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Antigenic Characterization and Pathogenicity Study of Recent Field Isolates of Infectious Bursal Disease Viruses

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Epidemiological investigation of severe outbreaks of infectious bursal disease (IBD) occurred among vaccinated chicken flocks during the period 2002-2003 revealed that all IBDV outbreaks occurred at 18-33 days of age, 70% (21/30) of these outbreaks were characterized by typical clinical signs and gross lesions. The mortality rates in these flocks ranged between 9.4-12.8 % (average 11.1 %); 16.4-30 % (average 23.2 %) and 12-23.5 % (average 17.75 %) in commercial broilers, commercial layer replacement pullets and native baladi varieties, respectively. On the other hand, 30% (9/30) of these outbreaks occurred at 18-21 days of age and the mortality rates ranged between 1.4-4.9% (average 3.15%) in commercial broilers and native baladi varieties. IBDV was detected in 100% of the tested bursal homogenates from acutely affected birds using agar gel precipitation test (AGPT) and antigen capture- enzyme linked immuno-sorbent assay (AC-ELISA). Antigenic typing of IBDV from selected outbreaks were carried out by monoclonal antibodies-based AC-ELISA and the pathogenicity of some representative infectious bursal disease virus (IBDV) field isolates were studied. Of the selected 22 IBDV-positive bursal samples, 59.1% (13/22) were typed as classic IBDVs and 40.9% (9/22) were of variant IBDVs. The majority of IBDV variant antigens detected (89% of IBDV variants) were related to IBDV Del/E variant strain and one sample (11% of IBDV variants) was related to RS593 variant strain. The pathogenicity study of representative IBDV field isolates in 49-days-old egg-type chickens revealed that the highest mortalities were 53.3% and 36.6% in birds infected with the two field isolates which typed as classic IBDVs. Whereas, in birds infected with three variants IBDVs related to Del/E (two) and RS593 (one), the mortalities were 10%, 13.3% and 10%, respectively. Moreover, the pathogenicity of such IBDV field isolates indicated that IBDV related to variants Del/E or RS593 caused severe bursal atrophy (BF), as judged by bursal index (BI), in comparison to the classic vvIBDV in susceptible infected chickens. However, the high incidence of variant IBDV antigens detected in tested samples indicates the existence of circulating variant IBDVs despite of vaccination of flocks with classical IBDV vaccines. Regardless of the antigenic differences detected by AC-ELISA and pathogenicity test in the representative isolates, they share antigenic sites with other serotype-1 IBDV strains, as they cross-reacted in AC-ELISA and AGPT. Although, the epidemiological investigation and antigenic typing by AC-ELISA test as well as pathogenicity study suggested that IBD field isolates are in the majority of highly virulent pathotype producing acute disease with severe clinical picture, the current study presents evidence of two variant isolates existing in commercial broiler and native baladi farms with history of problems associated with IBDV.

INTRODUCTION

Infectious bursal disease is one of the most important viral infections occurring in young chickens. The disease is caused by an *Avibirnavirus* from the *Birnaviridae* family designated as *infectious bursal disease virus* (IBDV) (Lukert and Saif, 2003).

Two serotypes (serotype 1 and serotype 2) have been recognized that naturally infect chickens. However, only IBDV strains belonging to serotype 1 are considered pathogenic for chickens (Jackwood et al., 1985). IBDV is a lymphotropic pathogen with a special predilection for differentiating cells in the bursa of Fabricius. Infection can induce B- cell apoptosis, necrosis, and bursal atrophy with a concomitant suppression of the humoral response (Sivanandan and Maheswaran, 1980; Muller, 1986; Rodenberg et al., 1994; Jungmann et al., 2001). Damage to the bursa may occur with a severe inflammatory response such as the one described of standard IBDV strains (Lasher and Shane, 1994; Tanimura et al., 1995). However, atrophy of this organ may be induced with little or no inflammation (Allan et al., 1972; Faragher et al., 1974; Tanimura et al., 1995).

Differences in the relative pathogenicity of IBDV that are apparently related to the host system utilized have been previously reported (Muller et al., 1986; Becht & Muller, 1991; Tanimura et al., 1995; Yamaguchi et al., 1996; Fussell, 1998). However, these authors did not correlate changes in relative pathogenicity with either antigenic or immunogenic differences.

Several investigators, especially in the USA (Saif, 1984; Rosenberger and Cloud, 1986) have reported antigenic variation among the isolates of serotype-1 IBDV. These antigenic variants were also reported through the use of a selected panel of neutralizing monoclonal antibodies (Mabs) (Snyder et al., 1992). Mabs were used in Enzyme linked immunosorbant assay (ELISA) and agar gel precipitation tests (AGPT) (Snyder et al., 1992). Furthermore, viral strains of serotype-1 can also be classified according to their virulence into classical virulent (IBDV), very virulent (vvIBDV) or variant (vIBDV) strains (Van den Berg 2000).

In the summer of 1989, severe outbreaks of very virulent IBD (vvIBD), similar to those reported in European countries in both vaccinated and non-vaccinated flocks, were recorded in several Egyptian provinces, and were associated with drastic mortalities up to 70% in replacement layer pullets and 30% in meat-type birds (El-Batrawi, 1990; Ahmed, 1991 and 1993; Khafagy et al., 1991; Sultan, 1995 and Hassan et al., 2002). In addition, antigenic variant strains have been detected in chicken flocks with IBDV infections (El-Sanousi et al., 1994; Sultan, 1995 and Metwally et al., 2003). Recently, IBDV variant strain was isolated from commercial broiler chicken associated with proventriculitis and stunting syndrome and the isolated virus was typed by monoclonal antibodies-based AC-ELISA as Del/E variant strain of IBDV (Hussein et al., 2003). The incidence of IBDV infection and its associated disease problems were still common in Egypt in spite of the routinely applied vaccination program. Therefore, the present studies were directed towards some aspects related to the IBDV diversity, antigenic characterization and pathogenicity of the recent IBDV

field strains isolated from IBD-infected flocks.

MATERIALS AND METHODS

Epidemiological Investigation of the examined chicken farms

A total of 30 chicken farms naturally affected with IBD were investigated in the present study, they included 14 commercial broiler farms, five commercial layer pullet farms, 11 native (baladi) variety farms, located in five governorates. The capacity of the affected farms ranged between 3,000 and 15,000 chickens and the age of the affected birds ranged between 20-30 days for broilers, 28-55 days for commercial layer replacement pullets, 18-25 days for native birds. All affected farms were floor reared and had history of vaccination against IBD with commercial live vaccines via drinking water route. The history of the investigated farms is shown in table (1).

Samples for IBDV detection

Postmortem examination was performed on a variable number of freshly dead birds which succumbed to the disease after onset of mortalities on the examined farms. Gross lesions were observed and recorded. The birds with typical IBD gross lesions, mainly gross bursal changes, were used for virus detection and typing. For this purpose 6-8 cloacal bursae per farm were collected at the acute stage of the disease. Five bursae were collected as one pool and processed as described by Hirai and Shimakura (1972) to prepare a bursal homogenate for antigen detection, the bursal homogenates were frozen and thawed three times and finally clarified by centrifugation at 3000 rpm for 15 minutes. The supernatant fluid was used for antigen detection by AGPT. Normal bursal homogenates were prepared in the same manner from uninfected 6-week-old chickens to serve as negative antigen control.

Reference antigens and antisera

Known positive and negative precipitating antigens in the form of bursal homogenates and known positive and negative precipitating reference antisera against IBDV obtained from Intervet, Inter. B.V.Boxmeer, Holland, were used for the AGPT.

IBD viruses

a- A local field isolate of vvIBDV isolated and identified by Sultan (1995) in the form of bursal extract was diluted 1: 10 in phosphate buffer saline, which killed 72 % of 7-week-old susceptible commercial male chickens, was passed once in 7-week-old susceptible egg-type male chickens for propagation and was used in pathogenicity studies as challenge virus and was designated as (S-95).

b- Four field isolates from the present study, three from each of IBDV variants (no. 12, 14 & 22) and one IBDV classic (no.1), were used in pathogenicity studies.

ELISA

Commercial ELISA kits ProFlock supplied by Synbiotics Corporation, 11011 via Frontera, San Diego, CA 92127. The kits were used for measuring maternal

antibody decline to determine the accurate the time of pathogenicity study. Application and interpretation of the test were according to the kits manufacturer.

Antigen Capture ELISA (AC-ELISA)

Commercial AC-ELISA kits were supplied by Synbiotic Corporation, 2 rue A. Fleming, 69007 Lyon, France. The test was used for rapid detection and typing of classic and variant strains of IBDV serotype 1 by using a panel of 4 neutralizing Mabs; Mab (#8), Mab (B69), Mab (R 63) and Mab (#10). The Mab (#8) was used for the initial screening of samples because it reacts with viral protein 2 (VP2) epitopes that conserved on both classic and variant IBDV. The remaining Mabs were used for subtyping according to the sample reactivity patterns. IBDV antigens that did not react with Mab (B69) were considered variants (Snyder *et al.* 1992). It was carried out according to Lamichhane *et al.* (2000) .This test was used to detect IBDV-antigen(s) in the cloacal bursa of the affected chickens and IBDV vaccines.

Interpretation of results of AC-ELISA

An optical density (OD) reading of 0.600 or higher indicates the presence of IBD viral antigen (+). Optical densities less than 0 600 are indicative of the absence of IBD viral antigen (-). The Antigen-Capture ELISA results interpretation are shown in the following table:

AC-ELISA reactivity patterns of IBDV antigens

Virus type	Mabs			
	#8	B69	R63	#10
Classic	+	+	+	+
Gls/Y2k	+	-	-	+
Del-E	+	-	+	-
RS593/AL2	+	-	-	-

Agar gel precipitation test

The test was used to demonstrate the presence of antibodies to IBDV in examined chicken sera and for detection of IBDV antigen(s) in the cloacal bursa of affected chickens as described by Wood *et al.* (1979).

Pathogenicity studies of recent IBDV field isolates

For this purpose, sufficient one-day-old, commercial egg-type male chicks(Bovans) were produced from a commercial hatchery (Fat Hens company), which possessed maternal antibodies against IBD, acquired from their parents that were vaccinated with live and inactivated oil emulsion IBDV vaccines according to a specific vaccination program, were used. The maternal antibody waning in those chicks was followed up at different intervals starting from 7 days up to 49 days of age. They were examined individually by AGPT and ELISA. At day 49 of age, six groups, 30 chicks each, were subjected to ocular and nasal challenge with 100-µl of field isolates of IBDV in the form of bursal extract and observed for 14 days post-infection (PI) according to the experimental design in the following table:

The experimental design of pathogenicity studies with IBDV field isolates

Groups No.	IBD challenge virus*			Criteria adopted for evaluation of pathogenicity		
	Isolate No.	Isolate year	IBDV antigenic type**	Observation for 14 days Pch[1]	Serology[3]	Antigen detection
1	1	2002	Classic	1-clinical signs. 2-Mortality percent. 3-Gross lesions. 4-B: B Index[2] for survivors at day 7 Pch.	1-Follow up of maternally derived antibodies (MDA). 2-Seroconversion 14 days Pch.	Pool of bursal homogenates of dead birds.
2	12	2002	Variant Del.E			
3	14	2003	Variant RS 593			
4	22	2003	Variant Del.E			
5	vvIBDV isolate (S-95)	1995	classic			
6	Non-challenged control					

*Challenge= Oculo-nasal challenge at 49 days of age with 100 µl/bird of IBDV field isolates. ** According to AC-ELISA. Pch[1]= Post-challenge. B: B[2] Index= Bursal body weight index (Lucio and Hitchner, 1979). Serology[3]= AGPT and ELISA were used.

Assessments of pathogenicity study

1-Clinical signs; mortality percentage and postmortem gross lesions were recorded.

2- Detection of IBDV antigen(s) in the cloacal bursae of dead birds.

3- Bursa: body weight ratio, bursal index and bursa: body weight index were calculated by the formulas given, respectively, by Sharma *et al.* (1989) and Lucio and Hitchner (1979) as follows:

-Bursal index = Bursal weight / Body weight X 1000

-Bursa: body weight index = bursa/body weight ratio of infected chickens / Mean bursal body weight ratio of uninfected chickens. Chickens with bursa: body weight index lower than 0.7 were considered by Lucio and Hitchner (1979) to have bursal atrophy.

RESULTS

Epidemiological Investigation of the examined chicken farms

During the period 2002-2003, 30 IBD outbreaks were investigated, which occurred in five governorates (Kafr El-Sheikh, El-Gharbia, El-Dakahlia, El-Menofia and El-Kalubia) and included different types of chickens (14 commercial broiler farms, 5 commercial layer pullet farms and 11 native baladi variety farms). All of these IBD outbreaks occurred at 18-33 days of age. From the investigated outbreaks, 70% (21/30) outbreaks were characterized by the presence of clinical signs, gross lesions and mortality rates as well as the mortality pattern that were characteristic to IBD. The mortality ranged between 9.4-12.8 % (average 11.1 %) in commercial broilers farms, 16.4-30 % (average 23.2 %) in commercial layer replacement pullets and 12-23.5 % (average 17.75 %) in native baladi varieties. On the other hand, 30% (9/30) of the outbreaks were characterized by low mortality, a mortality pattern that were dissimilar to that is characteristic for IBD with no obvious signs except for the severely atrophied Bursa and sometimes thickening of the proventriculus. The mortality ranged between 1.8-4.9 % in commercial broilers farms and 1.4-2.7 % in native baladi varieties with total mortality ranged between 1.4-4.9% (average 3.15%) in these outbreaks. Out of 30 IBD outbreaks, 80% (24/30) outbreaks were characterized by a course of the disease about 7-10 days and mortality peaked rapidly in the 4^{th} or 5^{th} day after the onset of the disease, to fall rapidly to normal during the next 4-5 days. In 20% (6/30) of the investigated IBD outbreaks, the course of the disease did not behave the characteristic course of IBD.

Affected birds showed profuse watery yellowish white diarrhea with soiled vent feathers, vent picking, anorexia, depression, trembling, and prostration and finally died. The gross lesions were mainly dehydration, extensive hemorrhages on the muscles of the legs, breast and wings, swollen pale or congested kidneys with prominent tubules and distension of the ureter with urates. The Bursa was constantly involved, and was either enlarged, edematous, yellowish-pink or hemorrhagic and contained blood in the lumen, or reddish in color and atrophic. In advanced stages, the bursa was sometimes almost normal in size

but filled with creamy or caseous exudates and sometimes the plicae showed petechial haemorrhages.

All investigated flocks have been vaccinated several times between 10-28 days of age using various classes ("intermediate" and/or "hot") of standard serotypes of live IBD vaccines administered via drinking water route. The history and locality of examined chicken farms are shown in table (1) and the characteristic summary of classic and variant IBDV outbreaks in the examined chicken farms are tabulated in table (4). The gross lesions of some investigated cases are shown in Fig 1.

Results of pathogenicity studies of commercial white egg-type male chickens challenged with IBDV field isolates:

Table (4) shows the maternal antibody waning in commercial white-egg-type male chickens of one hatch as judged by AGPT and ELISA. The maternal precipitins were not more demonstrable at 28 days of age, whereas negative ELISA titers were found at 42 days of age. Results of experimental infections of the groups of chickens with 100 µl/ bird of recent field isolates IBDV (No. 1,12, 14 & 22) and vvIBDV reference isolate (S-95) via oculonasal route at 49 days of age are presented in Table (5).

Birds were observed for 14 days post-challenge. The highest mortality was 53.3% and 36.6% inbirds infected with vvIBDV field isolate (S-95) and local field isolate typed by AC-ELISA test as vvIBDV, respectively. In birds infected with variants related to IBDV, the mortalities were 10%, 13.3% and 10%, respectively (Table 5). In all cases, all mortalities occurred 3-5 days post-infection and birds inoculated with IBDV field isolate No. 1 and S-95 showed hemorrhagic gross lesions typical of IBDV infection. However, the birds challenged with IBDV local field isolates No. 12, 14 and 22 showed bursal atrophy and other birds showed typical gross lesions of IBDV as in Fig. 1. IBDV antigen was demonstrated in all bursal homogenates of dead birds. The most important findings of the pathogenicity study is the induction of lesions in the proventriculus with both classic and IBDV-related to variant (Del/E or RS593).

The seroconversion via AGPT was 100%(5/5) in all infected birds and 0% (0/5) in the control non infected birds. The ELISA titers were 1215±218, 1297±160, 1214±198, 1377±264, 1309±317 and 0.0 in all infected and non infected control groups, respectively. BI was 0.371, 0.230, 0.211, 0.220, 0.301 and 1.0 in all infected and non infected control groups, respectively. Using the formula of Lucio and Hitchner (1979) for determining the bursa: body weight index markedly lower values than the reference value of 0.7 were found at 7 days PI, denoting bursal atrophy with different degrees in all affected groups, as shown in table (5).

Table (1): Epidemiological investigation of the examined chicken farms

Code No.	Date	Governor-ate	Bird type	Bird variety	chicks No. No.	Age/day at disease Onset.	Total Mortality	
							No.	%
1	Aug.2002	Kafr El-Sheikh	B	Hubbard	5000	26	640	12.8
2	Oct.2002	Kalubia	B	Hubbard	5000	21	102	2.4
3	Oct.2002	Kalubia	B	Hubbard	7000	27	770	11
4	Sep.2002	Kafr El-	B	Hubbard	5000	20	150	3.0
5	Oct. 2002	Gharbia	B	Ross	5000	23	620	12.5
6	Nov.2002	Gharbia	B	Ross	5000	19	180	3.6
7	Nov.2002	Gharbia	B	Ross	4500	28	440	9.7
8	Nov.2002	Gharbia	B	Ross	5000	30	490	9.8
9	Oct.2002	Kalubia	B	Hubbard	5000	19	90	1.8
10	Oct.2002	Kalubia	B	Hubbard	5000	28	470	9.4
11	Dec.2002	Dakahlia	B	Hubbard	5000	27	502	10.04
12	Dec.2002	Dakahlia	B	Hubbard	10000	18	320	3.2
13	Dec.2002	Monofia	B	Hubbard	3000	22	380	12.6
14	Dec.2002	Monofia	B	Hubbard	4000	20	196	4.9
15	Jan.2003	Kalubia	C.L	LSL**	10000	32	2400	24
16	Jan.2003	Kalubia	C.L	LSL**	7000	33	2100	30
17	Jan.2003	Dakahlia	C.L	Bovans	15000	29	2460	16.4
18	Nov.2002	Gharbia	C.L	LSL**	10000	33	2310	23.1
19	Nov.2002	Gharbia	C.L	Bovans	10000	30	2380	23.8
20	Jan.2003	Gharbia	N.B	Baladi	10000	24	2160	21.6
21	Jan.2003	Gharbia	N.B	Baladi	8000	26	1440	18
22	Jan.2003	Gharbia	N.B	Baladi	6000	18	130	2.1
23	Jan.2003	Gharbia	N.B	Baladi	5000	27	950	19
24	Jan 2003	Gharbia	N.B	Baladi	15000	27	3300	22
25	Jan.2003	Gharbia	N.B	Baladi	12000	26	2820	23.5
26	Jan 2003	Gharbia	N.B	Baladi	10000	20	140	1.4
27	Jan.2003	Gharbia	N.B	Baladi	5000	20	120	23.2
28	Jan 2003	Gharbia	N.B	Baladi	7000	21	189	2.7
29	Feb 2003	Dakahlia	N.B	Baladi	10000	31	1200	12
30	Feb.2003	Dakahlia	N.B	Baladi	10000	29	1670	16.7

Table (1): continued.

Pattern of mortality per day*							IBD vaccination Schedule		
1	2	3	4	5	6	7	Frequency	Age/day	Vaccine strain
18	72	250	190	60	33	17	2x	11,22	D-78
16	18	18	20	13	12	5	2x	11,22	D-78
70	160	280	200	25	20	15	1x	12	2512
15	26	24	22	22	21	20	2x	12,23	D-78
22	92	224	154	72	40	16	2x	10,23	D-78
26	24	24	28	24	28	26	2x	10 20	D-78 228-E
10	65	168	109	48	28	12	1x	14	228-E
15	72	182	113	53	37	18	1x	13	2512
15	11	16	17	8	12	17	1x	12	2512
14	68	175	108	52	35	18	1x	11	2512
20	75	186	117	58	41	23	2x	11,22	D-78
17	50	55	52	51	55	40	2x	10,23	D-78
10	60	148	80	43	26	13	1x	14	228-E
27	31	28	33	38	21	18	2x	12,22	D-78
44	190	1200	490	260	176	40	2x	18 27	D-78 228-E
60	175	1155	380	185	80	65	2x	14 22	D-78 228-E
52	198	1210	500	268	184	48	2x	13 21	D-78 228-E
32	178	1185	475	248	164	28	2x	20,28	D-78 228-E
40	186	1194	486	259	175	40	2x	14 21	D-78 228-E
65	180	1170	395	190	87	73	2x	10 22	ALS's ST-12 228-E
20	131	844	252	96	61	36	2x	10 22	D-78 228-E
18	19	17	18	20	20	18	2x	10 22	D-78 228-E
15	40	520	230	105	26	14	2x	10 22	D-78 228-E
42	194	1930	740	293	77	24	2x	10,22	D-78
85	264	1150	894	310	77	40	2x	10,18	D-78
21	19	18	22	20	17	23	2x	10,18	D-78
38	65	333	127	48	29	20	2x	10 22	ALS's ST-12 D-78
27	33	24	25	22	26	32	2x	12,22	D-78
68	116	485	278	105	86	62	2x	10,20	D-78
51	114	970	325	100	67	43	2x	10,18	D-78

** = Lohmann Selected layer B= Broiler C.L.=Commercial layers
N.B =Native breed *No. = Number* *X = Vaccination number.*
* = Recorded number from onset of IBD mortality

Table (2): Results of IBDV antigen(s) detection and typing of examined flocks by AC-ELISA

Flock No.	Age/days	AC-ELISA reactivity pattern				IBDV-typing
		Mabs				
		#8	B69	R63	#10	
1	26	+	+	+	+	Classic
2	21	+	--	+	--	Delaware E
3	27	+	+	+	+	Classic
4	20	+	--	+	--	Delaware E
5	23	+	ND	ND	ND	--
6	19	+	--	+	--	Delaware E
7	28	+	+	+	+	Classic
8	30	+	+	+	+	Classic
9	32	+	--	+	--	Delaware E
10	28	+	ND	ND	ND	--
11	27	+	+	+	+	Classic
12	18	+	--	+	--	Delaware E
13	22	+	+	+	+	Classic
14	20	+	--	-	--	R S 593
15	32	+	+	+	+	Classic
16	33	+	ND	ND	ND	--
17	29	+	+	+	+	Classic
18	33	+	+	+	+	Classic
19	30	+	ND	ND	ND	--
20	24	+	+	+	+	Classic
21	26	+	ND	ND	ND	--
22	28	+	--	+	--	Delaware E
23	27	+	+	+	+	Classic
24	27	+	+	+	+	Classic
25	26	+	ND	ND	ND	--
26	24	+	--	+	--	Delaware E
27	20	+	+	+	+	Classic
28	21	+	--	+	--	Delaware E
29	31	+	ND	ND	ND	--
30	29	+	ND	ND	ND	--
31 (ALS's ST-22)	--	+	+	+	+	Classic
32 (D-78)	--	+	+	+	+	Classic
33 (2512)	--	+	+	+	+	Classic
34 (228)	--	+	+	+	+	Classic
35 (Positive control)	--	+	+	+	+	Classic
36 (Negative control)	--	--	--	--	--	--

ND = Not done
AC-ELISA= antigen-Capture enzyme-linked immunosorbant assay.
Mabs= monoclonal antibody.
IBDV= infectious bursal disease virus.

Table (3) Characteristic summary of classic and variant IBDV* outbreaks in the examined chicken farms

Flocks		Incidence Age / day	Mortality %	Gross lesions	IBDV AC-ELISA Typing
Type	No.				
-Commercial broiler farms-Native baladi variety farms	6 3	18-32 21-28	1.8-4.9 1.4-2.7	-Severely atrophied bursa of Fabricus. Thickening and hemorrhage of the proventriculus.	-Related to variant Delaware E and RS 593 strains.
Total	9	18-32	1.4-4.9		
-Commercial broiler farms -Commercial layer farms -Native baladi variety farms	8 5 8	22-30 29-33 20-31	9.4-12.8 16 4-30 12-23.5	-Enlarged and/or hemorrhagic BF. -Petechial and/or early hemorrhage on thigh and/or pectoral muscle.	-Classic IBDV.
Total	21	20-33	9.4-23.5		

-BF= Bursa of Fabricius.

DISCUSSION

Infectious bursal disease (IBD) is a major virus-induced immunosuppressive condition of young chickens, which has spread worldwide since the early seventies (Box, 1989; Van den Berg *et al.*, 1991). Because the vaccination is a basic method for controlling IBDV, IBD-induced immunpsuppression has long represented a major cause of economical losses to poultry industry (Lasher and Shane, 1994). However, since 1987, acute IBD caused up to 30-60 % mortality in broiler and pullet flocks, respectively. These have been related to the emergence of pathotypic variants of IBDV known as very virulent virus (Box, 1989; Chattel *et al.* 1989; Van den Berg *et al.*, 1991). IBD outbreaks with these characters appeared in Egypt and occurred since 1989 and have caused serious economic losses despite vaccination (El-Batrawi, 1990; Khafagy *et al.*, 1991; Sultan, 1995, Metwally *et al.*, 2003 and Abdel-Alim, 2003). In the present study, the investigation covered 30 IBDV suspected outbreaks, which involved different types of chickens in five governorates over the period 2002-2003. These investigations showed that (100%) of these outbreaks occurred at 18-33 days of age: 70% (21/30) of these outbreaks were characterized by typical clinical signs and gross lesions, similar to those described by Box (1989); Van den Berg *et al.* (1991), and Hassan *et al.* (2002). Moreover, the mortality rates in these flocks, ranged between 9.4-12.8 % (average11.1%); 16.4-30% (average 23.2%) and 12-23.5% (average 17.75%) in commercial broilers, commercial layer replacement pullets and native baladi varieties, respectively. On the other hand, 30% (9/30) of these outbreaks occurred at 18-21 days of age an the mortality rates ranged between 1.4-

4.9% (average 3.15%) in commercial broilers and native baladi varieties. Similar results were obtained previously by Rosenberger *et al*, (1987); Saif *et al*. (1987); Ismail *et al*. (1990); Lukert and Saif (1991), and Jordan and Pattison (1996). The differences in age susceptibility and mortality rates have been previously reported (Giambrone *et al*., 1982; Box, 1989, and Van den Berg, 2000).

Of 80% (24/30) of the investigated outbreaks, the coarse of the disease was acute lasting 7-10 days with most of the mortalities occurred within 3-5 days after the onset of the disease. In contrast, the course of the disease and the mortality pattern did not have the pattern of the classic IBD outbreaks in 20% (6/30) of the investigated outbreaks. This was probably related to the protective level of the IBD-vaccine strain used during vaccination and/or the virulence of IBDV challenge strain. Similar results were observed by Riks *et al*. (2001). Indeed, IBDV was confirmed by detection of IBDV antigen(s) in bursal homogenates from acutely affected birds as previously reported (Cheville, 1967; Cullen and Wyeth, 1975; Fadly and Nazerian, 1983).

All investigated flocks have been vaccinated several times between 10-28 days by various classes (intermediate and/or hot) of standard serotype1 live IBDV vaccines. However, in all these cases vaccine failure and IBD outbreaks occurred. The possible causes of vaccination failure could be due to the high field virus exposure, timing of IBDV vaccination, application of vaccine and IBDV challenge strain; has been reported by documents (Wyeth, 1980; Wood *et al*., 1981; Giambrone *et al*., 1982; Solano *et al*., 1985)

IBDV undergoes antigenic variation in nature (Rosenberger and Cloud, 1986; Snyder *et al*., 1988; Vakharia *et al*., 1994). Its diverse antigenic structure has been demonstrated in serotype 1 and 2 (Mcferran *et al*., 1980; Jackwood and Saif, 1987) and subtypes of serotype 1 (Rosenberger and Cloud, 1986; Jackwood and Saif, 1987). The molecular basis for this antigenic variation is associated with VP2 neutralizing and non-neutralizing epitopes on VP2 and VP3 proteins of IBDV using monoclonal antibodies (Mabs) (Fahey *et al*., 1989; Lee, 1990; Whetzel and Jackwood, 1995). The VP2 epitopes have also been recognized as confirmation dependent, confirmation independent and cross-reactive depending on their location on VP2 protein (Eterradossi *et al*., 1998, 1999; Sellers *et al*., 1999; Boot *et al*., 2000; Yu *et al*., 2001; Brandt *et al*., 2001; Cavanagh, 2001; Hoque *et al*., 2002; Kataria *et al*., 2001; Toroghi *et al*., 2001; Van Loon *et al*., 2002).

Characterization of the antigenic diversity of IBDV field isolates in certain locality is important in order to develop an effective vaccination program to control IBD. This important stem from the fact that antigenic variants escape an immune response induced by vaccination

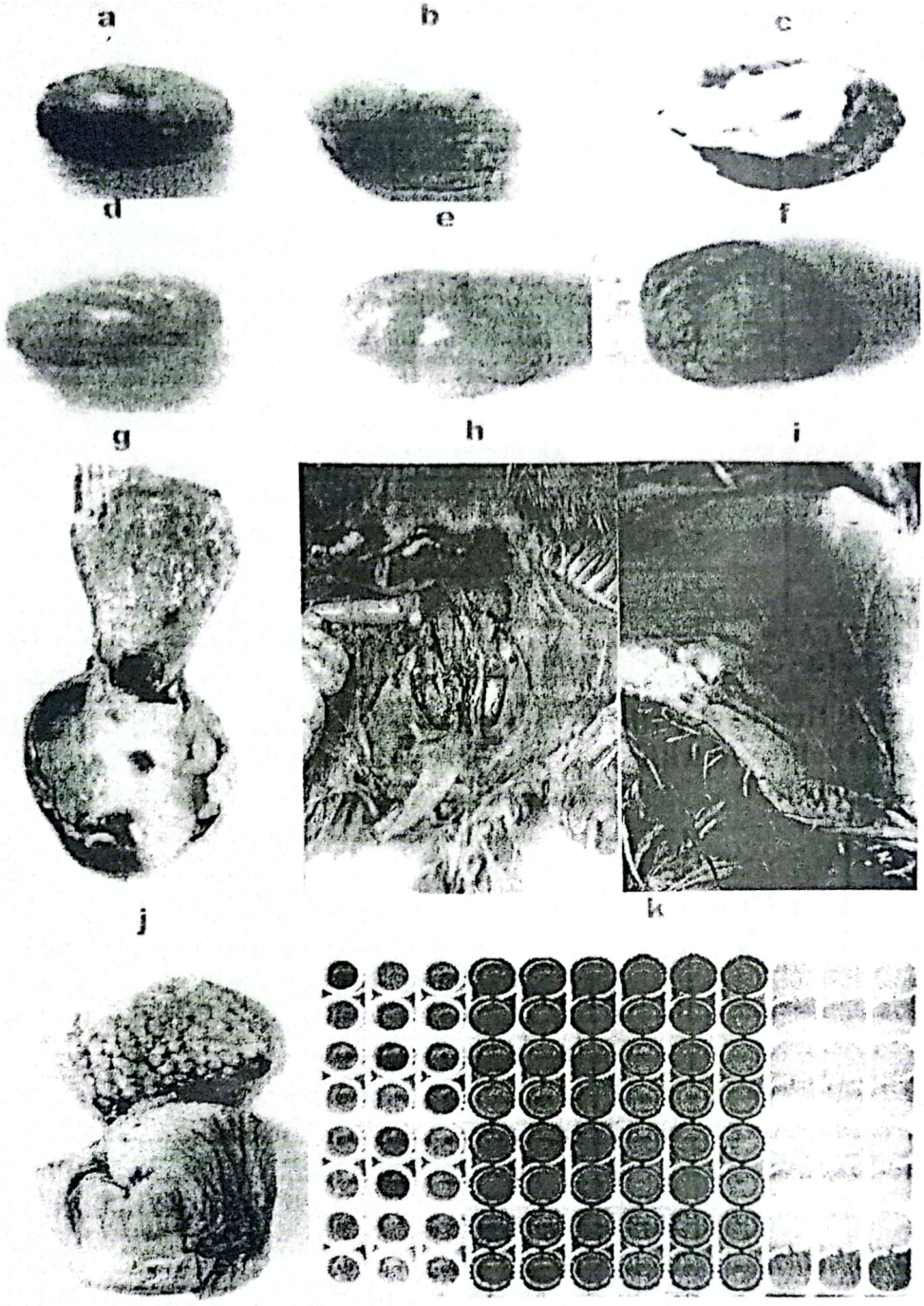

Fig. (1): A and F: Severe hemorrhagic bursae at 3 days PI of 49-days old white-egg-type chickens experimentally infected with vvIBDV local field isolate No. 1 . B and D: Edematous enlarged bursa at 4 days PI of 49-days old white-egg-type chickens experimentally infected with vvIBDV local field isolate No. 1 in comparison with non-infected control (E). C: Caseous material filled congested bursae at 5 days PI of 49-days old white-egg-type chickens experimentally infected with vvIBDV local field isolate S-95. G: Severe congested edematous proventriculus gland with hemorrhages at the proventricular-gizzard junction (Naturally infected chickens). H. Severe kidney swelling and the ureter distended with urates (Naturally infected chickens). I: Severe hemorrhagic strips on the thigh muscle (Naturally infected chickens). J: Thickening of proventriculus 7 days PI of 49-days old white-egg-type chickens experimentally infected with vvIBDV local field isolate no. (12). K: AC-ELISA of IBDV field isolates derived from bursa of Fabricius showing the pattern of reactivity against Mabs (B69; R63 and # 10).

Table (4): Waning of maternally derived antibody in commercial white egg-type male chickens used for pathogenicity studies of some recent IBDV field isolates

Age/days	Serological tests			
	AGPT (Positives No./examined No.)		ELISA	
	No.	%	Mean Titer ±Sd	%CV
7	20/20	100	8832±977	9.527
14	15/20	75	5030±427	20.330
21	3/20	15	2852±674	38.220
28	0/20	0	1385±881	35.010
35	0/20	0	660±730	39.640
42	0/20	0	0.0	--
49	0/20	0	0.0	--

AGPT=Agar gel precipitation test. ELISA=Enzyme linked immuno-sorbant assay. Sd = Standard deviation. %CV = Coefficient of variance.

Table (5): Results of pathogenicity studies of commercial white egg-type male chickens challenged with representative IBDV field isolates and vvIBDV (S-95) at 49 days of age

Groups No.	IBDV field isolates	Mortality		BI	Precipitinogen detection in dead birds (Positives No./examined No.)	Seroconversion at 14 days PI	
		No.	%			AGPT (Positives No./examined No.)	ELISA (Mean Titers±Sd)
1	1	11/30	36.6	0.371	11/11	5/5	1215± 218
2	12	3/30	10	0.230	3/3	5/5	1297± 160
3	14	4/30	13.3	0.211	4/4	5/5	1214± 198
4	22	3/30	10	0.220	3/3	5/5	1377± 264
5	S-95	16/30	53.3	0.301	16/16	5/5	1309± 317
6	Non-infected control	0/30	0	1.00	---	0/5	0.0

AGPT=Agar gel precipitation test. ELISA=Enzyme linked immunosorbant assay. *vvIBDV isolated and identified in 1995 (Sultan, 1995). Sd = standard deviation. BI = Bursal index. No. =Number.

ith classical standard IBDV vaccines (Rosenberger *et al.*, 1985). A new AC-ELISA is used for detection of IBDV antigens directly from bursal samples using a panel of monoclonal antibodies reactive to a selected group of VP2 epitopes (Lamichhane *et al.*, 2000). This assay utilized the

selective binding affinity of neutralizing monoclonal antibodies to differentiate between classic and variant IBDV isolates (Snyder *et al.*, 1988-a & b; Snyder *et al.*, 1992 and Lamichhane *et al.*, 2000).

In this study, variant IBDV antigens detected in 40.9% of the total IBDV positive samples (Table 3). The high incidence of variant IBDV antigens detected in tested samples indicates circulation of variant IBDVs despite of vaccination of flocks with classical IBDV vaccines. Variant IBDVs escape in the presence of classical IBDV vaccination has been previously reported (Rosenberger *et al.*, 1987 and Snyder, 1990). Infection of chicken with variant IBDV before 3 weeks of age is possible because variant IBDV can escape high level of maternal antibodies produced against classical IBDV (Snyder *et al.*, 1992 and Van den Berg, 2000 and 2002). This age of susceptibility explains why variant IBDV antigens were detected in samples from nine flocks collected between 18-32 days of age (Table 4). Similar results were previously reported in Egypt by El-Sanousi *et al.* (1994); Sultan (1995) and Metwally *et al.* (2003). On the other hand, classical IBDVs typically appeared at 3-5 weeks of age (Table 4) (Lukert and Saif, 1991). The majority of IBDV variant antigens detected (89 % of IBDV variants) were classified as Del/E. One sample (11% of IBDV variants) was RS593 variant IBDV (Table 3). In the support of other studies (Elankumaran *et al.*, 2002), it also provides evidence of the persistence of variant IBDV in vaccinated flocks. In similar study, Davison (2003) found that the intra-serotype-common neutralization site defined by the R63 Mab was present in IBDV isolates and laboratory strain tested. However, the neutralization site defined by the B69 Mab was found only on classic or older serotype-1 strains but not on the recently isolated variants of serotype-1 IBDV. He suggested that a major antigenic shift in IBDV had occurred in the field, which was probably favored by selection pressure due to the intensive use of vaccination based on classical serotype-1 strains.

The pathogenicity of recent IBDV isolates in birds with maternal derived antibodies (MDA) might generate information leading specifically to a more effective control of IBD in the field. The highest mortality was 53.3% and 36.6% in birds infected with vvIBDV field isolate (S-95) and field isolate typed by AC-ELISA test as classic IBDV, respectively. These results simulate those reported by Amer *et al.* (1985); Sultan (1995) and Eid (2000). In birds infected with variants related to IBDV, as tested by AC-ELISA, the mortality was 10%, and 13.3% (Table 5) like those reported by others (Lukert and Saif, 2003). Yet, infection of chickens derived from vaccinated parents with the field isolates at 49 days of age having level of MDA revealed variable mortality rates ranging between 10%and 53.3%. Differences in mortality rates could be attributed to variable level of the residual MDA

at time of infection (El-Batrawi and El-Kady, 1990 and Van den Berg, 2000).

In all cases, all mortalities occurred 3-5 days post-infection with field isolate No. 1 and S-95 and the infected birds showed hemorrhagic gross lesions typical of IBDV infection. On the other hand, birds, challenged with IBDV field isolates No. 12, 14 and 22 showed typical gross lesions or bursal atrophy (Fig.1). Positively, IBDV antigen could be demonstrated in all bursal homogenates of dead birds by AGPT.

The most important findings of the pathogenicity study include the induction of gross lesions in the proventriculus (Fig. 1) with both classic and IBDV-related to variant (Del/E or RS593) as previously emphasized by studies conducted on proventriculitis (Skeels and Newberry, 2000) which indicated that the presence of IBDV in proventriculitis is a contributing factor in the incidence of proventriculitis, moreover, the appearance of this virus corresponds to the natural decline of IBDV maternal antibody within three weeks of age of commercial broiler chickens. In addition, their studies indicated that both classic (STC) and variant (Del/E) IBDV could induce proventriculitis in chickens. However, the significance of IBDV as a cause of the proventricular abnormalities that have plagued the broiler industry in the recent years remains to be established but if multiple factors are required to result in the problem then certainly IBDV must be considered as having a possible role (Hussein, *et al.*, 2003).

This study presented evidence of two variant isolates of IBDV prevalent in commercial broilers and native baladi farms with history of problems associated with IBDV. The variants designed Del/E and RS593 caused severe atrophy of the BF of the susceptible chicks with low mortality percentages in comparison to classic vvIBDV strains as shown in Table (6).These results are similar to the findings of other researchers (Rosenberger & Cloud, 1985 and Rosenberger *et al.*, 1987).

The results of IBDV-typing by AC-ELISA and the pathogenicity test of some recently isolated IBDV, probably, suggesting that there are major antigenic differences between strains isolated in different poultry producing area in Egypt. A hypothesis to explain the existence of antigenically different IBDVs in the field referred to serotype I variant strains (Rosenberger and Cloud, 1985) or subtype (Jackwood and Saif, 1987). Nevertheless, although the marked increase in acute IBD in different of the world dominates the field picture, strain of different virulence still co-exist, warranting the need for a rapid discrimination between circulating strains. So far, no Mab specific for the very virulent strains have been obtained but , as previously mentioned , a modified neutralizing epitopes has been identified by Eterradossi *et al.*(1997). VP_2 can also used as a molecular testing like

reverse transcription- polymerase chain reaction (RT- PCR) followed by restriction enzyme digestion or restriction fragment length polymorphism (RFLP) analysis of the amplified fragment was helpful (Jackwood *et al.*, 2001). However, molecular characterization of VP2 by sequence analysis will help in the identification of the minor difference in the circulating strains and its impact in the developing an effective vaccination strategies.

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