Immunosuppressive Potential of Acute Caecal Coccidiosis as well as Anticoccidial Vaccine on Antibody Titers Induced by Newcastle Disease and Infectious Bursal Disease Viruses Vaccines in Broiler Chickens.

Shaban, KH. S

Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Egypt

Abstract: This study was designed to evaluate the effect of the most prevalent and highly virulent coccidial infection (caecal coccidiosis) as well as anticoccidial vaccination on haemagglutinating antibody titers of Newcastle Disease virus (NDV) and Infectious Bursal Disease Virus (IBDV) antibody ELISA titers resulted from vaccination program in broiler chickens. For this purpose, 180 day old Hubbard broiler chicks were randomly divided into 3 equal groups (G1, G2 and G3), each of 60 chicks. At 3rd day of age birds of G1 was vaccinated with anticoccidial vaccine via crop, and at 14th day of age birds of G2 was infected with high dose (50000) of sporulated E. tenella oocysts intra crop, while birds of G3 remain as control (Non coccidia vaccinated or infected). At 14th, 21th and 28th days of age, mean HI antibody titers of G3 were higher than G2 and G1 groups. At 21th and 28th days of age, mean HI antibody titers for NDV as well as mean ELISA antibody titers for IBDV of G2 were significantly lower than other groups ($p \leq 0.05$). At 35th and 42th days of age HI titers of G3 and G1 were higher than G2, but the differences were not significant ($p > 0.05$). The mean HI antibody titers for NDV as well as mean ELISA antibody titers for IBDV of G1 had non-significant lowering values than those of G3 at all ages ($p > 0.05$). It was concluded that coccidial infections as well as anticoccidial vaccination are able to reduce humeral immunological reactions of broiler chickens as indicated by the significant reduction in mean HI antibody titers for NDV and mean ELISA antibody titers for IBDV as well as the significant lowering in the protection percentage against challenge with VVND and virulent IBDV in these chickens. Since this effect was more prominent in infected group than in anticoccidial vaccinated one, in the cases of coccidiosis outbreaks in the farm with higher levels of coccidial infections, involvement of mixed virulent species (E. tenella, E. necatrix, E. acervulina, E. maxima….. ect.) and other environmental stressors, more severe and prolonged immunosuppresion are expected.

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1. Introduction

Coccidiosis is one of the most important protozoal diseases of poultry which is caused by genus Eimeria. Midst coccidial infections and during different stages of Eimeria life cycle which takes place in the mucosal layer of the intestine, destruction of the epithelial lining of the intestine takes place and is often accompanied by some degree of inflammation, resulting in local pathological changes (*Vermeulen, et al. 2000*), dehydration and diarrhea (*Augustin, et al. 1997*), interruption of feeding and digestive processes, blood loss and absorption of many nutrients (proteins, vitamins, minerals…) which are important in immunological reactions are reduced (*Larry R., 2003*). For instance the level of sodium, calcium, phosphorous and iron had been lower in coccidial infected birds (*Koinarski and Kamburov, 1985*). Coccidial infection also reduced the level of plasma albumin, iron, glucose, phosphorous and sodium (*Padmavathi and Muralidharam, 1986*). Basal metabolism in birds infected by coccidia had been lower than healthy birds (*Padmavathi and Muralidharam, 1986*). Infection with coccidia also reduced the level of Zn in the liver (*Southern and Baker, 1983*). Plasma Iron and bound Iron reduced in acute coccidiosis (*Southern and Baker, 1982*). Coccidial infection also reduced the plasma level of some amino acids (Arg. Thr, Ser, Pro and Gly) (*Elchiev, 1980*). Reduction of plasma carotenoids has been reported in coccidial infections (*Augustin and RUFF, 1983*). In coccidial infections the level of plasma ascorbic acid reduced (*Singh, et al, 1979*). It is also well established that coccidial infection causes reduction of growth and feed efficiency (*Bafundo, 1985*). Also the developmental forms of the protozoan parasite Eimeria tenella were demonstrated in the bursa of fabricius of birds infected with E. tenella (*Mohammed, et al. 1974; Anderson, et al. 1976 and Mohammed, et al. 1980*). Since certain role of many nutrients such as vit A, Amino acids, proteins, vit E, Selenium, vit C, vit B12, Zn, Fe has been well documented on the efficiency of cellular and humeral immune responses, therefore it logically sounds right, that coccidiosis which negatively alter the absorption of these nutrients as well as had a possible destructive effect on bursa of fabricius causes poorer immune responses in coccidial infected groups. Therefore

poorer weight gain and FCR as well as imunosuppresion are normal consequence of the disease. Since many of these nutrients are important in immunological reactions as well as the involvement of bursa of fabricius, a study was designed to clear out the role of coccidial infection as well as anticoccidial vaccination on the post vaccinal immune response of broilers against IBDV & NDV vaccines.

2. Materials and methods

Experimental chicks: A total of 200, one day-old meat-type broiler chicks (Hubbard breed) which obtained from private hatchery as hatched were used.

Anticoccidil vaccine:

(Coccivac-B) A live oocyst vaccine for broiler vaccination comprised four species of the wild type of Eimeria (E. acervulina, E. mivati, E. maxima and E. tenella) (Schering Plough Animal health Corp., Millsboro, Delaware, USA) was used.

E. tenella inoculum:

The inoculums of sporulated oocysts were prepared from a local strain of E. tenella which isolated from field outbreaks of broiler caecal coccidiosis and identification based on site of infection, pathognomonic lesions and shape of oocysts *(Joyner and Long, 1974)*. After sporulation it was tested for pathogenesity in a group of 10 birds, 14 days old, and then the recovered oocysts from the caecal portions were subjected for sporulation and then calculation by McMaster technique *(Khelfa, 1983)*. The inoculum infecting dose was given intra crop.

Viruses and vaccines:

1- Infectious Bronchitis (IB) virus vaccine:

Nobilis IB 120, live freeze dried virus vaccine (Intervet International, B.V. BOXMEER - Holland) against Infectious Bronchitis serotype Massachusetts (strain 120) was used for vaccination of birds.

2- NDV vaccines:

Live attenuated NDV vaccines, Intervet Company, Hitchener B1 strain and Clone 30 of titer $10^{6.5}$ EID_{50} / dose were used for vaccination of birds.

3- IBDV vaccine:

Live attenuated freeze-dried vaccine Nobilis Gumboro 228E (Intervet International, B.V. BOXMEER - Holland), grown on embryonated eggs was used. Each dose contains at least 2.0 $\log^{10}$ EID_{50} of the Gumboro strain 228E. It was given by eye-drop as recommended by the Manufacturer Company.

4- Avian Influenza (AI) virus vaccine:

Inactivated H5N2 Avian Influenza vaccine (Intervet Company) was used for vaccination of birds against Avian Influenza by subcutaneous route.

5- NDV challenge strain:

A lyophilized velogenic viscerotropic strain of NDV (VVND) characterized by *Sheble and Reda (1976)* was kindly obtained from ND department of Serum and Vaccine Research Institute, Abassia, Cairo, Egypt. The velogenicity of the virus was checked in 10 chickens by intramuscular injection of $10^{6.8}$ EID_{50} / ml / bird and resulted in 100% mortality rate.

6- Virulent infectious bursal disease virus:

A bursal homogenate containing highly pathogenic pathotype of local virulent IBDV isolate that has been characterized earlier by RT-PCR-RFLP *(Abdel-Alim et al., 2003)* was kindly obtained from Dr. G.Abdel-Alim, Poultry Diseases Department, Fac. Vet. Med., Cairo Univ., Egypt. Virus titration in chicken embryos was made by serial 10-fold dilution of the bursal homogenate and inoculation onto the chorioallantoic membrane as described by *Hitchner (1970)*. The titer is expressed as the 50% embryo infective dose (EID_{50}) per ml and was calculated by the method of *Reed and Muench (1983)*.

Experimental design:

A total of 180 day old Hubbard broiler chicks were randomly divided into 3 equal groups (G1, G2 and G3), each of 60 chicks. The birds were placed on floor pens in separated rooms, which previously disinfected, electrically lightened and heated and the litter was wheat straw, they fed balanced commercial ration *ad libitum*. Chickens of all groups were vaccinated as follow: The IB disease virus vaccine, strain 120, was given at 1st day of age via spray, and then they vaccinated against NDV with B1 strain at 7th day of age and with clone 30 strain at 15th day of age and both of them were given via eye drop, while the IBDV vaccine (228E) was given at 14th day of age by eye drop instillation and AI virus vaccine was given via sub cut injection in the back of the neck at 7th day of age. At 3rd day of age birds of G1 were vaccinated with anticoccidial vaccine via crop, and at 14th day of age birds of G2 were infected with high dose (50000) of sporulated E. tenella oocysts intra crop, while birds of G3 remain as control (nither coccidia vaccinated nor infected).

Blood sampling:

Birds of all groups were bled at 7th, 14th, 21th, 28th, 35th and 42th days of age. Blood samples were collected from wing veins by syringes, transferred to laboratory tubes and left for 1hr at 25^{oc} then centrifuged for ten minutes at 1500 rpm. Serum samples were preserved at -20^{oc} up to end of the experiment for HI and ELISA tests to be done altogether.

Evaluation of immunosuppressive effect:

To investigate the possible immunosuppressive effect of Eimeria on humeral immune response, immunoassay was carried out. For this purpose, the collected blood samples at the previously mentioned different intervals were subjected to HI test for determining antibody titers against NDV vaccination and also the blood samples collected at 21th and 28th days of age were subjected to ELISA test for determining antibody titers against IBDV vaccination.

Haemagglutination inhibition (HI) test:

Beta procedure HI test was used to measure the serological response to NDV vaccines using 4 haemagglutinating units (*Beard and Wilkes, 1973*), HI titers were determined in all chickens and the geometric mean titer (GMT) was calculated for each group.

ELISA test:

BioCheck ELISA kits were used to determine the IBDV antibody titers at 21th and 28th days of age for all groups. Samples dilution, test procedure, validity, and interpretations of the employed assays were carried out according to the manufacturer instructions.

Bioassay against NDV and IBDV:

At 28th day of age each group was then subdivided into 2 subgroups (a and b) each of 30 chicks, the chicks of subgroup (b) in all groups were then sub divided into another 2 subgroups (b-1 and b-2) each of 15 chicks, the chicks of sub group (b-1) in all subgroups (b) were challenged with VVND by intramuscular injection of $10^{6.8}$ EID_{50} / ml / bird and the chicks of subgroup (b-2) in all subgroups (b) were challenged with virulent IBDV by intraocular instillation of $10^{3.5}$ EID_{50} / ml / bird, both were kept under observation for further 2 weeks and the mortalities in both subgroups (b) were recorded.

Statistical analysis:

HI and ELISA antibody titers were statistically analyzed using analysis of variance and comparing between groups was performed using least significant difference (LSD) at P≤ 0.05 according to *Petrie and Waston (1999)* and computerized using *SPSS (1999).*

3. Results and discussion

At 7th and 14th days of age the difference among mean HI of all groups was not significant (table 1). Blood sampling at 7th day of age was performed before first ND vaccination and HI titers at this age were associated to residual of maternal antibodies. HI titers at 14th day of age are associated to antibody levels just prior to infection with Eimeria. It seems that at this age the first ND vaccination with B1strain had not been effective so much to raise the level of antibodies. At 21th and 28th days of age (1st and 2nd weeks after administration of Eimeria) the highest NDHI and IBDV ELISA antibody levels were related to G3 and the lowest antibody levels were related to G2 and the differences between G3 and G2 and between G2 and G1 were significant (p≤0.05), while the differences between G1 and G3 were not significant (table 1&2) indicating that coccidia induced severe immunosuppression in infected chicken group, while the anticoccidial vaccination had milder effect. The same result were obtained after challenging of coccidia vaccinated and infected chicken groups with the VVND and virulent IBDV, the protection percentages were 70% against VVND and 75% against virulent IBDV for coccidia infected group and 95% against VVND and 92% against virulent IBDV for coccidia vaccinated group compared to 100% in control group against both challenging viruses (table 1&2). At 35th and 41st days of age the differences among mean NDHI antibody titers of all groups were not significant (P>0.05) (table 1).

Table -1- Comparison of the mean NDHI titers and protection percentages against VVND in different groups (mean ± SEM)

Group \ Age in(days)	7	14	21	28	35	42	Protection %
G3 (Control)	$4.5^{\pm0.5a}$	$4.1^{\pm0.2a}$	$4.8^{\pm0.4a}$	$5.8^{\pm0.3a}$	$6.6^{\pm0.3a}$	$7.1^{\pm0.3a}$	100%
G2 (coccidia infected)	$4.7^{\pm0.6a}$	$3.5^{\pm0.3a}$	$3.3^{\pm0.4b}$	$4.4^{\pm0.2b}$	$5.8^{\pm0.2a}$	$6.3^{\pm0.3a}$	70%
G1 (Coccidia Vaccinated)	$4.3^{\pm0.4a}$	$3.7^{\pm0.2a}$	$4.3^{\pm0.3a}$	$5.5^{\pm0.3a}$	$6.0^{\pm0.2a}$	$6.7^{\pm0.2a}$	95%

Values in the same column with different superscripts are significantly different (p≤0.05).

Table -3- Comparison of the mean IBDV ELISA antibody titers and protection percentage against virulent IBDV in different groups

Group \ Age (days)	21	28	Protection %
G1 (Coccidia Vaccinated)	2868^{a}	1201^{a}	92%
G2 (coccidia infected)	1417^{b}	624^{b}	75%
G3 (control)	3285^{a}	1536^{a}	100%

Values in the same column with different superscripts are significantly different (p≤0.05).

Since coccidiosis is one of the most important protozoal disease facing poultry industry, it is very important to know about different aspects of the disease such as the possible suppressive effect of the disease on the function of the immune system. Generally, suppression of the immune system reduces the resistance of the body to infectious diseases as well as induction of suboptimal response to vaccines, normally used to build a resistance and immunity to some diseases. There are some infectious diseases which are well known of having mechanisms of suppressing the immunity such as IBD, CIA, Marek's disease, etc. Regarding coccidiosis, although some suppressive effects on immune system had been noticed by some researchers (*El-Wanis, 1991 a&b and Orbay, et al, 1994*), but the possible mechanisms has not been well discussed. *Mohammed, et al. 1974 and 1980*, proved the replication of the protozoan parasite E. tenella within the bursa of fabricius in chicks experimentally inoculated with oocysts via the natural route of infection and with sporozoites injected directly into the burse of fabricius, where a limited and delayed development of the parasite occurred in the epithelial cells of the bursa of fabricius following inoculation via both routes. No morphological differences were seen between the developmental stages seen in the bursa of fabricius and in the ceca. Oocysts harvested from the bursa of fabricius were as infective as those collected from the ceca. Definite pathological changes were seen in the epithelial lining of the bursa. The first stages of the parasite were accompanied with a clear heterophilic infiltration between the epithelial cells. On 7^{th} day of infection a clear hyperplasia of the epithelial cells was seen. Later a mucous degeneration and subepithelial haemorrhages were observed. Some cases showed a complete necrosis of the epithelial lining of the bursa. Other cases revealed severe degeneration in the epithelial cells resulting in severe erosion; this may explain the mechanism of poor immune response in coccidian infected chickens. Also coccidiosis is essentially an enteric disease, which cause poor digestion and absorption of nutrients, therefore mechanism of reduction of antibody levels could also be related to this issue. For instance the level of sodium, calcium, phosphorous and iron had been lower in coccidial infected birds (*Koinarski and Kamburov, 1985*). Coccidial infection also reduced the level of plasma albumin, iron, glucose, phosphorous and sodium (*Padmavathi and Muralidharam, 1986*). Basal metabolism in birds infected by coccidian had been lower than healthy birds (*Padmavathi and Muralidharam, 1986*). Infection with coccidia also reduced the level of Zn in the liver (*Southern and Baker, 1983*). Plasma Iron and bound Iron reduced in acute coccidiosis (*Southern and Baker, 1982*). Coccidial infection also reduced the plasma level of some amino acids (Arg. Thr, Ser, Pro and Gly) (*Elchiev, 1980*). Reduction of plasma carotenoids has been reported in coccidial infections (*Augustin and RUFF, 1983*). In coccidial infections the level of plasma ascorbic acid reduced (*Singh, et al, 1979*). It is also well established that coccidial infection causes reduction of growth and feed efficiency (*Bafundo, 1985*). Since certain role of many nutrients such as vit A, Amino acids, proteins, vit E, Selenium, vit C, vit B12, Zn, Fe has been well documented on the efficiency of cellular and humeral immune responses, therefore it logically sounds right, that coccidiosis which negatively alter the absorption of these nutrients as well as had a possible destructive effect on bursa of fabricius causes poorer immune responses in coccidial infected groups. It should be noticed that in this study the observed effect on NDHI and IBDV ELESA antibody titers was more prominent in the infected group. Since in the field situation, infection with higher doses of Eimeria and mixed virulent species, such as E. tenella, E. necatrix E. acervulina and E. maxima is prevalent, therefore, more severe suppressive effects on immune response are expected in the field situation. In a parallel study, the effect of experimental challenge with E. maxima on the immune responses to NDV vaccination was studied, a significant difference was noted between NDHI antibody titers of the infected and control group, (*El- Wanis, 1991 a&b*). In another study the negative effect of infection with E. necatrix on NDV vaccine immune responses was observed (*El- Wanis, 1991 a&b*), infection with E. necatrix, before, after and at the time of vaccination with live NDV vaccine induced immunosuppression for one week. According to the results of our study and other studies mentioned above, it is concluded that coccidiosis has negative effect on the vaccinal immune response.

Corresponding author
Shaban, KH. S
Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Egypt

References:

1. **Abdel-Alim,** G.A., Awaad, M.H.H., and Y.M. Saif (2003). Characterization of Egyptian field strain of infectious bursal disease virus. Avian Dis., 47: 1452-1457.
2. **Anderson**, W.I., Giambrone, J.J, Fletcher, O.J., Jr. Eidson, C.S. and Reid, W.M. (1976). Demonstration of Eimeria tenella in Bursa of fabricius of chickens. Avian Diseases, 20: pp. 752-755.

3. **Augustin**, P.C., J.L. McNaughton, E. Virtanen and L. Rosi (1997). Effect of Betaine on the growth performance of chicks inoculated with mixed culture of Avian Eimera tenella and Eimera acervulina in vitro and in vivo. Poult. Sci., 76: pp. 802-809.
4. **Augustin**, P.C. and RUFF, M.D.(1983). Changes in carotenoid and vitamin A levels in young turkeys infected with E. meleagridis or E. adenoides . Avian Diseases. 27 (4): pp. 963-971.
5. **Bafundo**, K.W.(1985). Trace elements inter relationships and zinc status of the chicken as affected by E. acervulina infection. Dissertation-Abstract- International, B, 45 (7): pp. 2066-2071.
6. **Beard**, C.W. and Wilkes (1973). A simple and rapid micro- test procedure for determining Newcastle heamagglutination inhibition (HI) antibody titers. Proc. 77th Ann. Meet. US Anim. Health Assoc. pp. 596-600.
7. **Elchiev**, Y. (1980). Biochemichal assessment of the efficacy of coccidia in chicken coccidiosis. Parasitology. 14 (5): pp. 452-456.
8. **EL- Wanis**, N.A. (1991a). Effect of infection with E. necatrix on the response of chickens to NDV vaccines using lentogenic Hitchner B1 (HB1) strain of NDV. Assiut Veterinary Medical Journal, 25 (50): pp. 80-88.
9. **EL- Wanis**, N.A. (1991b). Effect of infection with E. necatrix on the response of chickens to NDV vaccines using mesogenic komarov "K" strain of NDV. Asssiut Veterinary Medical Journal, 26 (51): pp. 124-137.
10. **Hitchner,** S.B. (1970). Infectivity of infectious bursa disease virus for embryonating eggs. Poult. Sci. 49: 511-516.
11. **Joyner** L.P. and Long P.L. (1974). The specific characters of the Eimeria with special reference to the coccidia of the fowl. Av. Pathol. 3 (3): 145-157.
12. **Khelfa** D.G. (1983). Further studies on coccidiosis in poultry. Ph.D. Thesis Poult. Dis. Fac. Vet. Med. Cairo Univ.
13. **Koinarski**, V. and kamburov, P. (1985). Changes in some blood indices in turkey poults with experimental E. adenoids infection. Vetrinar Nom Edit Sinsk Nauk, 22 (6): pp. 20-26.
14. **Larry** R. McDougald, (2003). Protozoal Infection. In: Diseases of poultry Y. M. Saif, 11th Edn., CD-ROM Iowa State Press, pp. 973-991.
15. **Mohammed**, M.A. Abbasi, k.H. and Sokkar, S.M. (1974). Histopathological examination of bursa fabricius as a mean for the evaluation of coccidiostats tested against Eimeria tenella. Assiut Vet. Medical Journal. 1, NO.1&2: pp. 41-45.
16. **Mohammed**, M.A., Sokkar, S.M. and Abbasi, K.H. (1980). The replication of Eimeria tenella in the bursa of fabricius of the domestic fowl. Arch. Geflugelk, 44, 22-25. ISSN 0003-9098. © Eugen Ulmer Gnb H & Co. Stuttgart.
17. **Orbay**, F.A., Edris, G.O. and Hegazy, M. (1994). Possible immunosuppressive effect of E. grenieri infected guinea fowl, the effect on vaccination with Newcastle disease virus K strain. Annals of Agricultural Science Moshtohor, 32 (4): pp. 2205- 2211.
18. **Padmavathi**, p. and muralidharam, S.R.G. (1986). Studies on the alteration in the serum metabolites during the E. tenella infection in chicken. Indian Veterinary Journal. 63 (7): pp. 530-535.
19. **Petrie,** A. and Waston, P. (1999). Statistics for Vetrenary and animal science. 1st Ed., pp. 90-99, the Blackwell Science Ltd, United Kingdom.
20. **Reed,** L.J., and H.A. Munech (1983). Simple method for estimation fifty percent end-point. Am. J. Hyg. 27: 493-497.
21. **Sheble,** A. and Reda, I.M. (1976) Cited by Khaphagy, A.K. (1977). M. V. Sc. Thesis, Fac. Vet. Med., Cairo University.
22. **Singh**, C.V., Joshi, H.C. and Shah, H.L. (1979). Biochemical studies on intestinal coccidiosis of poultry. Pannayar Journal of Research. 1(1): pp. 63-66.
23. **Southern**, L.L and Baker, D.H. (1982). Iron status of the chickens as affected by E. acervulina infection and by variable iron ingestion. Journal of nutrition, 112 (12): pp. 2353-2362.
24. **Southern**, L.L. and Baker, D.H. (1983). Eimeria acervulian infection and the zinc-copper inter relationship in the chick. Poultry Science, 62 (2): pp. 401-404.
25. **SPSS** (1999). "Statistical package for social science, Spss for windows Release 11.00" Standard version copyright Spss Inc., 1989 2002, All rights Reserved, copyright ® Spss Inc.
26. **Vermeulen**, A.N., P.M. Schetters and T.C. Schaap, (2000). Control of coccidiosis by vaccination. Paper presented at the XXI World Poultry Congress. Montreal, Canada.