cells around the adenoid sheath arteries lymphoid depletion and oedema. Kidney lesions showed infiltration of heterophils, oedema and degeneration. These findings were in agreement with the findings previously reported and reviewed by Faragher (1972) and Okoye et al. (1984). Application of either live commercial or inactivated oil based Gumboro vaccine, strain CU-IM for SPF chicks, resulted in postvaccination specific humeral immune response as detected

Table (7): Protection of IBD-immunized 12-day-old SPF chicks with TAD inactivated Gumboro oil based vaccine administered intramuscularly with recommended field dose (0.5 ml) and challenged 16 days post-vaccination with 1 : 10 dilution of pooled bursal homogenated isolates using 0.2 ml intra-conjunctival + 0.2 ml intracloacal/bird.

Status	Vaccinates (0.5 ml dose)	Non vaccinated
Antibody titer before challenge (Mean ELISA)	332	4
Mortality	0/20 ǂ	16/20

ǂ : Dead/challenged .

Table (8): Bursal body weight ratios (BBWR) and bursal weight reduction (BWR) of 42 days old white Lohmen cockerels inoculated intraocularly with 100 ul bursal homogenates with selective IBDV isolates .

Isolate code	BBWR	BWR
1	1.06	2.4
2	0.92	2.9
3	0.66	3.9
5	1.14	2.2

Table (10): Histopathological changes for bursae resulted from intraoccular infection with selective IBDV isolates (100 ul bursal homogenate per bird) at 42 days or survivors white Lohman cockerels.

Isolate code	Lymphoid necrosis	Lymphoid depletion	Reticulo-endothelial hyperplasia	Fibrosis	Haemorrhage	Oedema	Cystic formation	Hetrophil. infiltration
3	2/2	2/2	2/2	0/2	1/2	2/2	2/2	2/2
1	3/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3
5	3/3	3/3	3/3	3/3	0/3	3/3	3/3	3/3
2	2/2	2/2	2/2	0/2	0/2	2/2	2/2	2/2
Control	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4

Table (11): Histopathological changes of spleen resulted from intraocular infection with selective IBDV isolates (100 ul bursal homogenate) at 42 days old of survivors white Lohman cockerels.

Isolate code	Splenic lesions					
	Congestion	Lymphoid depletion	Reticulo-endothelial hyperplasia	Haemorrhage	Oedema	Hetrophilic infiltration
3	0/2	2/2	2/2	2/2	2/2	0/2
1	0/3	3/3	3/3	0/3	3/3	2/3
5	1/3	3/3	3/3	1/3	3/3	3/3
2	0/2	2/2	2/2	0/2	2/2	0/2
Control	0/4	0/4	0/4	0/4	0/4	0/4

Table (12):Histopathological changes of kidney resulted from intraocular infection with selective IBDV isolates (100 ul bursal homogenate) at 42 days of survivors white Lohman cockerels.

Isolate code	Kidney lesions					
	Congestion	Haemorrhage	Oedema	Degeneration	Glomerular hypercellularity	Hetrophilic infiltration
3	2/2	0/2	2/2	2/2	2/2	2/2
1	3/3	3/3	3/3	3/3	3/3	2/3
5	3/3	2/3	3/3	3/3	3/3	2/3
2	2/2	0/2	2/2	2/2	1/2	0/2
Control	0/4	0/4	0/4	0/4	0/4	0/4

by ELISA, AGP, and SN tests (Table, 6&7) which was 100% protective as measured by challenge against VVIBDV isolates thus indicated that the VVIBD viruses were standard type, 1, IBDV and they could be neutralised by standard type 1 antibody as the same reported by Box (1989).

Antigenicity study of VVIBD viruses using the AGP test previously applied by Weyth and Chettle (1988) revealed that all the examined VVIBDV isolates were the normal classical Gumboro strain, Faragher, so no variants.

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SUMMARY

This study presents evidence of isolation, identification and characterization of 9-field isolates of very virulent infectious bursal disease virus(VVIBDV) prevalent in 9 chicken farms within 9-Egyptian governorates with history of problems essociated with clinical infectious bursal disease (IBD) and unusual high mortalities up to 70%.

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