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EFFECT OF BOTH MYCOPLASMA GALLISEPTICUM (MG) AND INFECTIOUS BRONCHITIS (IB) CLASSICAL AND VARIANT STRAINS ON THE EGG PRODUCTION OF THE BROILER BREEDER FLOCKS

BY

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INTRODUCTION

It was later observed that the IB was common in semimature and lying flocks as well and caused a marked losses in egg production. Prior to 1956 avian infectious bronchitis was considered to be caused by a single antigenic type of virus (pathogenic Massachusetts M_{41}, and the Beaudette non pathogenic embryo lethal strain M_{42}, since that time a number of isolants have been identified from outbreaks that are different antigenically from the original Massachusett type Winterfield et al., 1971 and Hopkins, 1974).Variant strains most frequently found to be involved in IB problem herds, belonged to two main groups namely (D_{207} and D_{212}) which have poor immunogenic properties. A field isolate called D_{274}, however belonging to the D_{207} serotype, proved to induce cross-protection with D_{3896} and to a lesser extent D_{3128}. Also, in the D_{212} group haemagglutinating strains were found, of which D_{1466} was found to be more immunogenic than D_{212}.

Mycoplasma gallisepticum [MG] infected chickens have been reported to suffer from a drop in egg production [Carpenter et al., 1979; Carpenter et al., 1981; Lin and Kleven, 1982]. MG infected hens have been observed to lay an average of 15.7 fever eggs/hens housed than MG free hen over a 45 week laying period [Carpenter et al., 1981]. The aim of this work was to demonstrate and evaluate the role of IB variant strains either alone or combined with Mycopeasma gallisepticum on the egg production of layer breeder flocks.

MATERIALS AND METHODS

1. Mycoplasma gallisepticum antigen:

A coloured antigen for plate agglutination test was prepared as described by Adler (1954) and modified by Attia (1988). Sensitivity and specificity of the antigen were tested by the serum plate dilution test using standard hyperimmune serum against Mycoplasma gallisepticum.
(MG) kindly supported by Dr. Ali El-Ebeedy (nimal Health Research Institute Dokki, Giza) and compared with

(MG) antigen Nobilis. Prepared by Intervet. By Boxmeer-Holland.

2. Agar gel percipitating antigen:

a) Chorioallamtoic membrane (CAM) from embryos infected with 10^4 EID_{50} of the egg adapted Beaudette strain of IBV was harvested, pooled and homogenized, then centrifuged at 3000 r.p.m. for 30 minutes. The supernatant was collected and mixed with tween 80 (0.05 ml/wl) shaken well, distributed in small vials and kept at-20°C.

b) IB (M_{41}, D_{274} and D_{1466}) antigens were kindly supplied by Dr. Magda M. Ali (Fac. of Vet. med. Mostoher, Banha and prepared as follws:

After treatment of IB [M_{41}, D_{274} and D_{1466}] antigens with phosphatase C and then inactivated with betapropiolactone, the infectious bronchitis virus agglutinates chickens hemates, the reaction inhibition allows titrating IB specific antibodies present in poultry serum [Alexander et al., 1976, Alexander and Chettle, 1977].

3. Phsyological buffered saline Ph 7.2.
4. 1% of 7 weeks old chickens erythrocytes PH 7.2.
5. Positive control sera.

   Kindly supplied by Dr. Magda M. Ali.
6. Sera treatment:
   Inactivation of collected serum samples to be tested and control sera by heating 30 minutes at 56°C.
7. Nobil Agar (Difco).
8. **Birds:**

Three broiler breeding farms (FA_1, FA_2, and FA_3) previously vaccinated against IB by H_{120} and H_{52} since 17 weeks tested to avoid precipitating antibidies against vaccinal strains [Carporale and Semproni, 1972]. but not vaccinated against MG with normal ND antibody titer ranging from Log_2 M 8-9, Allover the examined period the birds suffered from drop in egg production, abnormalities in egg shell and colour. FA_1 and FA_2 comprised 36000 birds each at Kalubia Govèrnorate. While FA_3 had 18000 birds at Al-Tahreer, province. Each farm is formed from three floor each one consisted from two deptartments, random serum samples were collected from each farm at 30 and 33 weeks old except at FA_3 the serum samples were collected at 30 weeks old only. Another control flock was free from MG and IB antibodies allover the examined period and showed normal egg production curve.

9. SPA [Slide plate Agglutination test against MG].

Rapid serum plate test was done according to Adler (1954) the serum samples were tested before freezing.

10. AGPT:

This test was done according to Woernle and Brunner (1960).

11. HI test:

Haemagglutination inhibition test was carried-out after King and Hopkins [1984] using the three prepared antigens. (M_{41}, D_{274} and D_{1466}).

RESULTS

The results of farm FA_1 which are tabulated in Table (1) showed that chickens in 5 departments (A_1, B_1, D_1 E_1 and F_1) had negative AGP antibodies against IB while those in department C were 100% positive, but 3 weeks later at the age of 33 weeks chickenin department No. B_1, C_1 and F_1 showed positive results by 50% while the other departments were still negative. At the same time test against IB (Classical and variant strains showed mean titres of 8.36, 7.23 and 5.61 for M_{41}, D_{274} and D_{1466} respectively at 33 weeks old). Concerning SPA test against MG chickens in all departments gave 100% positivity either at the age of 30 or 33 weeks.

Chickens in FA_2 [Table 2] showed negative results against IB when AGPT was used at both ages of 30 and 33 weeks, while the HI test showed high titre at the same time MG antibodies were recorded only in chickens of department A_2 (100%) at the age of 30 weeks, while at the age of 33 weeks chickens in all departments showed 100% seropositivity.

The birds in FA_3 (Table 3) showed negative AGPT against IB in all departments and high HI titre, concerning MG antibodies, at only chickens in department A_3 and B_3 gave 100% positive reaction while the others showed negative results.

Effect of both Mycoplasma gallisepticum.....

Table (1): Relationship between MG and IB seropositivity in FA_1 at 30 and 33 weeks old.

	30 weeks age						33 weeks age					
	MG		IB				MG		IB			
FA_1	SPA	AGPT	10^9_2 M HI titer				SPA	AGPT	10^9_2 M HI titer			
	the%	+ve%	M_{41}	D_{274}	D_{1466}		the%	+ve%	M_{41}	D_{274}	D_{1466}	
A_1	100	0	8.0	7.0	5.0		100	0	8.0	7.7	6.0	
B_1	100	0	8.0	7.0	5.0		100	50	7.5	7.5	5.5	
C_1	100	100	8.0	6.5	4.5		100	50	8.7	7.0	6.0	
D_1	100	0	10.5	6.0	3.5		100	0	8.3	6.0	4.7	
E_1	100	0	8.3	5.3	3.0		100	0	9.5	7.5	6.5	
F_1	100	0	8.0	4.0	4.0		100	50	8.7	7.7	5.0	
Mean	100%	16.62	8.46	7.63	4.25		100%	25%	8.36	7.23	5.61	

SPA = Slide plate agglutination test. AGPT = Agar gel precipitation test.
HI = Haemagglutination - inhibition test. FA_1 = Farm No^{-1}

Table (2):

Relation ship between MG and IB seropositivity in FA2 at different ages.

	30 weeks age					33 weeks age				
MG		IB				MG	IB			
FA2	SPA	AGPT	Log2 M HI titer			SPA	AGPT	log2 M HI titer		
	+ve%	+ve%	M41	D274	D1466	+ve%	+ve%	M41	D274	D1466
A2	100	0	9.5	8.0	9.5	100	0	9.5	6.0	8.0
B2	0	0	7.7	6.0	7.0	0	0	9.3	9.0	7.0
C2	0	0	6.3	7.0	6.7	100	0	9.0	6.0	5.0
D2	0	0	8.0	6.3	7.3	100	0	11.0	8.0	8.0
E2	0	0	6.5	5.0	6.5	100	0	8.5	7.0	8.0
F2	0	0	8.0	7.3	5.3	100	0	9.3	8.0	7.7
Mean	16.6%	0%	8.0	6.6	7.05	83.3%	0%	9.43	7.33	7.38

FA2 = Farm 1402
SPA = Serum plate asslutination test.
AGPT= Ayer bellpheciptation tes.

Table 3. Relationship between MG and IB Seropostivity in FAs

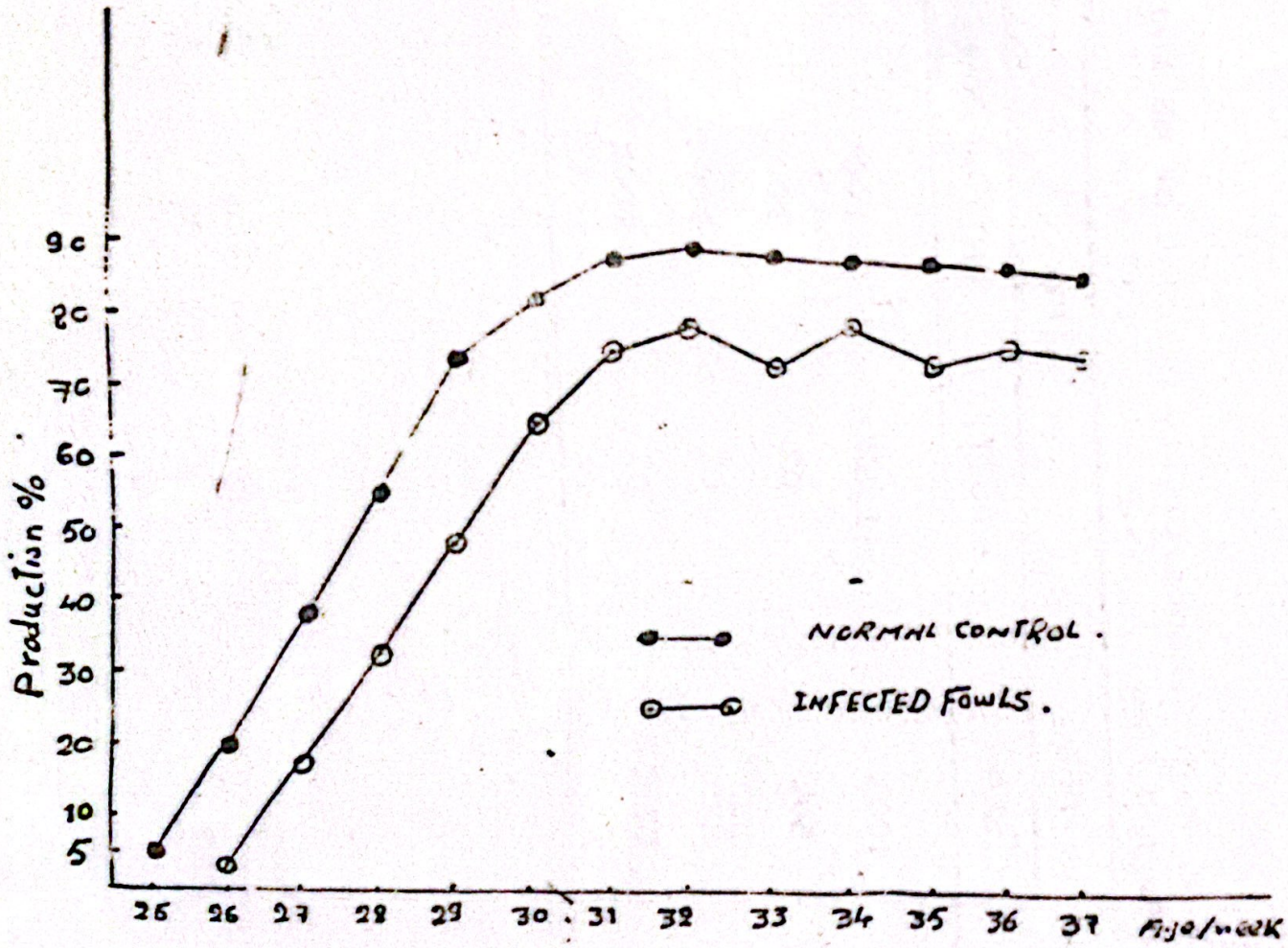
	30 weeks age				
	AG	IB			
FAs	SPA	AGPT	Log2 M HI titer		
	+ve%	+ve%	Mes	Dave	Dsese
As	100	0	10.0	8.7	7.3
Bs	100	0	8.0	10.0	9.3
Cs	0	0	8.0	9.0	6.3
Ds	0	0	9.0	11.0	6.7
Es	0	0	10.0	9.5	8.0
Fs	0	0	10.5	10.0	9.0
Main	33.3%	0%	9.25%	9.75%	7.76

SPA = Slide plate agglutination test.
FAs = Farm no. 3.

Table 4. Main Seropositivity percent of MG and IB in the FA_1.FA_2 and FA_3.

Farm	MG%	IB%	
	SPA	AGPT	HI(variants)
FA_1	100%	41.6%	100%
FA_2	50%	0%	100%
FA_2	50%	0%	100%
FA_3	33.3%	0%	100%

Fig :. Mean egg production % of the three Broiler Breeder flocks (FA_1, FA_2 and FA_3) naturally infected with MG & I.B in comparing with normal curve.



The figure showed the decrease in egg production in the three farms (mean%) compared normal with the control farm products, at the same time the egg production started at the 26th week.

Table (4) recorded the mean% seropositively against MG and IB in the three farms.

DISCUSSION

Generally, the results of AGPT indicated that chickens in 3 departments out of the 30 examined departments were positive. In one of them the results were 100% and in the other two departments. This result , agrees with that of EL. Kady [1989]. Who recorded that AGPT gave 52% positive results in the sera of breeder flocks. High HI antibody titre was detected in all examined serum samples (100%) against the classical and variant strains of IB virus (M_{41}, D_{724} and D_{1466}). These titres were not less than the $10g_2M$ 7 in most of the departments. Few departments gave 3.5-6 against M_{1466} variant. Davelaar et al., [1984]. Could detect a positive IB varient in serum samples collected from breeder flocks in Egypt.

The incidence of MG seropositivity in the serum samples examined in the three farms differed in each farm (100%, 50% and 33.3%) in FA_1, FA_2 and FA_3 respectively (Table 4). Also, from that Table it is clear that MG seropositivity was not always 100%, also AGPT was not enough to diagnose IB infection as it was zero in both FA_2 and FA_3 but when HI test was used, it indicated high titres against classical and variant serotypes of IB virus which caused similar problem [Hopkins, 1974].

The effct of MG and IB on egg production is well known, as recorded by Carpenter et al., [1981], Lin and Kleven [1984] & 1985] and Develaar et al., [1984] from the FA_1 it is clear that the mean egg production of the three farms was lower than the normal control one by about 10% and the egg production started later by one week.

SUMMARY

A surver was made of *Mycoplasma gallisepticum* (MG) and infectious bronchitis classical and variant strains. Blood samples were collected at the age of 20 and 33 weeks having a $\log_2$ M 9-10 ND HI antibody titers allover the examination period. SPA test against MG gave 100%, 50%, and 33% positive in farm 1 (FA_1), FA_2 and FA_3 respectively. AGPT against IB recorded a result of 41.6%, 0% and 0%, while the result of HI test against variant IB strains (M_4, D_{274} and D_{1466}) was 100% in all the three examined farms indicating higher titer against M_{41} than D_{274} and the lowest was D_{1466}. The egg production curve through the period of 26-37 weeks was found to be decreased by about 10% than the control farm (negative MG and IB, Classical or variant). This decrease in egg production may be due to the infection with either MG amixed with IB or IB alone.

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