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SEROLOGICAL STUDIES ON FLOCKS SHOWING DEPRESSED EGG PRODUCTION

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SUMMARY

A serological investigation was undertaken on flooks with depressed egg production. This syndrome is characterized either by a failure to attain predicted production targets or by a fali in egg numbers. The depression in production could reach over 30%. It might or might not return to normal. For a short perod the eggs produced in some flocks were smaller, lose colour, have poor egg shell strength and many soft shelled eggs are laid. Also, some flocks were suffering form mild respiratory symptoms and increase in mortality with decrease in feed intake. The results revealed that no obvious correlation was found between antibody to adenovirus, (BDS76 and CELO), infectious bronchitis virus (I.B.V), infectious larengotracheitis virus (I. L. T. V.), avain encephalomyelitis virus (A. E. V.), Newcastle disease virus (N. D. V.), reovirus or infectious bursal disease virus (I. B. D. V.) and this syndrome.

The ELISA results of turkey rhinotracheitis (TRT), showed only and positive cases out of 38 samples from four flocks, while three gave high positive results. When the same serum samples were examined by Ornithobacterium rhinotracheale (ORT) ELISA 23 of 38 serum samples were positive to ORT and 11 serum samples ± and only 3 serum samples were negative.

These results indicate that ORT might be the cause of this syndrome. This is the first serological evidence of its presence in Egypt. There was a high correlation between results of immunocomb IgG and HI for ND virus antibody titers for all serum samples tested. Immunocomb IgG proved very sensitive in detecting antibodies against IB, IBD, when it was compared with AGPT.

INTRODUCTION

Depressed egg production occurs in broiler breeders where the syndrome affects the birds at the peak of production or soon before entering production, mostly between 24th and 52nd weeks of age (Van Eck et al., 1976).

The severity of this syndrome is extremely variable because it is influenced by many factors such as poor management, inadequate ventilation, high stocking density, poor litter condition, poor hygiene, high ammonial level and concurrent disease. Debeaking or vaccination with live

Newcastle disease virus if done at a critical time (Hafez, 1994).

During viruological investigation of these outbreaks a number of agents were recognized as egg drop syndrome 076 (Khafagy and Hamouda, 1991). EDS Giza 1991 (Ahmed, 1995), turkey rhinotracheitis (TRT), and other microorganisms were designated as Pasteurellia-like organism (ORT). Vandamme et al., (1994) carried out further identifications on this microorganism using genetic taxonomic methods and designated that bacterium as Ornithobacterium rhinotracheale gen, nov sp. (nov. in the rRNA-superfamily V).

The purposes of the present study were

1- Detection of specific antibodies to some common viruses that have been incriminated as causes of depressed egg production.

2-Evaluation of the application of the immunocomb bursal disease - Newcastle - Bronchitis antibody test kit as a quick sensitive test which detects antibody levels in the serum and compare its results with the results of HI and AGPT.

MATERIAL AND METHODS

Serum samples:

Blood was collected from broiler breeders (30 t 51 weeks old), their details are shown in Table (1). These broiler breeders were in flocks vaccinated against . Marek's disease, infectious bronchitis, Newcastle disease, Reodisease, infectious laryngotracheitis, infectious bursal disease and avian encephalomyelitis. The blood was refrigerated at 4°C overnight, the serum was then separated by centrifugation at 1500 rpm for 10 mins, the clear serum was then collected and kept in the deep freeze at -20°C.

Haemagglutination (HA) and haemagglutination inhibition (HI) tests : The tests were conducted by microtiter method of ND acording to Beord and Wilkes (1973) and for EDS76 according to Mcferran (1989).

Agar gel diffusion tests (AGDT): The test was carried out according to the method of Hitchner et al., (1975) to detect the antibodies against IB, IBD, AE and Celo virus. Positive and negative sera included in the test were kingly supplied by (Doorn Institute, Holland)

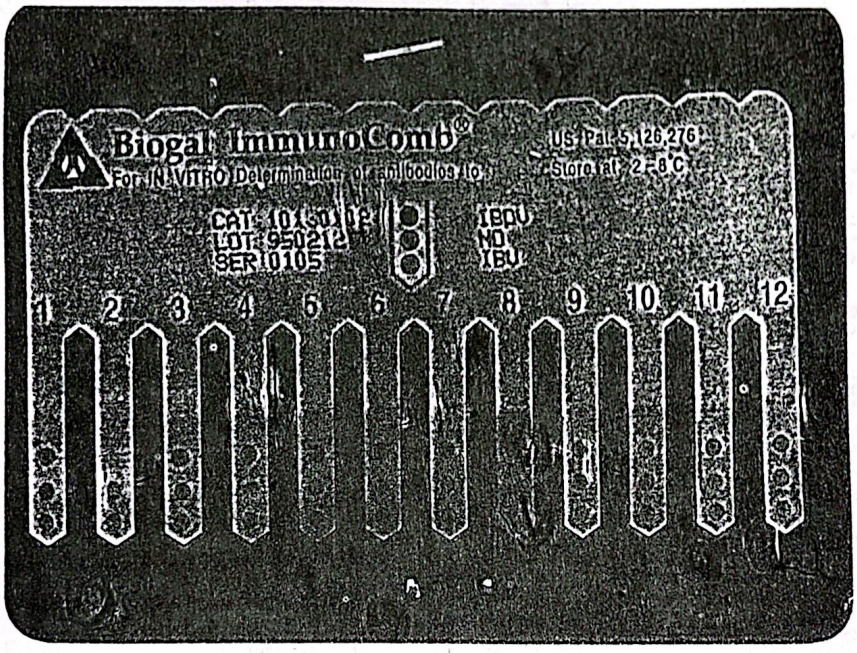
Immunocomb solid-phase imunoassay kit: manufactured by commercial Biogal galed Labs. (Israel) . This kit is designated to determine chicken serum IgG antibody titers to bursal disease-Newcastle-bronchitis (Rivetz et al., 1985). Results of immunocomb were compared with HI titer for NDV and AGD for IB and IBD viruses.

Indirect ELISA test: The test was carried out according to Hafez and Sting (1995). Serum samples from 38 broiler breeders suffering from depressed egg production and respiratory symptoms were sent to State Veterinary Laboratory. Stuttgart, Germany. Serum samples were examined for the presence of antibodies against TRT virus, and antibodies to ORT bacterium using self made indirect ELISA

prepared from Stuttgart ORT-turkey-isolate GGD 1269/91.

RESULTS

Clinical signs: As shown in Table (1), the reduction percentage in gee number varied from 10-38 . In broiler breeders the disease affects the birds at the peak of production or soon before entering production, mostly between 30th weeks of age. Before the main symptoms are detected a slight increase in mortality in some flock's and decrease in feed intake may be observed plus the respiratory manifestation. The symptoms are generally accompanied with a drop in egg production, decrease in egg size and poor shell quality, fertility and hatchability are affected in many cases.



* 1 = + ve
** 2 = - ve

Fig. (1): The immunocomb is a plastic card shaped like a comb on which purified IBDV, ND and IVB antigens are attached. This kit is designed to determine chick serum IgG antibody titers to these viruses. The upper spot on the immunocomb tests for IBDV, the second spot tests for NDV and the lower spot for IBV.

* To determine its titer, compare the specimens colour intensity with that of the positive control (+) included in the kit and comparing with scale score.
* Specimens with an identical or higher colour intensity than the positive control are considered positive.
* The negative control consists of non immunized sera and should be read as zero (S-0).

Table (1): History of examined flocks for drop in egg production.

Flocks	Breed	House capacity	House type	Source of day old chicken	Age at fall (weeks)	Average egg fall%	Maximum egg fall %	Time for maximum fall (weeks)	Time for maximum production	Egg quality	Sings	PM	Mortality	Remarks
House 4	Ross	3900	Closed system	Local	38	30-62	32	13	-	-	-	-	-	
Flock 4	Avian	7000	Closed system	Local	30	0-10	10	10	-	-	Facial oedema			
Flock 3	Avian	7700	Closed system	Local	33	0-38	38	12	-	Colour-less, shell-less loose shell	Facial oedema	Petechial haemo-rrhage on the heart	High	
House 5	Ross	2900	Closed system	Local	38	30-62	32	13	-	-	-	ulcera-tion in the intestine	high	

Hatchability and fertility were greatly reduced

Serological results: Sera were tested for antibody to ND and EDS 76 as shown in Table (2). HI titer in Log2 ranged from 8 to 10 for ND and all sera showed negative results by HI for EDS 76 disease. The detection of antibodies for AE, Reo, ILT and adenovirus by AGD test showed that most of the sera were positive. The measurable antibodies of all viruses showed the normal levels according to the vaccination (Table 3).

Table (4) showed that the comb score increased with the increase in HI titer for ND. Some samples gave negative results to IB and IBD with AGDT, while gave positive results with immunocomb kit..

Table (5) showed clearly that antibodies to ORT were detected by ELISA in 23 sera sampled from broiler breeder flocks (60.5%) and antibodies to TRT were detected in 6 samples (15%).

Table (2): Results of tested serum by haemagglutination inhibition test (HI).

	Age (weeks)	No. of samples	Virus	Log_2 HI titers									
				1	2	3	4	5	6	7	8	9	10
Hous1	51	20	ND									1	19
			EDS	-	-	-	-	-	-	-	-	-	-
Flock 4	30	25	ND										25
			EDS	-	-	-	-	-	-	-	-	-	-
Flock 3	38	17	ND									2	15
			EDS	-	-	-	-	-	-	-	-	-	-
house 4	51	15	ND										15
			EDS	-	-	-	-	-	-	-	-	-	-

Table (3): Detection of antibodies to IB, ILT, IBD, Adeno, AE and Reo viruses by AGDT.

	Age (weeks)	No. of samples	No. of positive serum samples by AGDT					
			IB	ILT	IBD	Adeno	AE	Reo
House 4	51	20	12	10	14	-ve	7	10
Flock 4	30	25	15	13	13	4	8	12
Flock 3	38	17	10	6	10	2	8	6
House 3	51	15	10	8	8	4	5	10

Table (4): Comparative study between results of immunocomb and HI to ND and AGD for IBD and IBV.

Flocks	No. of samples	HI titer ND	Immunocomb scale (S) value			AGDT	
			ND	IBD	IB	IBD	IB
House 4	1	1024	S 5	S 6	S 4	+	+
	2		5	6	3	+	-
	3		6	5	3	-	-
	4		6	5	4	+	-
	5		5	6	4	+	+
	6		5	6	4	-	+
	7		5	6	4	-	-
	8		6	6	3	+	-
	9		5	5	3	+	-
	10		6	5	4	-	+
Flock 4	1		5	5	3	-	+
	2		6	6	3	+	+
	3		6	6	4	+	-
	4		6	5	4	-	-
	5		5	5	5	-	-
	6		6	5	5	-	+
	7		6	5	3	+	+
	8		6	6	3	+	-
	9		6	5	3	+	-
	10		5	5	4	+	-
Flock 3	1		5	6	4	-	+
	2		5	6	4	-	-
	3		6	6	4	+	-
	4		6	5	3	+	-
	5		6	5	3	+	-
	6		6	5	5	+	+
	7		5	5	5	-	+
	8		5	6	5	+	-
	9		6	6	3	+	+
	10		6	5	4	-	+
House 5	1		5	6	3	+	-
	2		5	6	4	+	-
	3		6	6	3	+	-
	4		6	6	3	-	+
	5		5	6	4	-	+
	6		6	6	3	+	-
	7		5	6	3	-	-
	8		5	6	3	-	+
	9		6	6	3	+	+
	10	1024	5	5	3	+	-

Table (5): Examination of serum samples from broiler breeder flocks.

Flocks	No. of samples	TRT-ELISA			ORT-ELISA		
		+	±	-	+	±	-
House 4	10	3	0	7	10	0	0
Flock 4	15	0	2	13	4	8	3
Flock 3	5	3	1	1	2	2	1
House 5	8	0	0	8	7	1	0
Total	38	6	3	29	23	11	4

DISCUSSION

Drops in egg production cause heavy economic losses in the poultry industry. This syndrome is accompanied with reduction of egg shell quality, decreased fertility, decreased hatchability and increased mortality rates. The serological examination of all serum samples by different tests showed that the immune response of broiler breeders in all flocks was in the normal level as a result of vaccination (Tables 2 and 3). In the investigated broiler breeders the disease primarily affected the birds at the peak of production or before entering production between 30 and 52nd weeks of age. Before the drop of egg production, a slight increase in mortality and decrease in feed intake, mild respiratory symptoms followed by facial oedema (Table 1). The results of indirect ELISA showed that the broiler breeders with antibody to ORT were 100% in house 4, 87.5% in house 5, 26, 7% in flock 4 and 40% in flock 3. The antibody percentages to TRT were 30% in house 4, 60% in flock 3. This high positive results to ORT and the presence of antibody to TRT indicate that these flocks are exposed to natural infection to ORT and TRT because there is no vaccination policy adopted in Egypt against ORT and TRT. This means that the main cause of depressed egg production in the investigated flocks was ORT. Diagnosis of ORT based on clinical signs and pathological lesions is very difficult since many other infectious diseases such as pasteurellosis, chlamydiosis, can produce similar clinical signs and postmortem lesions. Diagnosis can only be confirmed by isolation and identification of the causative agent and detection of the specific Ab against it. The epidemiology of ORT has been discussed previously by Hafez (1994) who attributed the disease to mixed infection with Pasteurella-like organism. Vandamme et al., (1994) carried out further identification of isolates using genetic taxonomic mehtods and designated that bacterium as Ornithobacterium rhinotracheale gen, nov. in the rRNA-superfamily V.

The present study offers an excellent opportunity for a comparative investigation between the different serological tests, concerning their sensitivity and validity as diagnostic tools. Thus (4) shows as trial where the results of the different serological tests have been compared in relation to s valus of the immunocomb kits (the intensity of colour). The results of the tests showed that the HI titer of ND was parallel to S value of the immunocomb. On the other hand the low sensitivity of AGPT may explain the negative results to IBD and IB, this is attributed to the antibody response did not reach levels sufficiently high to give positive reaction. All samples were positive to IB and IBD when tested by the immunocomb a matter which denotes clearly the superiority of HI and the immunocomb over the AGPT. The immunocomb is a self-contained portable kit. A sensitive test which detects antibody levels in the blood, serum or egg yolk, the immunocomb provides results within 40 minutes.

In conclusion, the presence of considerable levels of antibodies against ORT and TRT virus must receive more attention for deeper and planned investigation to prove the isolateion and identification of the microorganism under field

conditions and to determine the role of these agents in the production of egg as well as its interaction with other microorganisms.

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