Vet.Med.J.,Giza. Vol.51, No.1. (2003) :105-119.

DIRECT DETECTION OF VARIANT INFECTIOUS BURSAL DISEASE VIRUS IN VACCINATED EGYPTIAN BROILER FLOCKS USING ANTIGEN-CAPTURE ELISA

A. M. METWALLY*, M. Z. SABRY, A. M. SAMY***, M. M. OMAR*, A. A. YOUSIF*** and I. M. REDA*****

* Private Poultry Diagnostic Laboratory, Giza, Egypt.

** Egytech, Dokki, Cairo, Egypt.

*** Virology Department, Faculty of Veterinary Medicine, Cairo University, Egypt.

Received: 5.12.2002.
Accepted:31.12.2002.

SUMMARY

Infectious bursal disease virus (IBDV) serotype I isolates result in serious economic losses to the worldwide poultry industry. Variant IBDV strains are usually isolated from flocks vaccinated using classical IBDV vaccines. Monoclonal antibody typing has been used to differentiate between classical and variant strains. A total of 277 bursal samples from 13 vaccinated broiler flocks were tested for IBDV subtypes. A panel of 4 monoclonal antibodies directed against VP2 epitopes was used to test the bursal homogenates using an antigen-capture ELISA (AC-ELISA). Of the total number of samples, 73.6% were IBDV-antigen-positive, of which, 41.7% were classical IBDV and 58.3% were variant IBDV. Del/E was the predominant variant detected in the tested flocks (51.3% of IBDV-positive samples). Moreover, 47% of the total variants were untypeable. The presence of variant IBDV in vaccinated Egyptian broiler flocks demonstrates the need for a revision of current control strategies and vaccination programs.

Key words: IBDV; Classical; Variant; Antigen-capture; Antigenic profile.

INTRODUCTION

Infectious bursal disease (IBD) is an acute highly contagious viral infection in young chickens. Infectious bursal disease virus (IBDV) infections lead to direct and indirect significant economic losses to the worldwide poultry industry. The direct economic impact of IBD is due to the high

mortality rates (Chettle et al., 1989; Van Den Berg et al., 1991), while the indirect impact is due to immunosuppression of infected birds (Allan et al., 1972). Immunosuppression following IBDV infection is due to virus-induced destruction of B-lymphocyte precursors in the bursa of Fabricius (Hirai et al., 1981). IBDV-induced immunosuppression is a leading cause of vaccination failure and bad performance in chicken (Giambrone et al., 1976; Giambrone et al., 1979). IBD viruses are non-enveloped, icosahedral members of the genus Avibirnavirus of Birnaviridae (Dobos et al., 1979; Hirai and Shimakura, 1974). The double stranded RNA genome of IBDV codes for five proteins. The major structural proteins of the virion are VP2 and VP3, both of which are constituents of the IBDV capsid. VP2 carries the major neutralizing epitopes (Azad et al., 1987; Becht et al., 1988).

Two distinct serotypes, I and II, have been identified (Jackwood and Saif, 1987; Jackwood et al., 1985 ; McFerran et al., 1980). All known pathogenic IBDV strains belong to serotype I. Serotype II viruses infect mainly turkey but have not been associated with clinical disease (Ismail, et al., 1988). Antibodies against serotype II do not protect against serotype I (Jackwood and Saif, 1985).

IBDV serotype I strains are substantially heterogeneous with respect to pathogenicity (Lukert and Saif, 1997) and antigenicity, as determined by virus neutralization tests (Jackwood and Saif, 1987; Snyder, 1988b). Pathogenic IBDV serotype I isolates are commonly grouped based on antigenic and pathogenic properties in one of three categories; classical, very virulent and antigenic-variant.

Antigenic-variant IBDV was first reported in the United States (Rosenberger et al., 1985; Rosenberger et al., 1986). Variant strains of IBDV are usually isolated from vaccinated flocks. These IBDV variants are antigenically different from classic strains of IBDV; they lack classical epitope(s) defined by neutralizing monoclonal antibodies (Snyder et al., 1988b; Snyder, 1990 Snyder et al., 1992). Most of these epitopes are located in the VP2 hypervariable region (Snyder et al., 1988a).

IBD was first reported in Egyptian flocks in the early seventies (El-Sergany et al., 1974). However interest in IBDV antigenic characterization was triggered by the appearance of the very virulent IBD in vaccinated Egyptian flocks (El-Batrawi, 1990; Khafagy, et al., 1991). Several reports have classified the Egyptian IBDV isolates as classical IBDV (Khafagy, et al., 1991; El-Sanousi, et al., 1992; Bekhit, 1996). On the other hand, some reports have provided partial evidence of the presence of antigenically variant IBDV strains in Egyptian flocks. El-Sanousi et al., (1994), were able to detect BK9 reactive epitopes in one out of 48 bursal homogenates; BK9 reactive epitopes are present on Del/E IBDV variants. Using

cross-neutralization, Sultan (1995), found 3 out of 8 IBDV isolates with a significant antigenic divergence from the classical IBDV (only 11.32% relatedness to classical IBDV). The current status of IBDV antigenic diversity in Egypt is yet to be determined.

Characterization of antigenic diversity of IBDV field isolates in a certain locality is important in order to develop an effective vaccination program to control IBD. This importance stems from the fact that antigenic variants escape an immune response induced by vaccination with classical standard IBDV vaccines (Rosenberger et al., 1985).

A new antigen-capture ELISA (AC-ELISA) is used for the 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 classical and variant IBDV isolates (Snyder et al., 1988a; Snyder et al., 1988b; Lamichhane et al., 2000).

In this study, bursal homogenates from selected Egyptian broiler flocks were tested using the new AC-ELISA for the presence of classical and variant IBDV antigens. The study was done to investigate the present antigenic profiles of Egyptian IBD viruses in the light of increasing field reports of IBDV-associated problems.

MATERIALS AND METHODS

Samples: Two hundred and seventy seven bursal samples from 13 broiler flocks were collected. Breeders of the tested broiler flocks were vaccinated with vaccines containing classical IBD viruses. Samples from examined broilers were grouped, according to the frequency of sampling of flock, into two categories: 1) Group 1: single-age-testing group (Table 1), and 2) Group 2: multiple-age-testing group (Table 2). Bursal samples of Group 1 were collected from live birds submitted for diagnostic services. Samples from flock (FN-7) of Group 2 were collected from live birds on a weekly basis, while samples from the remaining two flocks (FC-1 and FC-2) were collected from dead birds on a daily basis.

Sample preparation and test procedure: IBDV epitopes were detected using the antigen capture-ELISA test kit ProFlok(for detection and differentiation of IBDV in bursal tissue (Synbiotics Europe, Lyon, France). The AC-ELISA kit utilized 4 neutralizing monoclonal antibodies (MAb): MAb #8, MAb B69, MAb R63 and MAb #10. The MAb #8 was used for initial screening of samples because it reacts with a VP2 epitope conserved on both classical and variant IBDV. The remaining MAbs were used for IBDV subtyping according to the sample reactivity patterns (Table 3). IBDV antigens that did not react with MAb B69 were considered variants (Snyder et

al., 1994).

Sample preparation and test procedure was done according to the manufacturerís instructions using reagents provided in the kit. Briefly, bursal samples were homogenized using sterile sand and diluted in antigen dilution buffer. Homogenized samples were frozen at -20°C until testing. Bursal homogenates were clarified by low speed centrifugation, diluted and added to wells of the monoclonal antibody coated plates. After overnight incubation at 4-8C, plates were washed and chicken IBD positive serum was added. After a 30-min incubation period at room temperature, plates were washed and anti-chicken conjugated antiserum was added. Plates were incubated as before and then washed. The substrate solution was added and the plates were incubated for 15 min. Test plates were read using automated ELISA reader (BioTek ELX 800, USA) at 405 nm, after addition of the stop solution. Samples were considered positive when the obtained optical density (OD) values were equal to or greater than 0.600.

RESULTS

Group 1 results: One hundred and twenty two samples from group 1 (75.8%) were IBDV positive (Table 4). The IBDV-positive samples of Group 1 contained 34.4% and 65.6%, classical and variant antigens, respectively. The majority of IBDV variants (70%) were Del/E. Only 30% of the IBDV variants were untypeable; did not react with MAb B69 (Table 4).

In three flocks (FC-3, FN-1, and FN-6) only classical IBDV antigens were detected. In two flocks (FC-4 and FN-5) only variant IBDV antigens were detected (Table 4). While, in four flocks (FN-4, FN-3, FN-1A and FN-2) both classical and variant IBDV antigens were detected (Table 4).

Group 2 results: In two flocks (FC-2 and FC-1) in which samples were collected from daily mortality starting one day following the last vaccination date (Table 2), classical antigens were the predominant antigens detected after 3 weeks of age (Table 5, Fig. 1 and Fig. 2). Prior to three weeks of age the predominant antigens detected were IBDV variants (Table 5 and Fig. 1). In Flock FN-7, the predominant antigens detected throughout the duration of the study were variant IBDV. Antigen detection started at week three of the study (Table 5 and Fig. 3).

Results summary: IBDV antigens were detected in 73.6% of the 277 examined samples (Table 6). The majority of IBDV antigens detected were variants (58.3%) (Table 6). Del/E was the predominant variant detected in the examined flocks (51.3%). Only 1.7% of the variant antigens had the antigenic profile of RS593, AL2 or a mix of both variants (Table 6). Using the available monoclonal antibodies, 47.0% of the variants were untypeable.

Table 1. Group 1 (Single-age-testing group) samples' data

Flock	Agw (Days)	Vaccinations against IBD					Numere of sampels
		Vaccine	Type	Strain	Age (Days)	Method	
FC-5	6-7	-----	----	------	----	----	12
FC-4	15-16	IBD Blen	Living Standard Intermediate plus (Chicken embryo)	2512 strain	15	Drinking water	14
FN-5	18	Gumboro 228E	Living Standard Intermediate plus (Chicken embryo)	228E strain	14	Drinking water	8
FC-3	23-24	Bur-706	Living Standard Intermediate (Tissue culture)	S706 strain	8,15 and 21	Drinking water	14
FN-4	24-25	Univax-BD	Living Standard Intermediate (Tissue culture)	ASL's ST-12 strain	15	Drinking water	18
		Gumboro 228E	Living Standard Intermediate plus (Chicken embryo)	228E strain	19	Drinking water	
FN-1	26	Univax-BD	Living Standard Intermediate (Tissue culture)	ASL's ST-12 strain	14,22	Drinking water	20
FN-3	28-29	Univax-BD	Living Standard Intermediate (Tissue culture)	ASL's ST-12 strain	15	Drinking water	32
		Gumboro 228E	Living Standard Intermediate plus (Chicken embryo)	228E strain	19	Drinking water	
FN-1A	32	Univax-BD	Living Standard Intermediate (Tissue culture)	ASL's ST-12 strain	14,22	Drinking water	20
FN-6	33-34	Univax-BD	Living Standard Intermediate (Tissue culture)	ASL's ST-12 strain	14,22, 26	Drinking water	9
FN-2	34	Univax-BD	Living Standard Intermediate (Tissue culture)	ASL's ST-12 strain	14,22	Drinking water	14
Total							161

Table 2. Group 2 (Multiple-age-testing group) samples data

Flock	Agw (Days)	Vaccinations against IBD					Numere of sampels
		Vaccine	Type	Strain	Age (Days)	Method	
FN-7	07	Bursa Plex	Immuno-comples (Standard Intermediate virus + antibodies)	2512	Chicken Embryos of 18 days	In-ovo	7
	14						3
	21						10
	28						5
	35						8
	Total						33
FC-2	16-17	Bursine Plex	Living Standard Intermediate plus (Chicken embryo)	Lukert strain	14-15	Drinking water	10
	18-19						9
	20-21						6
	22-23						4
	24-25						3
	Total						32
FC-1	22-23	Bur-706	Living Standard Intermediate (Tissue culture)	S706 sttain	8,15 and 21	Drinking water	10
	24-25						10
	26-27						10
	28-29						11
	30-31						10
	Total						51

Table 3: AC-ELISA reactivity patterns of IBDV antigens

Vairus type		MAb # 8	MAb B69	MAb B63	MAb # 10
Classic		+	+	+	+
CLS	Y2K	+	-	-	+
Del/E		+	-	+	-
RS593	AL2	+	-	-	-

Table (4): Group 1 (Single-age testing group) results

Flock	Age (days)	Number of sampels	Total Positive		Total Classical		Total Variants		Identification of variants					
									Del/E		RS593 AL2		Untypeable	
			Number	%*	Number	%**	Number	%**	Number	%***	Number	%***	Number	%***
FC-5	6-7	12	0	0.0	0	0	0	0	0	0	0	0	0	0
FC-4	15-16	14	14	100.0	0	0.0	14	100.0	9	64.3	0	0.0	5	35.7
FN-5	18	8	6	75.0	0	0.0	6	100.0	6	100.0	0	0.0	0	0.0
FC-3	23-24	14	14	100.0	14	100.0	0	0.0	0	0	0	0	0	0
FN-4	24-25	18	18	100.0	2	11.1	16	88.9	13	81.2	0	0.0	3	18.8
FN-1	26	20	1	5.0	1	100.0	0	0.0	0	0	0	0	0	0
FN-3	28-29	32	31	96.9	4	12.9	27	87.1	15	55.6	0	0.0	12	44.4
FN-1A	32	20	16	80.0	5	31.3	11	68.7	7	63.6	0	0.0	4	36.4
FN-6	33-34	9	9	100.0	9	100.0	0	0.0	0	0	0	0	0	0
FN-2	34	14	13	92.9	7	53.8	6	46.2	6	100.0	0	0.0	0	0.0
Totals		161	122	75.8	42	34.4	80	65.6	56	70.0	0	0.0	24	30.0

* Positive percentages are relative to the total number of samples.
** Positive percentages are relative to the total number of positive samples.
*** Positive percentages are relative to the total number of variants.

Table (5): Group 2 (Multiple-age testing group) results

Flock	Age (days)	Number of sampels	Total Positive		Total Classical		Total Variants		Identification of variants					
									Del/E		RS593 or AL2		Untypeable	
			Number	%*	Number	%**	Number	%**	Number	%***	Number	%***	Number	%***
FN-7	07	7	0	0.0	0	0	0	0	0	0	0	0	0	0
	14	3	0	0.0	0	0	0	0	0	0	0	0	0	0
	21	10	10	100.0	4	40.0	6	60.0	3	50.0	1	16.7	2	33.3
	28	5	3	60.0	1	33.3	2	66.7	1	50.0	0	0.0	1	50.0
	35	8	4	50.0	1	25.0	3	75.0	0	0.0	0	0.0	3	100.0
	Total	33	17	51.5	6	35.3	11	64.7	4	36.4	1	9.1	6	54.5
FC-2	16-17	10	3	30.0	1	33.3	2	66.7	0	0.0	0	0.0	2	100.0
	18-19	9	7	77.8	2	28.6	5	71.4	0	0.0	0	0.0	5	100.0
	20-21	6	5	83.3	4	80.0	1	20.0	0	0.0	0	0.0	1	100.0
	22-23	4	4	100.0	4	100.0	0	0.0	0	0	0	0	0	0
	24-25	3	3	100.0	2	66.7	1	33.3	0	0.0	0	0.0	1	100.0
	Total	32	22	68.8	13	59.1	9	40.9	0	0.0	0	0.0	9	100.0
FC-1	22-23	10	9	90.0	1	11.1	8	88.9	1	12.5	0	0.0	7	87.5
	24-25	10	8	80.0	1	12.5	7	87.5	0	0.0	0	0.0	7	100.0
	26-27	10	7	70.0	6	85.7	1	14.3	0	0.0	0	0.0	1	100.0
	28-29	11	9	81.8	8	88.9	1	11.1	0	0.0	0	0.0	1	100.0
	30-31	10	10	100.0	8	80.0	2	20.0	0	0.0	1	50.0	1	50.0
	Total	51	43	84.3	24	55.8	19	44.2	1	5.3	2	5.3	17	89.4

* Positive percentages are relative to the total number of samaples.
** Positive percentages are relative to the total number of positive samaples.
*** Positive percentages are relative to the total number of variants.

Table (6): Summary results of IBDV antigen detection and typing in bursal homogenates.

Category	Number	%
Total samples	277	---
positive	204	73.6*
Classical	85	41.7**
Variants	119	58.3**
Del/E	61	51.3***
RS593 or AL2	2	1.7***
Untypeable	56	47.0***

* Positive percentages are relative to the total number of samples.
** Positive percentages are relative to the total number of positive samples.
*** Positive percentages are relative to the total number of variants.

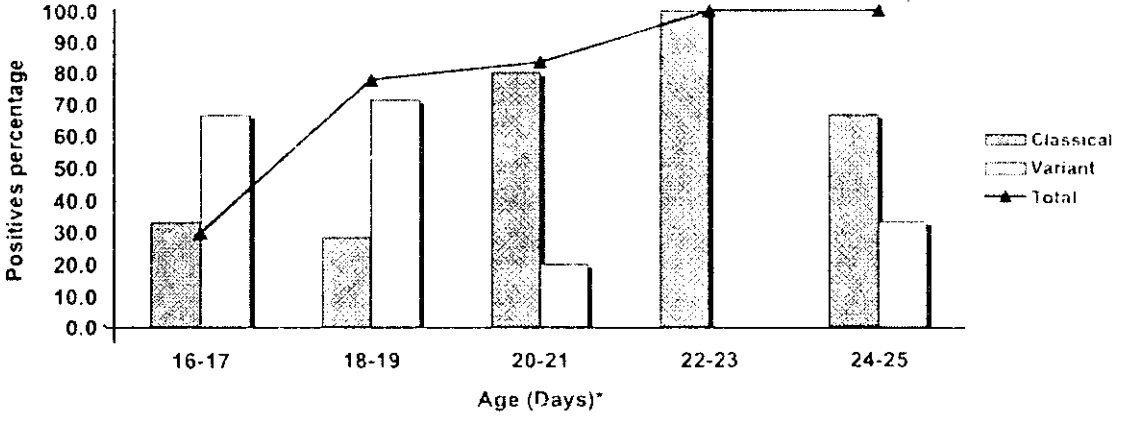

Fig. 1: The percentage of detection of classical and variant IBDV antigens in flock FC-2 samples compared to the percentage of IBDV positive samples.

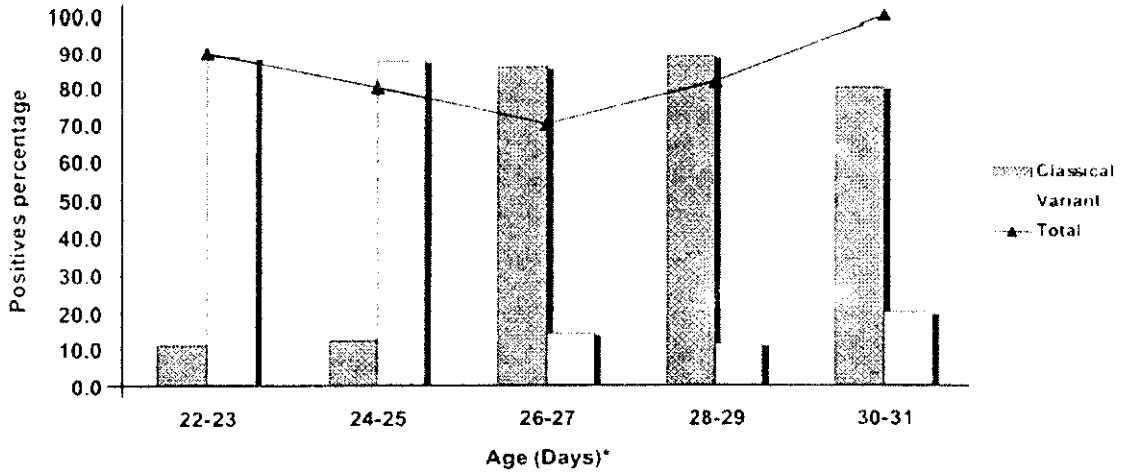

Fig. 2: The percentage of detection of classical and variant IBDV antigens in FC-1 samples compated to the percentage of IBDV positive samples.

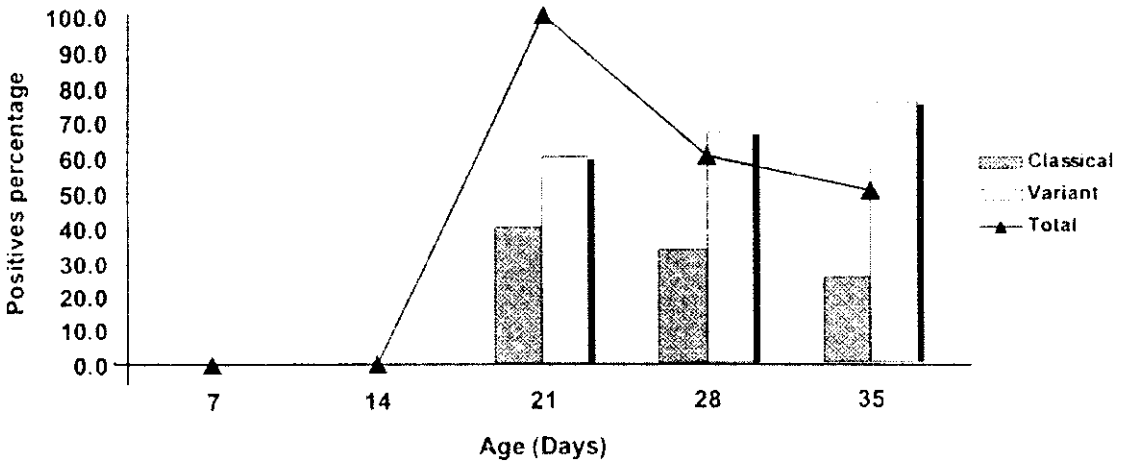

Fig. 3: The percentage of detection of classical and variant IBDV antigens in flock FC-7 samples compared to the percentage of IBDV positive samples.

DISCUSSION

The severe economic impact of IBDV infections in poultry flocks warrants the implementation of extensive control programs in Egypt. Vaccination of poultry flocks is the main component of most IBD control programs. Most Egyptian flocks are vaccinated using classical live and killed IBDV vaccines. This is done without proper investigation of the current genetic and antigenic diversity among IBDV field strains. Improper vaccine selection constitutes a weakness in any control program. IBDV-associated problems have been reported in flocks that were successfully vaccinated against classical IBDV. Some of these IBDV-associated problems were attributed to circulating variant IBDV field strains (Lukert and Saif, 1997). Moreover, maternal antibodies induced by IBDV vaccines designed to protect against challenge with classical IBDV isolates do not protect against challenge with antigenic variants (Rosenberger et al., 1985; Saif, 1984).

Field reports of increasing IBDV-associated problems in vaccinated flocks have triggered the present investigation of IBDV subtypes. The most feasible approach was the use of the monoclonal-based AC-ELISA (Snyder, 1988b). The AC-ELISA used in this study utilized a panel of 4 monoclonal antibodies developed against IBDV VP2 epitopes. Screening for IBDV VP2 epitopes was done directly on bursal homogenates. The basis for differentiation between classical IBDV and the most common IBDV variants was their reactivities with MAb B69 (Lamichhane et al., 2000; Snyder et al., 1994).

The most interesting finding of this investigation was that bursal samples from 9 of the 13 tested flocks had IBDV variant antigens (Tables 4 and 5). Variant IBDV antigens were detected in 58.3% of the total IBDV positive samples (Table 6). Another important finding is the detection of both classical and variant IBDV antigens in samples from the same flock. This phenomenon was present in 7 of the 13 sampled flocks (Tables 4 and 5). The high incidence of variant IBDV antigens detected in tested samples indicates circulation of variant IBD viruses despite vaccination of flocks with classical IBDV vaccines. Variant IBDV escape in the presence of classical IBDV vaccination has been previously reported (Rosenberger et al., 1986; Snyder, 1990).

Infection of chicken with variant IBDV before 3 weeks of age is possible because variant IBDV can escape high levels of maternal antibodies produced against classical IBDV (Snyder, 1992). This age of susceptibility explains why only variant IBDV antigens were detected in samples from flocks FC-4 and FN-5 (Table 4). The samples from these two flocks were collected at 18 days of age, or younger (Table 1).

On the other hand, classical IBD viruses typically appear at 3-4 weeks of age (Lukert and Saif,

1997). This may explain why classical but not variant IBDV antigens were detected from samples of the three broiler flocks FC-3, FN-1, and FN-6 (Table4). Samples from these flocks were collected from birds submitted for diagnostic services between days 23 and 34 (Table 2).

The age of susceptibility of classical IBDV may also explain the predominance of classical IBDV antigen-detection after 3 weeks of age in group 2 flocks FC-1 and FC-2 (Table 5, Fig. 1 and Fig. 2). Prior to that age, the predominant antigens detected were variant IBDV (Table 5, Fig. 1).

In only one flock from group 2 (FN-7), the predominant antigens detected throughout the duration of the study were variant IBDV (Table 5 and Fig. 3). This is probably due to the relatively high protection induced by in-ovo vaccination against classical IBDV (Metwally, 2000). Detection of variant IBDV up to 5 weeks in bursal tissues is probably due to antigen persistence. Variant IBDV antigens have been shown to persist in bursal tissues for 4-6 weeks post- infection (Elankumaran et al., 2001). Virus-antibody complexes cause localization of the IBDV to germinal centers. The presence of the virus for long periods in commercial broilers may aid in the spread of the virus to successive crops (Elankumaran et al., 2001).

We did not detect IBDV antigens in Flock FN-7 samples prior to week 3 of the study (Table 5 and Fig. 3). The reason for this may be that only living birds were submitted from this flock and a relatively small number of which were tested at week 2 (Table 2).

The majority of the IBDV variant antigens detected (51.3% of IBDV variants) were classified as Del/E (Table 6). Of the total IBDV variants detected, 47.0% were untypeable with the monoclonal antibody panel used in this investigation, including 2 samples that contained either RS593 or AL2 variants or a mix of both, (Table 6). Untypeable variants will be investigated further when more monoclonal antibodies become available.

The present study highlights the presence of circulating IBDV antigenic variants in broiler flocks in Egypt despite the implementation of extensive vaccination programs. In support of other studies (Elankumaran et al., 2001), it also provides evidence of the persistence of variant IBDV in vaccinated flocks. We propose reviewing the current vaccination programs to include IBDV strains representative of the IBDV antigenic diversity in Egypt. We also propose broadening the base of screening for variant IBDV in flocks with clinical problems suggestive of IBDV immunosuppression. Molecular and phylogenetic analysis of the detected Egyptian variant IBDV isolates is in progress to investigate their origins and evolution.

REFRENCES

Allan, W. H., J.T. Faragher and G.A Cullen (1972): Immunosuppression by the infectious bursal agent in chicken immunized against Newcastle disease. Vet. Rec. 90: 511-512.

Azad, A. A., M. N. Jagadish, M. A. Brown and P. J Hudson (1987): Deletion mapping and expression in ***Escherichia coli*** of the large genomic segment of a birnavirus. Virology 161: 145-152.

Becht, H., H. Müller and H. K. M¸ller (1988): Comparative studies on structural and antigenic properties of two serotypes of infectious bursal disease virus. J. Gen. Virol. 69: 631-640.

Bekhit, A. B. A. (1996): Antigenic characterization of IBD field virus isolates from sever outbreaks of the disease using a panel of monoclonal antibodies. Proc. 4th. Sci. Conf. Eyptian veterinary Poultry Association, pp: 65-81.

Chettle, N., J.C. Stuart and P .J. Wyeth. (1989): Outbreak of virulent infectious bursal disease in East Anglia. Vet. Rec. 125: 271-272.

Dobos, P., B. J. Hill, R. Hallet, D. T. C. Kells, H. Becht, and D. Teninges. (1979): Biophysical and biochemical characterization of five animal viruses with bisegmented double-stranded genomes. J. Virol. 32:593-605.

Elankumaran, S., Heckert, R. A., and Moura, L. (2001): Persistence and tissue distribution of a variant strain of infectious bursal disease virus in commercial broiler chickens. International Symposium on infectious bursal disease and chicken infectious anaemia, Rauischholzhausen, Germany. pp: 353-365.

El-Batrawi, A. M. (1990): Studies on sever outbreaks of infectious bursal disease in Egypt. Proc. 2nd. Sci. Conf. Egypt. Vet. Poult. Assoc. pp: 239.

El-Sanousi, A., Madbouly, H. Am., Saber, M. S., el-Bagoury, G. F., Abd-El-Bar, N. A., Bartrawi, A. and Reda, I. M. (1994): Infectious bursal disease virus infectiou among Egyptian poultry flocks: III. Antigenic characterization of IBDV by the antigen capture ELISA (AC-ELISA) using monoclonal antibodies (MAbs). Beni-Suef Vet. Med. Res., 4: 300-307.

El-Sergany, M., A. Moursi, M. Saber and M. Mohamed. (1974): A preliminary investigation on the occurance of Gumboro disease in Egypt. J. Vet. Sci.: 11: 7.

Giambrone, J. J. (1979): Effects of early infectious bursal disease virus infection on immunity to Newcastle disease in adult chickens. Poult. Sci. 58: 794-798.

Giambrone, J. J., C. S. Eidson. R. K. Page, O. J. Flitcher, B. O. Barger arid S. H. Kleven. (1976): Effects of infectious bursal disease agent on the response of chickens to Newcastle disease and Marek's disease vaccination. Avian Dis. 20: 534-544.

Hirai, K., T. Funakoshi, T NakaL and S. Shimakura. (1981): Sequential changes in the number of surface immunoglobulin-bearing B Lymphocytes in infectious bursal disease virus-infected chickens. Avian Dis. 25: 484-496.

Hirai, K. and Shimakura, S. (1974): Structure of infectious bursal disease virus. J. Virol. 14: 957-964.

Ismail, N. M., Y. M. Saif and P. D. Moorhead. (1988): Lack of pathogenicity of five serotype 2 infectious bursal disease viruses in chickens. Avian Diseases 32: 757-759.

Jackwood, D. H. and Y. M Saif. (1987): Antigenic diversity of infectious bursal disease viruses. Avian Diseases 31: 766-770.

Jackwood, D. H., Y. M. Saif and P. D Moorhead (1985): Immunogenicity and antigenicity of infectious bursal disease virus serotypes I and II in chickens. Avian Dis. 28, 100-116.

Khafagy, A. K., A. Sawy, M. B. Kouvenhoven, Vielitz, I. M. Ismail, A. A. Amer, H. Sultan and A. M. El-Gohary. (1991): Very virulent infectious bursal disease. Vet. Med. Giza, 39:299-317.

Lamichhane C.M, L. Jerome, and B. Adenikinji. (2000): ELISA for Detection and differentiation of infectious bursal disease virus. Presented at 49th Western Poultry Disease Conference, Sacramento, CA.

Lukert, P.D., and Y. M. Saif. Infectious bursal disease. (1997): In: Diseases of poultry. Editors Calnek, B. W., Barnes, H. J., Beard, C. W., Reid, W. M. and Y. M. Saif. 10th Edition. Iowa State Univ. Press. Ames Iowa, U.S.A. pp. 721-739.

McFerran, J. B., McNulty, M. S., McKillop, E. R., Connor, T. J., McCracken, R. M., Collins, D. S. and Allan, G. N. (1980): Isolation and serological studies with infectious bursal disease viruses from fowl, turkeys and ducks: demonstration of a second serotype. Avian Pathol. 9: 395-404.

Metwally, A. M. (2000): Studies on Virus-antibody Complex vaccine for Gumboro disease in poultry. M. V. Sc. Virology Department, Faculty of Veterinary Medicine, Cairo University.

Rosenberger, J.K., and S. S., Cloud. (1986): Isolation and characterization of variant infectious bursal disease viruses. Proc 12: 3rd Annu. Meet Am. Vet. Med Assoc., Abstr. 181.

Rosenberger, J.K., S.S. Cloud, J. Gelb, J.E. Odor and J.E. Dohms (1985): Sentinel bird survey of Delmarva broiler flocks. Proc. 20th Nat. Meet Poult. Health Condemn, Ocean City, MD, pp. 94-101.

Saif, Y. M. (1984). Infectious bursal disease virus types. Proc 19th Natl Meet Poult Health Condemn, Ocean City, MD, pp 105-109.

Snyder, D. B. (1990): Changes in the field status of infectious bursal disease virus. Avian Pathol. 19: 419-423.

Snyder, D. B., D. P. Lana, B. R. Cho, and W. W. Marquardt (1988a): Group and strain-specific neutralization sites of infectious bursal disease virus defined with monoclonal antibodies. Avian Dis. 32: 527-534.

Snyder, D. B., D. P. Lana, P. K. Savage, F. S. Yancey, S. A. Mengel, and W. W. Marquardt (1988b): Differentiation of infectious bursal disease viruses directly from infected tissues with neutralization monoclonal antibodies: evidence of a major antigen shift in recent field isolates. Avian Dis. 32: 535-539.

Snyder, D. B., P. K. Savage, S. A. Mengel, V. N. Vakharia and D. Lutticken. (1994): Molecular epidemiology of infectious bursal disease virus in the United States. International Symposium on infectious bursal disease and chicken infectious anaemia, Rauischholzhausen, Germany. pp: 65-70.

Snyder, D. B., V. N. Vakharaia, and P. K. Savage. (1992): Naturally occurring neutralizing monoclonal antibody escape variants defined the epidemiology of infectious bursal disease virus in the United States. Arch. Virol. 127: 89-101.

Sultan, H. A. (1995): Studies on infectious bursal disease in chickens. Ph. D. Thesis, Fac. Vet. Med., Alexanderia Univ.

Tanimura, N., and J. M. Sharma. (1998): In-situ apoptosis in chickens infected with infectious bursal disease virus. J. Comp. Pathol. 118:15-27.

Van Den Berg, T. P., M. Gonze and G. Meulemans (1991): Acute infectious bursal disease in poultry isolation and characterization of a highly virulent strain. Avian Pathol. 20: 133-143.