The effect of clindamycin oral administration on some immunological and biochemical parameters of Newcastle vaccinated broilers
Mohamed El-Dakrouy¹, Alshaymaa Darwish², Asmaa Darwish³.

¹Department of pharmacology, faculty of veterinary medicine, Matrouh University, Egypt.
² Department of biochemistry, Faculty of pharmacy, Sohage University.
³ Department of animal and poultry health, Desert Research Center, Materia, Egypt.

ABSTRACT
Present work aimed to study some immunological and biochemical effects of Clindamycin on broiler vaccinated against Newcastle virus. Three groups of one day old Hubbard chicks each of 50 birds were used in this study. Group (1) was control non treated group (CG). Group C20 and C40 were given Clindamycin (20mg/kg and 40mg/kg body. wt) respectively in drinking water at 14th, 15th and 16th days of age. All groups were routinely vaccinated against Newcastle virus at 7 and 18 day of age. On the 25th day of age, blood samples were collected from ten birds of each group for immunological and biochemical tests. Five birds from each group were slaughtered at the same age then the lymphoid organs (thymus, bursa and spleen) were carefully separated & weighed and each organ relative weight was determined. Number of dead birds was recorded throughout the experiment period (45 day age). It was observed that administration of Clindamycin decreased the leukocytic count haemagglutination inhibition antibody titer (HI), total protein, globulin and the relative weight of bursa in C20 and C40. It did not induce any significant changes in the differential leukocytic count, liver and kidney function tests, spleen relative weight in C20 and C40. While, relative weight of thymus and body weight gain significantly decreased in C40 and non-significantly changed in C20. While, feed conversion rate significantly increased in C40 only. In addition, C20 and C40 had higher mortality rates than CG. Conclusion: Clindamycin administration suppresses the chicken's immune response to Newcastle disease virus vaccine.

Keywords: Newcastle vaccine, immunological parameters, biochemical parameters, broilers.

1. Introduction
Newcastle disease is an obstacle hinders the poultry industry development all over the world. Avian paramyxovirus attacks the respiratory system of the bird causing several symptoms as gasping, coughing, sneezing and rales (Brown and Bevins 2017; Desouky et al., 2020). Sometimes it causes nervous symptoms like terrors and complete paralysis. In-appetence and watery greenish diarrhea are general symptoms also related to the disease. The disease usually caused high morbidities and mortalities (Brown and Bevins 2017; Desouky et al., 2020). Moreover, ND virus can life under the bird skin and resist the freezing for several months and cause public health hazard for poultry consumers. Vaccination is the only effective tool can control the virus (Dakouo et al., 2020). Unfortunately, several factors can lead the vaccination failure as pollution, chemicals, infectious agents, hormones and nutrition. Chemotherapeutic agents also play a critical role in stimulation or suppression of the immune response against the vaccine (Mund, 2017; Kim and Lillehoj, 2019).

Under field conditions, it is unusual to run a commercial poultry cycle without some medication as antibiotics & anticoedicids (Mund, 2017; Kim and Lillehoj, 2019). Clindamycin is a semi-synthetic lincosamide antibiotic, it is effective against anaerobic bacteria and gram-positive aerobic bacteria (including most Staphylococcus and Streptococci spp), and some protozoal infections (including Toxoplasma) (Wan et al., 2016; Dotel et al., 2019). It inhibits bacterial protein synthesis by binding to 23S RNA of the 50S subunit of the bacterial ribosome. It easily distributes in all body fluids and reaches bone. According to the dosage, it may be bacteriostatic or bactericidal (Okudo and Anusim, 2016). In comparison to other antibiotics it has a shorter half life time and easily eliminated from the bird body through the liver and kidneys (Sadek and Shabeneh, 2014). This makes Clindamycin a good choice during broilers breeding. The present work aimed to shed the light on the effect of clindamycin oral administration on some immunological and biochemical parameters of Newcastle vaccinated broilers.

2. Materials and Methods
2.1. Drugs
Clindamycin (Ato Clinda 20%) are product of Atchapharma. Experimental design
A total of 150 one day old Hubbard chicks were used in the present study. The chicks were divided into three equal groups, each of 50 chicks. First group was a control non treated group (CG). At 14th, 15th and 16th days of age, the second group (C20) and the third group (C40) were given Clindamycin (20 and 40mg/kg body. wt) respectively in drinking water as recommended by the producers. All groups were routinely vaccinated against Newcastle by Hitchner and Lasota at 7th and 18th day of age respectively. On 25th day of age, blood samples were collected from ten birds of each group for immunological and biochemical parameters evaluation. Five birds of each group were slaughtered at the same age then the lymphoid organs (thymus, bursa and spleen) were carefully separated & weighed and each organ relative weight was determined. Number of dead birds were recorded throughout the experiment period (45th day).

Laboratory examinations:
2.2. Immunological tests
Phagocytic activity was estimated according to Barry et al. (1989). Total leukocytic counts (TLC) were evaluated according to Natt and Herrick (1952). Blood filter was prepared and stained with Giemsa stain for differential leukocytic count (DLC) according to Schalm et al., (1975). haemagglutination inhibition titer (HI) was determined according to Takatsy (1956).

2.3. Biochemical tests
Serum biochemical tests were measured spectrophotometrically in a private laboratory using commercial kits of Biodiagnostic company® commercial kits, Cairo, Egypt. Serum globulin concentrations were calculated by subtracting the value of albumin from the corresponding value of total protein and A/G ratio by division of albumin on the corresponding globulin value.

2.4. Statistical analysis
SPSS program version 24 was used to compare between means of different statistical parameters (one-way ANOVA test) and estimate the post-hoc differences between means (a multiple comparison Tukey’s HSD test). A difference was considerable significant at P< 0.05.
3. Results
Table (1) displayed a significant (P<0.05) decline in TLC, HI antibody titer, total protein and globulin levels in C20, C40 When compared to CG. While, A/G significantly (P<0.05) decreased in C40 and non-significantly (P≥0.05) changed in C20 in relation to CG. On the other hand, no significant changes were observed between C20, C40 and CG in TEC (monocytes, lymphocytes, heterophils, eosinophils, basophils), phagocytic activity, albumin, liver enzymes (ALT, AST) and kidney function tests (uric acid, creatinine Cr).

Table (2) cleared a significant (P<0.05) decrease in bursa relative weight of C20, C40 when compared to CG. While, thymus relative weight, body weight gain significantly (P<0.05) depressed in C40 only and non-significantly (P≥0.05) decreased in C20 in relation to CG. On contrast feed conversion rate significantly (P<0.05) increased in C40 and non-significantly (P≥0.05) changed in C20 compared to CG. In comparison to CG number of dead birds and mortality rate increased in C20 and C40. The comparison between C20 and C40 revealed a significant (P<0.05) inhibition of HI antibody titer and weight gain in C40 in relation to C20 (table 1 and 2).

4. Discussion
Vaccination is a necessary process in all animal husbandry section, including poultry. It is the most reliable mean for pandemic-highly infectious diseases control (AlDubayghi et al., 2011). No doubt, the bird response towards the vaccine widely depends on his immune system soundness. Like mammals, avian immune system produces two types of immunity: innate immunity and adaptive immunity. Innate immunity (circularing leukocytes and macrophage) is non-specific. originated from conversion rate and adaptive immunity (immunoglobulin M, G, A developed in bursa of Fabricius from B-cells) and cell-mediated immunity (T-cell derived from thymus) (Birhan, 2019). Antibiotics administration usually restrains the immune system proper functions (Bystrzycka, et al., 2016; Jamal et al., 2017).

In this study, clindamycin affected both innate and adaptive immunity of the treated birds. Whereas, a pronounced depression was detected in C40 compared to C20. It was observed that clindamycin had a negative effect on innate immunity of C20 and C40 chicks. This was indicated by the noticed leukopenia in both groups members. This leukopenia may be attributed to the myelosuppressive effect of clindamycin due to its direct hematopoietic tissue injury (Morales et al., 2014; Birhan, 2019). In the same way, clindamycin inhibits humoral immunity of C20 and C40 boilers, through reduction of the bursa relative weight in both groups and subsequently inhibits B cells maturation and antibody formation (Suresh Kumar et al., 2013; Birhan, 2019). This was translated by the observed decrease in HI antibody titer and subordinate hypoglobulinemia, hypoproteinemia in C20 and C40 individuals and decreased A/G ration in C40 chicks. Similar results were reported before in Newcastle vaccinated boilers treated with antibiotics (Suresh Kumar et al., 2013). While, the cell-mediated immunity was only suppressed in C40 boilers because of the remarkable decline of thymus relative weight in this group chicks (Birhan, 2019).

In addition to the clindamycin immune-inhibitory effect in treated animals, they suffered from lower body weight gain, higher feed conversion rates, higher number of dead birds and mortality rates (Jamal et al., 2017). Rationally, the lowered HI antibody titer and body weight gain in C40 in relation to C20 clarified that the clindamycin immunomodulatory effect correlated with its dosage. On contrast, clindamycin has no effect on hepatic or renal function tests of C20 and C40 here. Although it is mainly metabolized and eliminated from the body through liver and kidney, it has a short-lived effect and rarely causes hepatic or renal injury (Moole et al., 2015; Subedi et al., 2017). These findings agree with Sadek and Shaheen (2014) results, who obtained physiological hepatic and renal functions in E. Coli experimentally infected boilers, within 2 weeks from clindamycin treatment.

5. Conclusion
It could be concluded that, Clindamycin oral administration interferes both innate and humoral boilers immune response against Newcastle disease virus vaccine and reduce body weight gain. So it is not recommended to use them few days before or after vaccination.

Acknowledgments
Stuff members of stuff members of animal and poultry health department, DRC, Cairo, Egypt.

Conflict of interest
There is no conflict of interest.

6. References
AlDubayghi, I.I.; Mohamed, M.J. Yahiya, B.M., 2011. Humoral immunity resulted from vaccination with Brucella melitensis Rev. 1 vaccine given subcutaneously and intraocularly in goats. Iraqi J Sci. 52(1), 20-29.
Barry, L., Ghannon, U., John, R., Gils-son, A., 1989. In vitro microbial activity of avian peritoneal macrophages. Avian Diseases. 23(1), 107-111.
Birhan, M., 2019. Systematic review on avian immune systems. J. Life Sci. Biomed. 9(5), 145-152; www.jsb.science-line.com
Brown, V.R., Bevins, S.N., 2017. A review of virulent Newcastle disease viruses in the United States and the role of wild birds in viral persistence and spread. Vet. Res. 48, 68-83. DOI:10.1186/s13567-017-0475-9.
Bystrzycka, W., Moskalik, A., Sieczkowska, S., Manda-Handlizk, A., Demkow, U., Ciepiela, O., 2016. The effect of clindamycin and amoxicillin on neutrophil extracellular trap (NET) release. Cent. Eur. J. Immunol. 41(1), 1-5, doi:10.5114/cej.i.2016.58811.
Dakor, M., Gil, P., Fofana, M., Nang, M., Coulibaly, M. K., Samakâ, K., Traoré, A., Diallo, B.O., Albina, E., Bougoudougou, F., de Almeida, R.S., 2020. Efficacy of Newcastle disease vaccines and vaccination protocols commonly used in Mali, West Africa. Journal of Veterinary Medicine and Animal Health. 12(2), 55-65.
Desouky, A.I., Saad, A.E., El Shorthany, M.A., Abodallah, S.E., 2020. Isolation and identification of new sub-genotypes of virulent ND virus in broiler chickens in Qalyobia governorate. Benha Veterinary Medical J. 39, 159-164.
Dotel, R., Tong, S., Bowen, A., Nelson, J.N., O’Sullivan, M., Campbell, A.J., McMullan, B.J., Britton, P.N., Francis, J.R., Eisen, D.P., Robinson, O., Manning, L., Davis, J.S., 2019. CASSITEF-clindamycin adjunctive therapy for severe Staphylococcus aureus treatment evaluation: study protocol for a randomised controlled trial. Trials. 20(1), 353, doi:10.1186/s13063-019-3452-y. PMID: 31196132; PMCID: PMC6567404.
Jamal, N., Shareef, M., Sajid, S., 2017. Lincomycin and tetracycline resistance in poultry. Review,” Matrix Science Pharma (MSP), Zibeline International Publishing, vol. 1(1), pages 33-38.
Kim, W.H., Lillehoj, H.S., 2019. Immunity, immunomodulation, and autonomic alternatives to maximize the genetic potential of poultry for growth and disease response. Animal Feed Science and Technology. 250, 41–50.
Moole, H., Ahmed, Z., Saxena, N., Puli, S.R., Dhillon, S., 2015. Oral clindamycin causing acute cholestatic hepatitis without ductopenia: a brief review of idiosyncratic drug-induced liver injury and a case report. J Community Hosp Intern Med Perspect. 5(5), 28746. Published 2015 Oct 11. doi:10.3402/jchimp.v5i5.28746.
Morales, M.P., Curvalo, A.P., Espinosa, K.A., Murillo, E.E., 2014. A young man with myelosuppression caused by clindamycin: a case report. J Med Case Rep. 8, 7-11. doi: 10.1186/1752-1977-8-7. PMID: 24387005; PMCID: PMC3917410.
Mund, M.D., Khan, U.H., Tahir, U., Mustafa, B., Fayyaz, A., 2017. Antimicrobial drug residues in poultry products and implications on public health: A review. International Journal of Food Properties. 20(7), 1433-1446, DOI:10.1080/10942912.2016.1212874.
Natt, M.P., Herrick, C.A., 1952. A new blood diluent for counting the red and white blood cells of the chicken. Poultry Science. 31: 335.
Okudo, J., Anusim N., 2016. Hepatotoxicity due to Clindamycin in Combination with Acetaminophen in a 62-Year-Old African American Female: A Case Report and Review of the Literature. Hindawi Publishing Corporation, Case Reports in Hepatology. Volume 2016, Article ID 2724738, 5 pages. http://dx.doi.org/10.1155/2016/2724738.
Schalm, O.W., Jain, N.C., Carroll, E.J., 1975. Veterinary Haematology, 3rd ED. Lea and Feibiger, Philadelphia.
Subedi, P., Chowdhury, A., Tanovic, K., Dunic, I., 2019. Clindamycin: An Unusual Cause of Acute Kidney Injury. Am J Case Rep. 20:248-251. doi:10.12659/AJCR.913779
Sureshkumar, V., Saratchandra, G., Ramesh, J., 2013. Effect of enrofloxacin on zootechnical performance, behaviour and immunohistopathological response in broiler chicken. Vet. World, 6(6), 337-342. doi: 10.5455/vetworld.2013.337-342.

Takatsy, G.Y., 1956. The use of spiral loop in serological and virological micromethods. Acta Microbiologica Acad. Sci. Hung, 3, 197.

Wan, H., Hu, Z., Wang, J., Zhang, S., Yang, X., Peng, T., 2016. Clindamycin-induced Kidney Diseases: A Retrospective Analysis of 50 Patients. Intern Med. 55(11), 1433-7. doi: 10.2169/internalmedicine.55.6084. Epub 2016 Jun 1. PMID: 27250048.

Table 1: comparison between some immunological and biochemical parameters of C20, C40 in relation to CG. Values are means ± SD.

| Parameters         | CG            | C20            | C40            |
|--------------------|---------------|----------------|----------------|
| TLC (x10^4/μl)     | 29.10±2.40^a  | 26.50±1.80^a   | 21.40±2.01^a   |
| Monocytes (%)      | 9.70±0.65^a   | 10.10±0.54^a   | 10.20±0.53^a   |
| Lymphocytes (%)    | 62.60±3.43^a  | 64.50±5.34^a   | 61.50±4.24^a   |
| Heterophils (%)    | 23.70±1.28^a  | 21.60±2.38^a   | 24.20±1.76^a   |
| Eosinophils (%)    | 2.50±0.26^a   | 2.50±0.25^a    | 2.60±0.33^a    |
| Basophils (%)      | 1.50±0.18^b   | 1.30±0.17^b    | 1.50±0.22^b    |
| Phagocytic activity (%) | 21.43±0.32^a | 22.67±0.25^a   | 21.75±0.31^a   |
| HI antibody titer (log_{10}) | 5.80±0.42^a  | 4.40±0.39^b    | 3.80±0.29^b    |
| Total protein (g/dl) | 4.77±0.46^a  | 4.30±0.35^b    | 4.14±0.42^b    |
| Albumin (g/dl)     | 2.30±0.19^a   | 2.11±0.24^a    | 2.24±0.26^a    |
| Globulin (g/dl)    | 2.49±0.07^a   | 2.03±0.07^a    | 1.90±0.08^a    |
| A/G                | 0.92±0.05^a   | 1.04±0.08^a    | 1.18±0.10^a    |
| AST (U/L)          | 21.63±1.64^a  | 24.14±0.85^a   | 23.67±1.38^a   |
| ALT (U/L)          | 99.36±7.42^b  | 105.00±5.39^a  | 107.55±8.21^a  |
| Uric acid (mg/dl)  | 11.72±0.57^c  | 11.47±0.65^c   | 12.21±0.68^c   |
| Cr (mg/dl)         | 1.13±0.17^a   | 1.10±0.14^a    | 1.07±0.19^a    |

Means in the same row bearing different letters differ significantly (P<0.05).
CG: Control group, C20: Clindamycin 20mg/kg bodyweight, C40: Clindamycin 40mg/kg bodyweight.
TLC: total leukocytic count, A/G: Albumin/Globulin ratio, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, Cr: Creatinine.

Table 2: comparison between the relative weights of bursa, spleen and thymus body weight, feed conversion rates, no of dead birds and mortality rates of C20, C40 in relation to CG. Values are means ± SD.

| Parameters         | CG            | C20            | C40            |
|--------------------|---------------|----------------|----------------|
| Bursa relative weight (%) | 2.13±0.16^a   | 1.75±0.15^b    | 1.65±0.18^b    |
| Spleen relative weight (%) | 1.34±0.14^a   | 1.36±0.13^a    | 1.33 ± 0.16^a  |
| Thymus relative weight (%) | 2.30±0.15^a   | 2.15±0.21^b    | 1.93±0.16^b    |
| Weight gain (gm)   | 1997±65.8^c   | 1879±61.2^a    | 1851±52.3^b    |
| Feed conversion (%) | 1.97±0.029^c  | 2.19±0.041^b   | 2.41±0.078^b   |
| Number of dead birds | 5             | 6              | 8              |
| Mortality rate (%) | 7             | 9              | 15             |

Means in the same row bearing different letters differ significantly (P<0.05).
CG: Control group, C20: Clindamycin 20mg/kg bodyweight, C40: Clindamycin 40mg/kg bodyweight.