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Detection of Maternal Immunity in Progenies from Breeders Vaccinated with Necrotic Enteritis Vaccine.

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ABSTRACT

Maternal immunity considered important factor that affecting vaccination program timing. Vaccination of breeders at 2 and 8 week old gives satisfactory levels of specific antibodies against alpha toxin of *C. perfringens* type A during the breeding period which is 4 and 2 IU when measured by mouse neutralization test and o.551 and 0.381 when measured by ELISA test. These results establish a base for high levels of specific antibodies during the laying period with boostering at 20 week old as results show 6 and 10 IU and 0.983 and 1.581 respectively. Consequently enough levels of specific antibodies transferred to the hatched chicks as results said 1 and 1.5 IU at day 1 and 3 o.242 and 0.399 respectively to protect the progenies during first days post hatching.

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INTRODUCTION

Necrotic enteritis is one of the most important poultry disease caused by *C. perfringens* type A, the disease has predisposing manegmental factors such as ration content, parasitic infestation such as coccidiosis resulting in high economic losses including affected feed conversion rate, decrease weight gain and increased mortality and morbidity in such flocks **[1].** Necrotic enteritis is a disease of poultry that well demonstrated since first being reported by **[**2]. It has been established that *C. perfringens* type A is the causative agent **[3].** Diets which high in energy and protein and infected by coccidia have been incriminated as predisposing the intestinal tract of chickens to an overgrowth by clostridial organisms **[**4]. Necrotic enteritis is a disease of broilers and hens either breeders or layers at different ages **[5]** starting from two weeks until 12 weeks old. **[6].**

The disease has two forms, clinical or acute form of necrotic enteritis which is characterized by high mortality among broiler flocks **[**7].While the subclinical form resulting in impaired productivity performance due to decreased digestion and absorption and consequently low levels of food conversion rates which lead to reduced weight gain **([8].** Also in the subclinical form, there is an increased number of condemnations in slaughters due to liver lesions as a result of cholangiohepatitis **[9].**

Global costs to the poultry industry all over the world have been estimated up to two billion dollars/year **[10]** Control of necrotic enteritis could be achieved by routin hygienic measures together with suitable therpuetic agents against both coccidiosis and *C. perfringens* type A in ration and water **[11]**

Therefore the risk of necrotic enteritis has increased in recent years because of the voluntary and legality required withdrawal of the use of feed and water antibiotics **[12]** and the use of antibiotics to control the disease is now being questioned in many countries **[13]**. This give rise to persistent need for alternative approach such as vaccination which is used nowadays.

One day old chicks has ill developed immune system depending mainly on maternal derived immunity and become sensitive to different pathogens, moreover this ill developed immune system resulting in weak antibody response to vaccinal program , this problem affect mass parentral vaccination at day one which need enhancing immune response as it is not applicable **[14] ,** hence the maternal antibodies is crucial means of infectious disease protection, As levels of antibodies (IgY) against alpha toxin of *C. perfringens* may play a role in protection against necrotic enteritis **[15]** . Therefore maternal derived antibodies via egg yolk play an important role in protecting newly hatched chicks from common pathogens in early weeks of life [**16].** Many reports in literatures assist specific antibody against different pathogens transfer from breeder hens to their newly hatched chicks egg yolk resulting in the protection in their early life time **[17].** The time at which the newly hatched chicks start to synthesize antibodies endogenously depends on the type of antibodies as it was found that IgY type of antibodies secreting B- cells are not detectable in chicks plasma until six days post-hatching **[18].** However some authors reported that maternal antibodies either from natural exposure or following vaccine stimulation, may represent a valid alternative way for protecting newly hatched chicks against many pathogens including pathologic effect of *C. perfringens* **[13] and [19].**

Two clinical field trials were conducted in Europe demonstrated that vaccination of hens with a *C. perfringens* type A toxoid as a necrotic enteritis vaccine prevented development of necrotic enteritis syndrome in their progenies as this vaccine when administered to the hens will convey the immunity against the necrotic enteritis disease to their progenies via passive transfer of maternal antibodies **[20].** Brioler breeders were injected I/M with the candidate vaccine based on *C. perfringens* type A and type C toxiods adjuvinated with aluminium hydroxide gel, resulted in a strong serum IgG response to *C. perfringens* alpha toxin and *C. perfringens* beta toxin in parent hens and these specific antibodies were transfermalered to their progenies **[19]** Therefore, this study was carried to monitor the level of specific IgG against alpha toxin of *C. perfringens* type A in both vaccinated hens during both the breeding and production period of age and also to monitor the levels of antibodies in newly hatched chicks during the first week of age.

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MATERIALS AND METHODS

Necrotic enteritis gel and oil vaccines were obtained from Veterinary Serum and Vaccine Research Institutes, Anaerobic Vaccine Department. A broiler breeder hen flock was selected from one Egyptian farm located at Monofia governorate was vaccinated by the vaccine as shown in the following table:

Blood samples were collected from the vaccinated flock as shown in the following table:

Hatched chicks were collected from eggs of vaccinated hens at 40 weeks age (peaks of egg production). Then they were bled for serum collection at day one and day three.

All samples either from vaccinated hens or hatched chicks were introduced to measure the specific IgG levels against alpha toxin of c. perfringens type A by both mouse neutralization test according to **[21]** and Enzyme Linked Immunosorbent Assay ELISA according to **[22].**

RESULTS

Table 1:Vaccination scheme for hens:

Vaccination	1st vaccination dose by gel vaccine	2nd vaccination dose by gel vaccine	Booster vaccination dose by oil vaccine
Age	2 weeks old	8 weeks old	20 weeks old

Table 2: blood sample collection

Sample	prevaccination samples	1st sample	2nd sample	3ed sample	4th sample
Age	At 2 weeks old (before vaccination)	At 10 weeks old (post 2nd vaccination with gel vaccine)	At 20 weeks old (before booster vaccination with oil vaccine)	At 30 weeks old (after booster vaccination with oil vaccine)	At 40 weeks old (at peak of egg production)

Table 3: Specific IgG titers in the sera of vaccinated hens by mouse neutralization test (SNT):

Test	Prevaccination	10 Weeks old	20 Weeks old	30 Weeks old	40 Weeks old
SNT	0U	4U	2U	6U	10U

Table 4: Specific IgG titers in the sera of vaccinated hens by ELISA:

Test	Prevaccination	10 Weeks old	20 Weeks old	30 Weeks old	40 Weeks old
ELISA	0.175± 0.04	0.551± 0.09	0.381± 0.06	0.983± 0.02	1.581± 0.08

mean ± SE cut off value = 0.193

Table 5: Specific IgG titers in the sera of hatched chicks by SNT:

Test	Day one old	Day three old	control
SNT	1U	1.5U	0U

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Table 6: Specific IgG titers in the sera of hatched chicks by ELISA:

Test	One day old	Three day old	control
ELISA	0.242 ± 0.07	0.399 ± 0.87	0.114± 0.01

Fig 1: Specific IgG titers in the sera of vaccinated hens by mouse neutrilization test

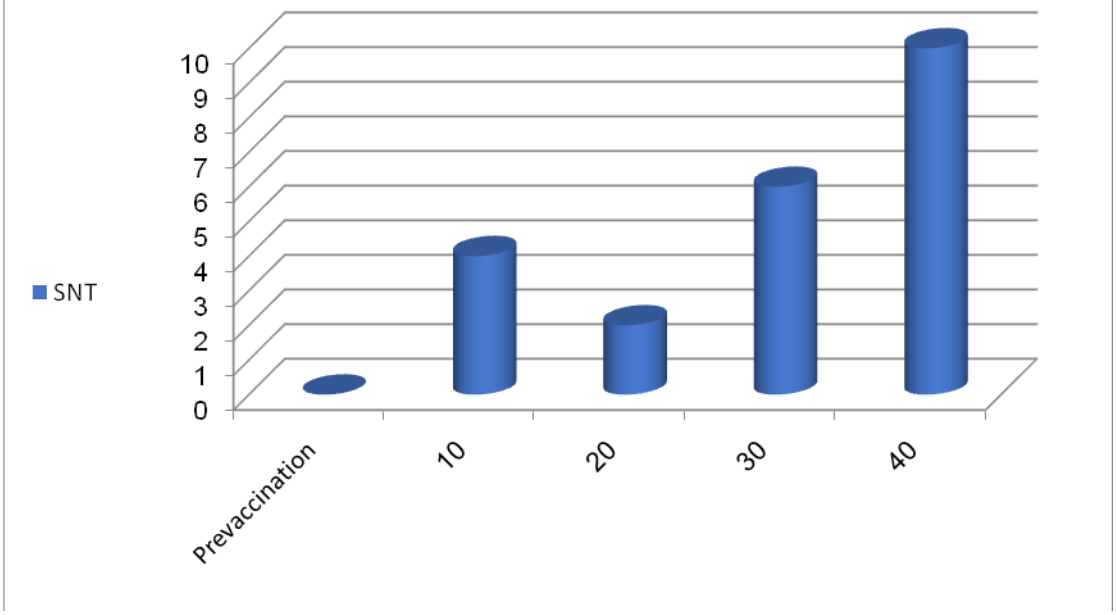

Fig 2: Specific IgG titers in the sera of vaccinated hens by ELISA

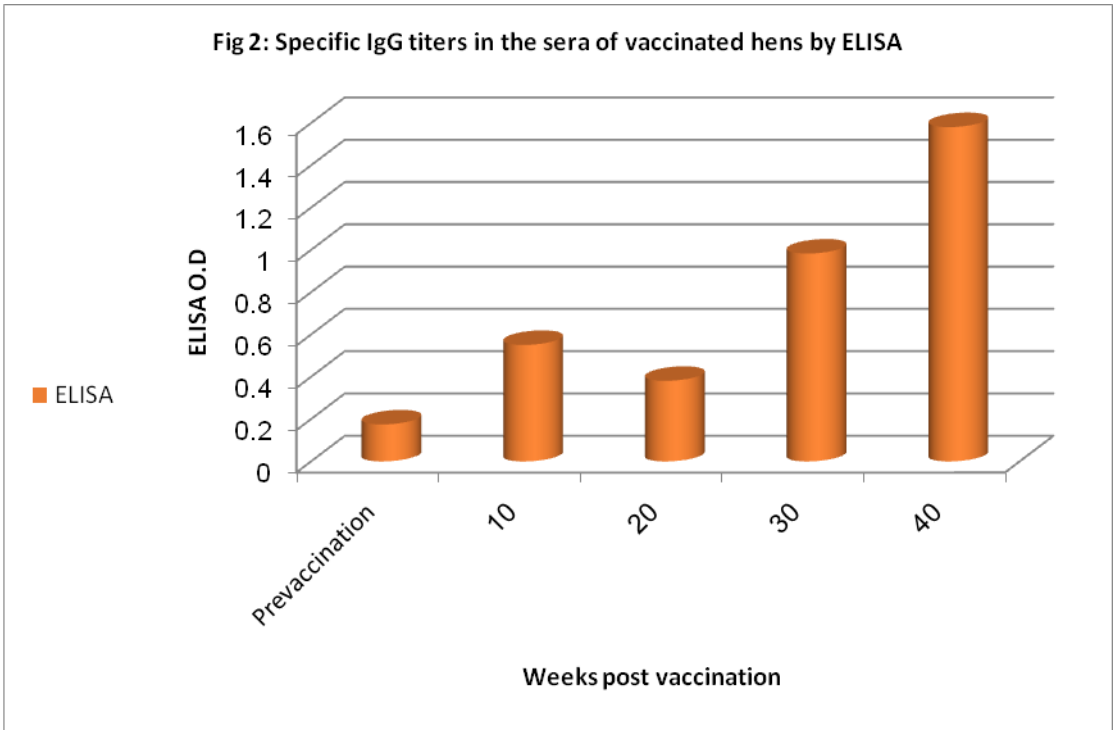

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Fig 3: Specific IgG titers in the sera of hatched chicks by SNT:

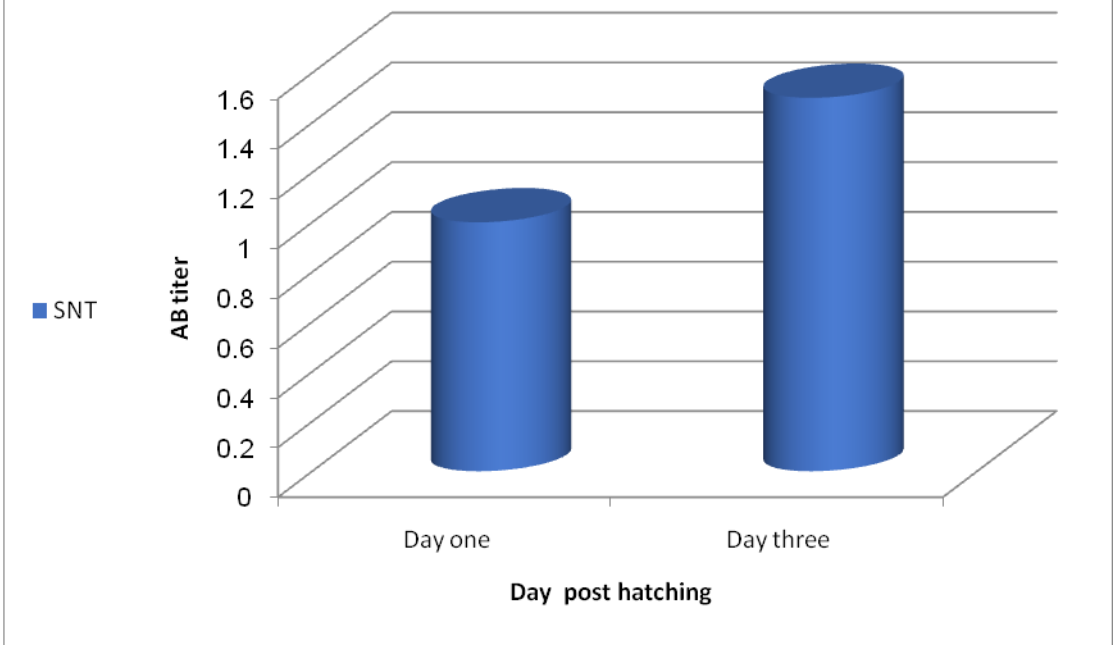
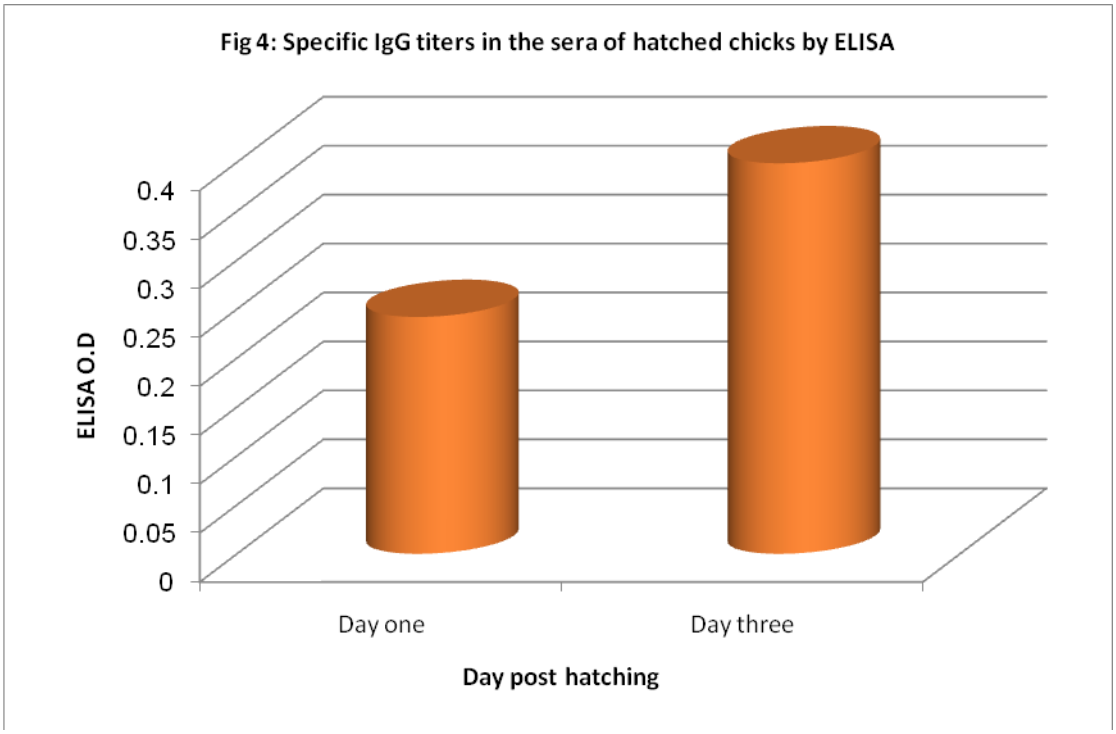


Fig 4: Specific IgG titers in the sera of hatched chicks by ELISA



DISCUSSION

Maternal antibody transfer is defined as the transfer of antibodies from breeder hens to hatched chicks via egg **[23].** There are three classes of antibodies in chickens that transferred by fertile egg, namely IgY (IgG), IgA and IgM. IgY is considered the avian antibodies equivalent to the mammalian IgG. In fertile egg, IgY is present predominately in egg yolk **[24]** while IgA and IgM are present in the egg white as a result of mucosal secretion in the oviduct **[25].**

In chickens the transfer of IgY from the breeder hens to her progenies takes place in a two step process. First step is IgY is taken up into the egg yolk via IgY receptors on the ovarian follicle from the breeder

hens blood **[26]** while The second step, IgY is transferred from the egg yolk to newly hatched chicks via the embryonic circulation. **[27]** reported that yolk IgY is transferred at a low rate across the yolk sac into the embryonic circulation as early as embryonic day 7. The rate of transfer started to increase by embryonic day 14 and by embryonic day 19 to day 21, there is a steep rise in the rate of transfer of IgY from the egg yolk to the embryonic circulation. **[28] and [29]** stated that expected transfer percentage for IgY of 30 % to chicks circulation, the maternal antibody transferred from dams to their chicks could be a good strategy to protect them in the first few days of age against specific pathogens. It was found that amount of IgY transferred to the egg yolk is proportional to breeder hens serum IgY concentrations **[30].** Also [28] reported that the maternal IgY in the hatched chick plasma were at the highest level at day one till day three post-hatching, therefore in this study the hatched chicks were bled at day one and day three of age.

In this study, the results recorded a protective level of IgY in the sera of hen breeders during the breeding period due to their vaccination by the necrotic enteritis gel vaccine in two doses as shown in tables (3) &(4) and figures (1) &(2).

These results was matched by **[31]** who reported that multiple vaccination dosages are necessary for a good immune response, and that booster vaccination against C. *perfringens* type A have been shown essential when inactivated and crude toxoid are used to provide protection compared with single immunization which showed little benefit.

These levels of IgY in hen breeder sera still protective until week 20-22 of age in spite of their decrease according to the results of the second samples which collected before booster dose as shown in tables (3)&(4) and figures (1)&(2).

Booster vaccination with necrotic enteritis oil vaccine with one dose at 20-22 week of age resulting in large increase in the specific IgY levels in the sera of hen breeders until 40 week of age which is the peak of egg production as shown in tables (3)&(4) and figures (1) &(2). Consequently the results reported that the specific IgY levels in the sera of hatched chicks at day one and three were satisfactory and protective as shown in tables (5)&(6) and figures (3)&(4).

These results were compatible with **[19]** who said that the immunization of broiler breeder hens against C. perfringens type A toxoid vaccine leads to higher levels of specific antibodies against *C. perfringens* type A toxoid in the sera of broiler breeder hens and consequently in the sera of their hatched chicks than their ones which were not vaccinated and their progenies. These specific levels gave protective effect to the progenies against subclinical *C. perfringens* infection associated necrotic enteritis.

Also these results agreed with the results stated in the evaluation discussion around the Netvax vaccine (oil vaccine for necrotic enteritis) produced by Schering Company in the conference of international Poultry Association in which the discussion reported that the benefits of Netvax are the active immunization of chickens in order to provide passive immunization against necrotic enteritis to their progenies during the laying period and the reduction of mortality and the incidence and severity of lesoinsns caused by *C. perfringens* type A. Also the discussion reported that the vaccination schedule consists of two doses, the first dose was given at 10-14 week of age and then the second dose was given at 18-20 weeks of age (at least 6 weeks before the hen breeders will start to lay eggs). The onset of passive transfer of immunity is begun 6 weeks after second dose and is lasting up to 51 week post second dose.

At the same manner **[1]** stated that vaccination of broiler breeder hens with *C. perfringens* type A toxoid (Netvax) formulated as an oil emulsion resulted in a significant increase in anti-alpha toxin antibodies in the sera of hen breeders and subsequently transferred to their progenies during the laying period via egg yolk this was parallel with results found in this study, moreover **[1]** found that the hatched chicks from eggs produced from vaccinated hens were shown to have reduced mortality when compared with control chicks which shows gross gut lesions associated with necrotic enteritis, and also he investigated that specific IgY levels which still persisted in the sera of hatched chicks until 7 day old progeny.

On the other hand, these results disagreed with **[32]** who suggested that the administration of hen egg antibodies of vaccinated hens by the alpha toxoid of *C. perfringens* type A did not reduce the level of *C. perfringens* intestinal colonization which resulting in clinical necrotic enteritis disease .

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REFERANCES

[1] Crouch, C.F. ; Withanage, G. S. K.; Haas, V. De.; Etore, F. And Francis, M.J. 2010 . Safety and efficacy of a maternal vaccine for the passive protection of broiler chicks against necrotic enteritis. Avian pathology. Vol. 39. P489-497.

[2] Parish, W.E. 1961. Necrotic enteritis in the fowl (Gallus gallus domesticus) Histopathology of the disease and isolation of a strain of *Clostridium welchii.* J. Comp. Pathol. 71:377-393.

[3] Al-Sheikhly, F and Truscott, R. B. 1977. The interaction of *Clostridium perfringens* and its toxin in the production of necrotic enteritis of chickens. Avian diseases, 21: 256-263.

[4] Maxey, B.W. and Page, R. K. 1977. Efficacy of lincomycin feed medication for the control of necrotic enteritis in broiler –type chickens. Poult. Sci. 56:1909-1913.

[5] Williams R.B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathology 34(3), 159-180.

[6] Broussard, C.T.; Hofacre, C.L.; Page, R.K. and Fletcher, O.J. 1986 . Necrotic Enteritis in Cage- Reared Commercial Layer Pullets. Avian Dis. 30:617-619.

[7] Khalhusdal, M. and Lovland, A. 2000. Necrotic enteritis (4): the economical impact of *Clostridium perfringens* is greater than anticipated. World Poultry, 16:50-51.

[8] Elwinger ib, K., Schneitz, C., Berndtson, E., Fossum, O., Teglof, B. And Engstom, B. 1992 . Factors affecting the incidence of necrotic enteritis, caecal carriage of *Clostridium perfringens* and bird performance in broiler chicks. Acta Veterinaria Scandinavica, 33: 369-378.

[9] Hofshagen, M. and Khaldhusdal, M. 1992 . "Barley inclusion and avoparcin supplementation in broiler diets. Effect on small intestinal bacterial flora and performance". Poultry Science, 71:959-969.

[10] Mc-Devitt, R.M., Brooker, J.D., Acamovic, T. And Sparks, N.H.C. 2006 . Necrotic enteritis: a continuing challenge for poultry industry. World's Poultry Science Journal, 62:221-247 .

[11] Ficken, M.D. and Wages, D.P. 1997 . "Necrotic enteritis" . In Diseases of poultry, 10th edn, edited by: Calnek, B. W., Barnes, H.J., Beard, C.W., McDougald, L.R. and Saif, Y.M. 261-264. Ames, IA: Mosby-Wolfe.

[12] Williams, R.B. (2005): Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathology, 34:159-180.

[13] Heier, B.T., Lovland, A., Soleim, K.B., Kaldhusdal, M. And Jarp, J. 2001. A field study of naturally occurring specific antibodies against *Clostridium perfringens* alpha toxin in Norwegian broiler flocks. Avian Diseases, 45:724-732.

[14] Dorien Mot, Leen Timbermont, Freddy Haesebrouck, Richard Ducatelle & Filip Van Immerseel (2014). Progress and problems in vaccination against necrotic enteritis in broiler chickens. Avian pathology. 43:4, 290-300.

[15] Lee, K.W.; Lillehoj, H.S.; Park, M.S.; Jang, S.I.; Ritter, G.D.; Hong, Y.H.; Jeong, W.; Jeoung, H.I.; An, D.J. & Lillehoj, E.P. (2012). *Clostridium perfringens* alpha- toxin & NetB toxin antibodies and their possible role in protection against necrotic enteritis and gangrenous dermatitis in broiler chickens. Avian Diseases, 56, 230-233.

[16] Hasselquist, D. and Nilsson, J.K. 2009. Maternal transfer of antibodies in vertebrates: Trans-generational effects on offspring immunity. 2009. Philos. Trans. R. Soc. London B: Biol. Sci., 364:51-60.

[17] Ahmed, Z., and S. Akhter. (2003): Role of maternal antibodies in protection against infectious bursal disease in commercial broilers. Int. J. Poult. Sci. 2:251- 255.

[18] Lawrence , E.C., F. Arnaud- Battandier, J. Grayson, I.R. Koski, N.J. Dooley, A.V. Muchmore, and R.M. Blaese. 1981. Ontogeny of humoral immune function in normal chickens: A comparison of immunoglobulin- secreting cells in bone marrow, spleen, lungs and intestine. Clin. Exp. Immunol. 43:450-457.

[19] Lovland, A.; Kaldhusdal, M.; Redhead, K.; Skjerve, E. And Lillehaug, A. (2004). Maternal vaccination against subclinical necrotic enteritis in broilers. Avian pathology. 33:83-92.

[20] Luciano Gobbi, Intervet / Schering Plough Animal Health Technical Manager for Poultry, Italy, Intestinal Health Center for Poultry

[21] Gadalla , M.S. ; Ikbal Farrage; Lotfy, O.; Mahmoud, M.S.; El Danaf, N.A.; Dorreya Sharaf and Hussein, M.(1971): The immunogenicity of alum precipitate multicomponent *clostridial* vaccine. J. Egypt. Vet. Med. Ass., 31(3/4): 135 – 150.

[22] Woods, K.R. (1991): An alternative to the toxin neutralization assay in mice for the potency testing of the *clostridium tetani*, *clostridium septicum*, *clostridium novyi* type B, and *clostridium perfringens* type D epsilon components of multivalent sheep vaccines. Biological 19(4): 281 – 286.
[23] Grindstaff , J. L., E. D. Brodie III , and D.K. Ellen. 2003. Immune function across generations: Integrating mechanism and evolutionary process in maternal antibody transmission. Proc. Biol. Sci. 270:2309-2319.
[24] Leslie, G.A., and W.L. Clem. 1969. Phylogeny of immunoglobulin structure and function. 3. Immunoglobulins of the chicken. J. Exp. Med. 130:1337-1352.
[25] Rose , M.E., E. Orlans, and N. Buttress. 1974. Immunoglobulin classes in the hen's egg: Their segregation in yolk and white. Eur. J. Immunol. 4:521-523.
[26] Loeken, M.R., and T.F. Roth, 1983. Analysis of maternal IgG subpopulations which are transported into the chicken oocyte. Immunology 49:21-28.
[27] Kramer, T.T., and H.C. Cho. 1970. Transfer of immunoglobulins and antibodies in the hens egg. Immunology 19:157-167.
[28] Kowalczyk, K., J. Daiss, J. Halpern, and T.F. Roth. 1985. Quantitation of maternal – fetal IgG transport in the chicken. Immunology 54:755-762.
[29] Ahmed, A. S. (2015): Assessment of Maternal Immunity against Newcastle disease in Offspring Chicks as affected by Parents Genetic Crossing. Asian Journal of Animal and Veterinary Advances, 10:35-42.
[30] Al-Natour, M.Q., L.A. Ward, Y.M. Saif, B. Stewart, and L.D. Keck. 2004: Effect of different levels of maternally derived antibodies on protection against infectious bursal disease virus. Avian Dis. 48:177-182.
[31] Mot, D.; Timbermont, L.; Delezie, E.; Haesebrouck, F.; Ducatelle, R. & Van Immerseel, F. (2013). Hatch vaccination is not protective against necrotic enteritis in broiler chickens. Avian Pathology. 42, 179-184.
[32] Wilkei , D.C.; Van Kessel, A.G.; Dumonceaux,T.J. and Drew, M. D. (2006): The effect of hen- egg antibodies on *Clostridium perfringens* colonization in the gastrointestinal tract of broiler chickens. Vet. Med.74:279-292.