



## **Enhancement of immune response against IBD and IB in antibiotic treated *Mycoplasma gallisepticum* serologically positive broiler chickens**

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**Abstract :** This study was carried out to study effect of antibiotics and or probiotics on immune response of *Mycoplasma gallisepticum* (MG) serologically positive broiler chickens to IBD and IB live vaccine. A total number of 210, 1 day old broiler chickens were used. Chicks were divided into seven equal groups and treated as follows: betaine, macroloids (tylosin), polymyxins (colistin), tylosin and betaine, colistin and betaine was given to group 1, 2, 3, 4 and 5; respectively. While group 6 and 7 was kept nontreated vaccinated and non vaccinated non treated (negative) control ; respectively. Chickens groups 1-6 were vaccinated with vector IBD and IB commercial vaccines simulating commercial field. Evaluating antibody titers against used vaccines was done using ELISA kits on sera collected at 0 day of life and every 10 days till the end of the experiments. Results revealed that the best means ELISA titer results against IBD vaccine by the end of the experiments was 7950 in both colistin group (3) and colistin and betaine group (5), followed by group (4) received tylosin and betaine which was 7800, followed by group (2) received tylosin which was 7750 followed by group (1) received betaine only which was 7700, followed by 7400 in group 6 (vaccinated non treated). The mean ELISA titers against IB live vaccine the highest was in group (5) received colistin and betain which was 1540, followed by group (3) received colistin only which was 1490, followed by group (4) received tylosin and betaine which was 1345, followed by group (1) received betaine only which was 1395, followed by group (2) which received tylosin which was 1300, while non treated vaccinated group 6 gives 1212. The recorded mean ELISA titers of MG, IBD and IB in sera of chicks at 1 day of life 5269, 11000 and 5790; respectively are due to maternal derived antibodies. Maternal derived antibodies to IBD and IB was gradually decreased in non vaccinated non treated group 7 to reach 2800 and 111 at the 30th day of life. While MG antibodies was markedly increased after live vaccine application, on the other hand non observed lesions was detected in antibiotic medicated group.

It could be concluded that both polymyxins (colistin) and macroloids (tylosin) antibiotics used in this study has positive impacts in controlling of *Mycoplasma galisepticum* and on immune response of broiler chickens to IB and IBD vaccines. Prebiotic (betaine) enhanced positively immune system to produce humoral immune response. A combination between antibiotic and prebiotic can be used to minimize the possible adverse effects of excessive use of antibiotic on vital organs.

**Keywords :** Broiler, IB and IBD vaccines, Elisa test, colistin, tylosin, Betaine.

## Introduction

Improvement of antibody titers against virulent field virus by vaccination is crucial specially against infectious bursal disease virus (IBD) and infectious bronchitis virus (IB) field challenge. IBD virus considered one of immunosuppressive disease in poultry farms<sup>1</sup> either clinical form causing severe mortalities reach over 90%<sup>2</sup> or subclinical disease with no clinical signs caused by variant strains resulting in severe immunosuppression<sup>3</sup>, protection against such disease fulfilled by live vaccine in broilers<sup>4</sup> taking inconsideration correct vaccination time ensuring decaying of maternal derived antibodies, know a day's new vector vaccines are used that not interfering with maternal derived antibody<sup>5</sup>. While IB virus is one of respiratory viruses that causing severe economic losses in poultry industries as it cause severe respiratory and kidney lesions<sup>6</sup>, however protection against such virus could be fulfilled by live vaccine, unfortunately vaccination failure occurs due to different causes including vaccine type and time of vaccination together with improper sanitation and hygienic programmes<sup>7,8</sup>. Protection against IB virus carried out by what it called protecto type phenomena<sup>9</sup> to increase number of neutralizing epitops against different field viruses that could infect broiler farms. Detection of antibody titer could be carried out by commercial ELISA kits against both IBD<sup>10</sup> and IB virus<sup>11</sup> any improvement of titers against this two viruses is of great value specially under our Egyptian field conditions including bad hygiene and improper vaccination programme. Many studies support improvement of ELISA titer humoral immune response by prebiotics against viral respiratory disease<sup>12</sup> and IBD virus<sup>13</sup>, one of used prebiotics is betaine which has many beneficial effects including improves immune response by enhancement protein and globuline in broiler serum together with improvement of lymphocyte proliferation<sup>14</sup>, many publications by different authors supports positive impacts of betaine on cellular immune response including phagocytosis, Nitric Oxide (NO) release, chemotaxis of monocytes toward chemotactic factors released by heterophils<sup>15,16</sup> moreover it was found that dietary supplementation of betaine resulting in improvement of one aspect of humoral immune response in the form of more IgA production in sera of broiler chickens<sup>17</sup>, as IgA considered mucosal immunoglobulin present on lining epithelium of respiratory and digestive tract preventing colonization and penetration of pathogenic infectious agents. On the other hand many antibiotics are widely used in poultry industry such as colistine sulphate which is a polypeptide antibiotic,<sup>18</sup> reported that colistin reduced individual lymphoid follicle proliferation resulting in decrease of circulating lymphocyte percentage particularly B-lymphocytes. <sup>19</sup> reported that the administration of polymyxins can decrease total protein due to hematuria and proteinuria, further, <sup>20</sup> mentioned that polymyxin B (PmB) stimulates monocytes to produce increased amounts of both complement factors and cytokines which are essential factors in local inflammatory response. Moreover, <sup>2</sup> stated that use of polymexins as vaccine adjuvants with protein antigens enhances specific humoral immunity in mucosal and systemic compartments and also induces immunological memory in mouse models, other author reported that colistin improves Anti- IB ELISA titer on chickens in Taiwan <sup>22</sup> other classes of antibiotics such as macroloids which are used in poultry industries also, is indeed capable of altering the proliferative capacity of immune cell <sup>23</sup>, promote production of pro-inflammatory cytokines such as interleukin 1(IL-1), interleukin 2 (IL-2), interferons (IFNs), and tumor necrosis factor alpha <sup>24</sup> this maybe due to positive effect of macroloids on macrophage. on the other hand other authors reported that macroloids found to improves spleenocytes proliferations in chickens but in the same time antibody ELISA titers against IB was lower when compared with colistin<sup>22</sup>, one of wildy used macroloids nowadays is tilmicosin which is bacteriostatic macrolide antibiotic synthesized from tylosin for veterinary use only has antibacterial spectrum mainly against atypical microorganism (*Mycoplasma* spp., *Pasterulla* spp. and many Gram- positive microorganisms<sup>25</sup>. Furthermore antimycoplasma drugs alone or in combination with prebiotic are used to improve productivity of broilers<sup>26,27,28</sup> as well as to improve immune response to Newcastle disease and Avian influenza<sup>29</sup>. Many publications ensure that tylosin improves cellular immune response at the time of respiratory viruses vaccination in chickens<sup>30</sup>. It was found that *Mycoplasma* infection in reared chickens resulting in either suppression or stimulation of B and T lymphocytes and inducing cytokines<sup>31</sup>, moreover live virus respiratory vaccine application in infected *Mycoplasma* chickens resulting in flaring respiratory conditions, due to enhancement of *Mycoplasma* in infected vaccinated birds<sup>32</sup> From the above mentioned data our trial were designated in order study effect of prebiotic (betaine) and /or antibiotic colistin or antibiotic tylosin on humoral immune response to vector IBD and live IB vaccines in *Mycoplasma gallisepticum* (MG) serologically positive broiler chickens.

## Material and methods

### 1- Experimental Chicks:

A total number of 210 *Mycoplasma gallisepticum* (MG) serologically positive broilers chicks were divided into 7 equal groups; 30 chicks in each. The used chicken groups were kept in clean disinfected pens and given ration and water adlibdem.

### 2. Ration

Commercial starter and grower broiler chicken ration were given till 21 and 32 days of age, respectively. The used commercial balanced ration based on yellow corn or soyabean that met the National Research Council (NRC, 1984) broiler chicken requirements. The starter ration contained crude protein-not less than 21%, crude fat-not less than 2.94%, crude fibers-not less than 2.35%, metabolizing energy-not less than 3054 Kcal/kg ration and used for the first 3 weeks of age. The grower ration contained crude protein-not less than 17.15%, crude fat-not less than 2.5%, metabolizing energy-not less than 3020 Kcal/kg ration and used for the remaining of the experimental period. The ration contained only coccidiostat (Semiduramicin).

### 3- Prebiotic:

**Betaine Anhydrous 98%** : produced by Nanchang lifeng Industry and Trading Co., Ltd., Batch no. 20131106, exp. Date : june 2016. Dosage 1 gm/L drinking water.

### 4- Antibiotics:

\* **Colistin sulphate 6 MIU**: each gm contains 6000.000 IU colistine sulphate. Lot No. 150415 . Jordan Vet. and Agr. Med. Ind. Co – Amman – Jordan.

\* **Tylosin: Tylox®** water soluble powder 100gm - Lot. No. 150118. Jordan Vet. and Agr. Med. Ind. Co – Amman – Jordan.

### 5- Serum samples:

Blood samples for serum were collected start from day one of life and in 10 days intervals till the end of the experiments for serology test.

### 6- Vaccine Strains

a. Hitchiner B<sub>1</sub> with infectious bronchitis (IB) live vaccine , each vial contain virus titre of 10<sup>9</sup> EID<sub>50</sub> for ND and 10<sup>3</sup> EID<sub>50</sub> for IB virus vaccine was used for vaccination of experimental chicks via eye instillation route at 3<sup>rd</sup> days of life. Second IB vaccination with lives vaccine takes place at 13<sup>th</sup> days of life using IB live Vaccine Nobilis, strain H-120 (Massachusetts), 1000 dose via eye drop instillation while second ND vaccination times takes place at 16 days of life using La Sota live vaccine.

b. Infectious bursal disease (IBD) administered with VAXXITEK® HVT + IBD (a registered trademark of Merial ) vaccination takes place at 1 day of age according to the manufacturer's recommendation.

### 7- Serology test:

a. **IBD serology** :The sera obtained from blood of experimental chicks at various time points were tested for IBD antibodies using the PROFLOK® plus IBD Ab test kit (Symbiotics, San Diego, CA). The antigen used by this kit is a the antigen is purified extract from IBDV infected bursa tissue (Le Gros et al., 2009) It allows early detection, during the observation period of the chickens within the study, of the post-HVT-IBD vector vaccine vaccination antibody detection. Antibody titers were measured in ten days intervals basis The results were expressed in titer (Log<sub>10</sub>), as recommended by the diagnostic kit producer.

### b. IB serology:

The procedure used in this test was performed according to the manufacturer instructions listed in the PROFLOK® IBV ELISA Kit (Synbiotics-USA), which is a rapid serologic test for the detection of IBV

Antibody in chicken serum samples .It was developed primarily to aid in the detection of pre and post-vaccination IBV antibody levels in chickens.

### C. MG serology:

The procedure used in this test was performed using commercial ELISA kits for the presence of anti-MG antibodies ProfLOK®Mycoplasma gallisepticum Antibody Test Kit, Synbiotics Corp. - USA] according to the manufacturer's instructions

### 7- Experimental design:

Experimental design is shown in table (1), a total number of 210, 1 day old broiler chickens were used. Chicks were divided into seven equal groups and treated as follows: betaine, macroloids (tylosin), polymyxins (colistin), tylosin and betaine, colistin and betaine was given to group 1, 2, 3, 4 and 5; respectively. While group 6 and 7 was kept nontreated vaccinated and non vaccinated non treated (negative) control; respectively. Chickens groups 1-6 were vaccinated with vector IBD and IB commercial vaccines simulating commercial field. Evaluating antibody titers against used vaccines was done using ELISA kits on sera collected at 0 day of life and every 10 days till the end of the experiments. Antibiotics used three times 1<sup>st</sup> start from day one till end of day five of age, second start from day 15 till 20 of age and finally start from day 25 till day 30 of age, while betaine application start from days six of life till the end of experiment. The obtained results are shown in table (2) and figures (1 -3).

**Table (1) showing experimental design**

Type of treatment	Group
Betaine	1
tylosin	2
Colistin	3
tylosin + betaine	4
Colistin + betaine	5
Negative non treated vaccinated	6
Non treated non vaccinated	7

### Results and discussion

Poultry industries under Egyptian field condition is directed by two main factors including managmental or infectious and so control of one or more of this factors is of great value. Improves immune response against infectious poultry disease either immunosuppressive disease such as IBD virus or respiratory virus such as IB not only resulting in control of both diseases but also improves poultry performance including immune response to respiratory virus vaccine together with low post vaccinal reaction against live respiratory virus vaccine otherwise impaired humoral antibody response, suboptimal response to live vaccine and complicated respiratory disease CRD takes place<sup>33,26</sup>.

The recorded mean ELISA titers of MG, IBD and IB in sera of chicks at 1 day of life 5269,11000 and 5790; respectively are due to maternal derived antibodies. IBD and IB maternal antibodies gradually decreased in non vaccinated non treated group 7 to reach 2800 and 111 at the 30<sup>th</sup> day of life<sup>34,35</sup>, this normal decaying of mean ELISA titers antibody of maternal derived antibody in group (7) revealed that no challenge with field virus during whole experimental time in both groups received vector IBD vaccine or IB live vaccine. while in case of IB group ,it was found that in vaccinated non treated group (6) maternal antibody titers interfere with first vaccination response resulting in neutralization of antibody titers , this was clear at 10<sup>th</sup> day (titer was 618) , this was parallel with results found by<sup>36</sup> who stated that high level of maternal antibody can interfere with vaccination response, moreover<sup>37</sup> stating that in spite of that vaccination in the presence of high level of maternal antibody resulting in the development of local immunity but unfortunately it well cause depression of developing of high level of circulating antibodies, it is well known that protective local maternal antibody known to has a great role in preventing IB challenge while systemic maternal antibodies responsible for protection against spread of IB virus from respiratory tract to internal organs via blood stream<sup>38</sup>, mean antibody titer increase after second live IB vaccination in group 6 vaccinated non treated group , this result was parallel with<sup>39</sup> who reported huge amount of memory cells synthesitized for rapid produce of antibodies after second vaccination with the same antigen of first vaccination.

On the other hand use of IBD vector vaccine in non treated vaccinated group (6) did not cause neutralization of maternal antibodies as it can overcome maternal immunity with no interaction, moreover release of antigen according individual diminish of maternal derived antibodies , this results was matched with 40 .

Results of 10 days of age was nearly half of that recorded at that of the 1 day then generally marked increased in all vaccinated groups at the 20<sup>th</sup> day of life ,While MG antibodies was increased to reach 3832 at the 30<sup>th</sup> day of life this increase in *Mycoplasma gallisepticum* titers may due to use of live respiratory vaccine including IB twice and Newcastle vaccine lasota which considered as stress factors that enhance Mycoplasma multiplication resulting in increase MG ELISA titer , as it was found that use of live respiratory virus vaccine in Mycoplasma infected chickens resulting in enhancement of mycoplasma multiplication and respiratory lesions occurrence<sup>32</sup>. The activation of Mycoplasma by live vaccines has been detected by lesions as described by of mild CRD at the end of the expeiment in non treated groups<sup>26</sup> while non observed lesions was detected in antibiotic medicated group , moreover no clinical disease occurs this maybe due to lack of complicating factors including manegmental or infectious agents<sup>41</sup>, as *Mycoplasma gallisepticum* considered slow spreading infection and often infected birds remain healthy without showing any signs of the disease.

Regarding the effect of treatments on means ELISA titer against IBD virus vaccine was shown in table (2) and fig (2) . Best means ELISA titer results against vector IBD vaccine by the end of the experiments were group (3) received colistin and group (5) which received colistin and betaine which both were 7950, followed by group (4) received tylosin and betaine which was 7800 , followed by group (2) received tylosin which was 7750 followed by group (1) received betaine only which was 7700, followed by group (6) vaccinated non treated group which was 7400 , and finally the lowest was group (7) non treated non vaccinated group which was 2800.

The mean ELISA titers against IB live vaccine (Table 2 Fig 3) revealed that the highest was in group (5) received colistin and betain which was 1540, followed by group (3) received colistin only which was 1490, followed by group (4) received tylosin and betaine which was 1345, followed by group (1) received betaine only which was 1395, followed by group (2) which received tylosin which was 1300,while non treated vaccinated group 6 gives 1212.

**Table (2): Means ELISA titer against IBD , IB live vaccines and MG in broiler chicken groups given Betaine, Tylosin and/ or Colistin.**

Gr. No.	Vaccination IB and IBD	Treatment	Age / days	Means IBD ELISA titers	Means IB ELISA titers	Means MG ELISA titers
1	+	Betaine	10	5000	710	1373
			20	7500	510	1099
			30	7700	1395	2927
2	+	Tylosin	10	5400	655	1123
			20	7100	465	845
			30	7750	1300	685
3	+	Colistin	10	6100	715	1945
			20	7400	595	1204
			30	7950	1490	926
4	+	Tylosin + Betaine	10	5800	680	1761
			20	7400	530	919
			30	7800	1345	668
5	+	Colistin + Betaine	10	5150	725	2892
			20	6800	618	1446
			30	7950	1540	1608
6	+	-	10	4650	618	2961
			20	7200	315	3199
			30	6100	1212	4668
7	-	-	1	11000	5790	5269
			10	5700	1480	2412
			20	3500	540	2871
			30	2800	111	3832

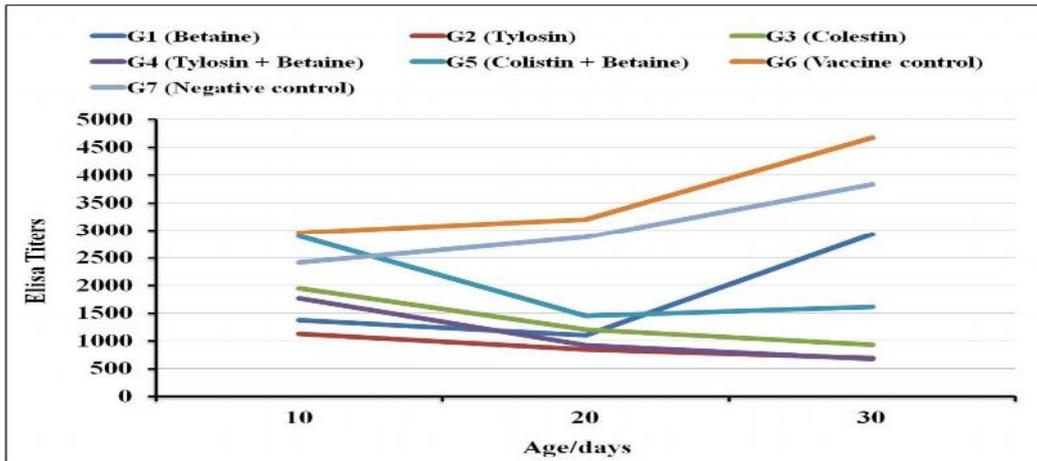


Fig (1): Levels of means ELISA titer against MG in broiler chicken groups given Betaine, Tylosin and/ or Colistin.

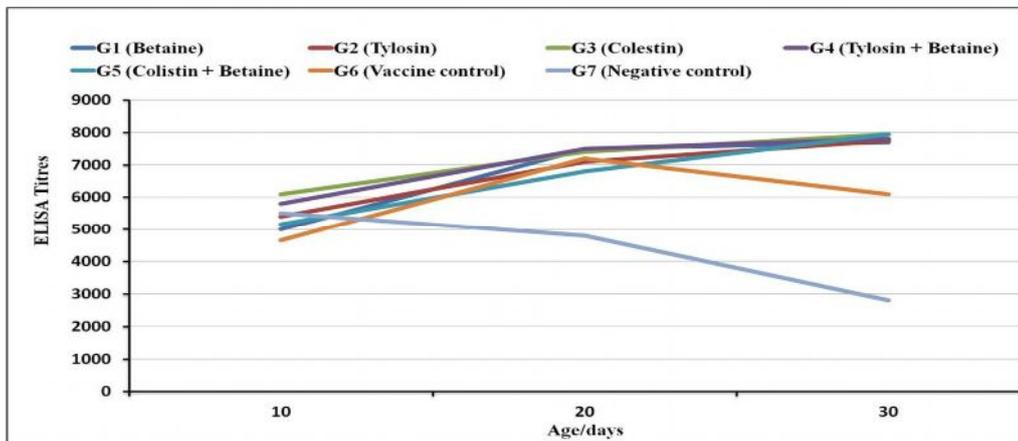


Fig (2): Levels of means ELISA titer against IBD live vaccines in broiler chicken groups given Betaine, Tylosin and/ or Colistin.

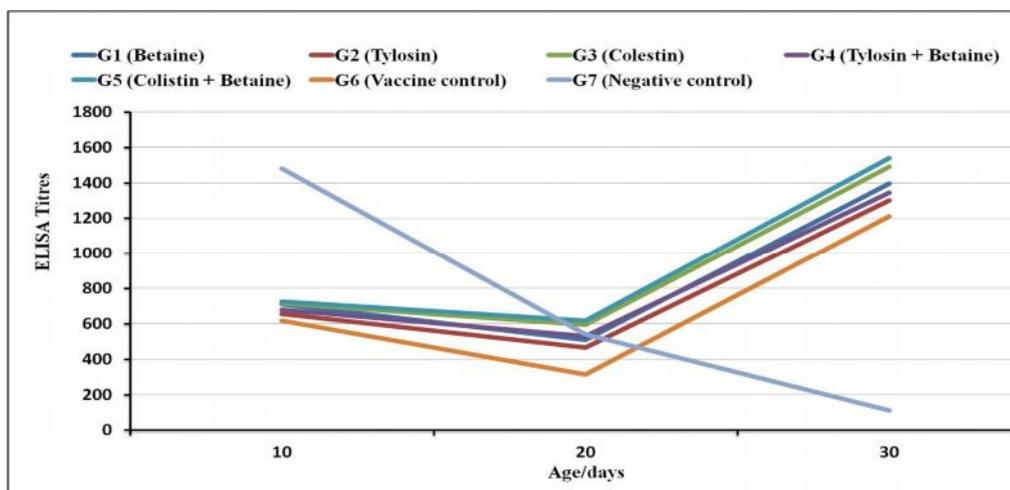


Fig (3): Levels of means ELISA titer against IB live vaccines in broiler chicken groups given Betaine, Tylosin and/ or Colistin.

Means ELISA titer was improved in all treated vaccinated groups than vaccinated non treated group. Improves of humoral immune response by colistin was parallel with results found by <sup>22</sup> who reported that use of colistin improves infectious bronchitis ELISA titer when compared with control group, also <sup>21</sup> stated that use of polymyxins resulted in enhances specific humoral immunity in mucosal and systemic compartments and also induces immunological memory when used as vaccine adjuvant, while titers improved by tylosin was matched with results found by <sup>23</sup> who reported that there was a significant increase in antibody production as well as in the numbers of antibody-producing cells in macroloids administered chickens compared with the untreated controls, also betaine found to improve immune response especially enhancing of globuline and protein <sup>14</sup> together with improving cellular immune response <sup>16</sup> and so use of combination of betaine with those antibiotics colistin and tylosin resulting in synergistic action <sup>28,29</sup>.

It could be concluded that both polymyxins (colistin) and macroloids (tylosin) antibiotics used in this study has positive impacts in controlling of *Mycoplasma galisepticum* and on immune response of broiler chickens to IB and IBD vaccines. Prebiotic (betaine) enhanced positively immune system to produce humoral immune response. A combination between antibiotic and prebiotic can be used to minimize the possible adverse effects of MG as well as excessive use of antibiotic on vital organs.

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