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Research Article
Protection of Neonatal Broiler by Using T Cell Lymphokines Prepared from Immunization with *Salmonella typhimurium* Against Field Local Newcastle Disease Virus Isolate

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Abstract

Objective: The current study aimed to use lymphokines from birds hyper-immunized against *Salmonella typhimurum* to enhance the immune response against Newcastle disease (ND) and to limit the use of vaccinal viruses that have been recently shown not to give absolute protection. **Materials and Methods:** Two groups of chicks were used: The first group was vaccinated with three doses of *Salmonella typhimurum* at 7, 14 and 21 days and the second group was not vaccinated and was considered a control group. Salmonella-immune lymphokines (S-ILK) were obtained from the T cells of the first group at 30 days, while non-immune lymphokines (N-ILK) were obtained from the T cells of the second group. Then, a total of 300 (Ross-308) 1 day old broiler chickens were randomly divided into 6 treatment groups (G1-G6) with 50 chicks in each group and treated as follows: G1: Treated with salmonella-immune lymphokines (S-ILK) and challenged with Newcastle disease virus (NDV). G2: Treated with non-immune lymphokines (N-ILK) and challenged with NDV. G3: Non-treated and challenged with NDV. G4: Non-treated and naturally infected with NDV. G5: Treated with salmonella-immune lymphokines S-ILK and not challenged. G6: Non-treated and non-challenged. **Results:** The results of immunity measured by ELISA and a hemagglutination inhibition test (HI) showed a significant decrease in the level ($p<0.05$) of maternal antibody titer (Abs) against ND on the seventh day after the challenge compared to the control group but on days 14 and 21, there was a slight increase in the level ($p<0.05$) of the antibody titer in G1 and G2 compared to the other groups in which no antibody titer was recorded. On the 28th day, the G3 and G4 groups recorded a significant increase ($p<0.05$) in the antibodies against ND and high mortality rates, while the G1 group revealed a moderate increase with no mortality. No titers were recorded in the G5 and G6 groups because they were not exposed to any challenge. **Conclusion:** The present study concludes that S-ILK provides absolute protection to chicks against NDV by enhancing their immune response and reduces virus isolation in living tissue following challenge with virulent local Newcastle isolate.

Key words: *Salmonella typhimurium*, T cell lymphokines, Newcastle disease, ELISA, viral load

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Newcastle disease (ND) is an important disease for poultry due to the expected economic effects on poultry production. The disease is caused by a virus belonging to the genus *Avulavirus*, of the *Paramyxoviridae*[1] family. Depending on the severity of the transmission of the disease in poultry, the virus is divided into three patho types (Velogenic, Mesogenic and Lentogenic). Unspecified signs of disease including ruffled feathers, depression, hyperthermia, gasping, decreased appetite and pre-death hypothermia can be observed[2]. Chickens infected with neurotropic vNDV are characterized by the development of nervous signs including ataxia, torticollis, paralysis of the legs or wings and usually the absence of gross lesions[3]. Despite the widespread use of vaccines against ND, neither attenuated vaccines nor inactivated vaccines used for the control of disease outbreaks have been able to fully prevent outbreaks against circulating virulent strains of NDV. This may result from the emergence of a highly virulent infectious patho type that shows antigen variations that may be responsible for the recent NDV outbreaks[4]. However, many reports have shown that most commercial vaccines against Newcastle disease are not properly implemented[5,6]. The development of an efficient immune response involves many complex interactions between the hematopoietic, inflammatory and lymphoid cells. Soluble molecules as well as many different resistance mechanisms, act as mediators in these reactions. These molecules are biologically effective, have small molecular weights and are usually composed of proteins and glycoproteins, known by the generic name of cytokines[7]. As a result of the intensity of this antigen exposure, vaccination programs and antibiotic treatment are not always practical and effective. In such circumstances, the use of cytokines as non-specific substances that contribute to increased immune capacity may be a solution with a greater protective effect against diseases caused by pathogens[8,9]. In the present study, the therapeutic effects of poultry lymphokine were analyzed against the negative impact caused by a local strain of virulent ND in chickens.

MATERIALS AND METHODS

Preparation of lymphokines: A primary isolate of *Salmonella typhimurium* from poultry was obtained from the Department of Pathology and Poultry Diseases-College of Veterinary Medicine-Baghdad University, Iraq (unpublished). Isolate growth in nutrient broth and peptone water was determined by the development of turbidity and a small white sediment 24 h after incubation at 37°C. Then, the isolate was grown on a selective culture media specific for Salmonella such as MacConkey agar and SS agar and biochemical identification was performed by API 20 E. A solution containing an appropriate concentration of bacteria (1×10^8 colony-forming units mL^{-1}) was prepared and the concentration of bacteria was spectrophotometrically determined by a standard curve at a wavelength of 625 nm[10].

In another step, two groups of broiler chicks were purchased from Al-Shakr Hatceery in Baghdad and placed in a poultry farm in the College of Veterinary Medicine-Baghdad University. The first group was vaccinated with a dose of 1 ml/bird of *Salmonella typhimurium* at 7, 14 and 21 days by oral inoculation; however, the second group was not immunized but was housed under similar conditions in a separate breeding unit and considered the control group. The S-ILK was obtained from the T cells of the first group at 30 days, while N-ILK was obtained from the T cells of the second group. The chickens were slaughtered and their spleens were removed, collected and teased a part by the use of forceps in a Petri dish containing PBS in order to obtain a lymphocyte culture from the two groups separately and allow for the production of S-ILK and N-ILK. The process of obtaining splenic cells and the preparation of the lymphokines were conducted according to previously described methods[11].

Viral inoculum: Local isolates of Newcastle disease virus (allantoic fluid) were provided by the Department of Pathology and Poultry Diseases-College of Veterinary Medicine-Baghdad University, Iraq (unpublished) and stored in a deep freeze (-20°C). This was later used for the challenge (100ELD50-10^5) according to previously published methods[12].

Experimental design: Three hundred broiler chicks (Ros 308, of Belgian origin) were purchased from AL-Shakr Hatceery in Baghdad and divided randomly in to 6 groups (G1-G6) with 50 chicks in each group: G1: Treated with (S-ILK) and challenged with (NDV). G2: Treated with (NILK) and challenged with NDV. G3: Non-treated and challenged with NDV (positive control). G4: Non-treated and naturally infected with NDV. G5: Treated with (S-ILK) and non-challenged. G6: Non-treated and non-challenged (negative control). On the first day, S-ILK and N-ILK were injected intraperitoneally at a dose of 0.5 mL and after 30 min the intramuscular challenge consisting of a virulent Newcastle virus isolate at a dose ELD_{50}(10^7) was administered. Clinical signs, mortality, lesions were observed and recorded during necropsy.

Sample collection: Five birds were selected from each group at 7, 14, 21 and 28 days, the birds were fasted for 6 h and approximately 3-5 mL of blood was taken from the jugular vein and collected in tubes (without anticoagulants). Then, the blood samples were centrifuged at 1000 rpm for 15 min to separate the serum, which was stored at -20 until use for ELISA and HI tests. At 7, 14, 21 and 28 days, 5 birds from each group were selected and slaughtered and tracheal samples were collected directly and rapidly frozen at -20 until use for RT-qPCR.

Evaluation: Clinical signs were recorded throughout the experimental period, including respiratory signs such as coughing, sneezing, rales and whistling, digestive signs including green whitish diarrhea, nervous signs including tremors, clonic spasms, ataxia, opisthotonos or torticollis and others including dehydration, depression, emaciation, lethargy, or prostration. Gross lesions were recorded throughout the experimental period, including respiratory lesions such as tracheal congestion and lung hemorrhage, digestive lesions including hemorrhage of the gastroenteric mucosa, nervous lesions including edema and hemorrhage of the brain and others including moderate hemorrhage, enlargement of the spleen and slight swelling of the bursa of Fabricius.

Real-time (RT-PCR) test: Viral load in tracheal tissue samples taken from chickens infected with NDV was analyzed as previously described[13]. The viral RNA extracted from the tracheal tissue samples of the birds were collected from each group by using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). For viral detection, specific primers and TaqMan probes against NDV RNA were selected[14]. Real-time RT-PCR was performed using the Light Cycler® 480 real-time PCR system (Roche Diagnostics Deutschl and GMbH, Mannheim, Germany) by using the One Step PrimeScript RT-PCR Kit[13].

ELISA test: Detection of the NDV antibody in chickens was accomplished using a quick serological test (ELISA), which was conducted according to the instructions of the manufacturing company ProFlock® NDV ELISA kit[15].

Hemagglutination (HA) and Hemagglutination Inhibition (HI) test

Procedure of the Hemagglutination test: The Procedure of the HA test was performed according to the method previously described[16]. The procedure of the Hemagglutination Inhibition test (HI test) was conducted as previously described[17].

Statistical analysis: The SAS is the statistical analysis system adopted to evaluate the influence of various factors in the parameters of the study[18]. Multilevel least significant difference (LSD) testing was used in order to determine the significance level. Differences of $p<0.05$ were considered statistically significant.

RESULTS

Newcastle disease immunity: To determine the maternal immunity against Newcastle disease, ten serum samples were randomly selected from the 300 1-day-old chicks pre-divided into groups. The results of the ELISA and the HI test revealed a good immune response with an average level of 8652 ± 276 and 231 ± 23, respectively. The present study targeted the limitation associated with the efficiency of salmonella-immune lymphokines (S-ILK) to improve the immune response against Newcastle disease by administering it 30 min before challenge with a local field ND (100ELD50 10^5) isolate. Table 1 shows the results of antibody titer against ND using an ELISA . At 7 days, all groups showed a decrease in maternal immunity, while at 14 and 21 days, a significant increase was observed ($p<0.05$) in the antibody titer against ND in the first group, while a slight increase was seen in the second group. No titers were recorded against ND in the other groups. However, at 28 days, both the third and fourth group recorded a highly significant increase ($p<0.05$) in antibody titer against Newcastle disease in comparison to the first and second groups, which recorded a moderate increase, while the fifth and sixth groups did not record any titers.

Table 2 shows the results of the antibody titer against ND by the HI test as identified with an ELISA . At 7 days, there was a decrease in maternal immunity in all groups, while at 14 and 21 days, a significant increase was observed ($p<0.05$) in antibody titer against ND in the first group and a slight increase in the second group, whereas, antibody titers against ND were not recorded in the other groups. However, at 28 days, the third and fourth group recorded a highly significant increase ($p<0.05$) in antibody titer against Newcastle disease in comparison to the first and second groups, which recorded a moderate increase, while the fifth and sixth groups did not record any titers.

Viral load: The results of the RT-qPCR after challenge with a local isolate (100 ELD_{50} 10^5) of NDV at one day of age (Table 3) showed that the number of RNA copies of NDV in tracheal tissues showed that RNA copies in chickens treated with S-ILK in the G1 group showed the lowest RNA copies (low replication) at the different periods (7, 14, 21 and 28 days

Table 1: Effect of (Salmonella-immune lymphokines) on antibody titer (Means±SE) against a challenge with a local strain of Newcastle disease (100 ELD50 10^5) in broiler chickens at different periods by ELISA

	ND antibody titer means±standard error			
	Periods			
Groups	7 days	14 days	21 days	28 days
G1	4299.2±197.2^{B}	2199.2±128.4^{A}	4799.2±223.8^{A}	6499.2±247.0^{D}
G2	3743.4±146.3^{C}	1237.8±111.1^{B}	2137.8±210^{B}	11226.4±333.0^{C}
G3	3729.2±161.3^{C}	0±0^{C}	0±0^{C}	18672.3±289.3^{A}
G4	5513.5±197.7^{A}	0±0^{C}	0±0^{C}	16826.0±584.2^{B}
G5	5649.1±187.6^{A}	0±0^{C}	0±0^{C}	0±0^{E}
G6	5538.6±207.8^{A}	0±0^{C}	0±0^{C}	0±0^{E}
LSD	522.28	196.49	355.36	893.74

Number of samples: 5 from each group. G1: Treated with salmonella-immune lymphokines and challenged with NDV. G2: Treated with non-immune lymphokines and challenged with NDV. G3: Non-treated and challenged with NDV. G4: Non-treated and naturally infected with NDV. G5: Treated with salmonella-immune lymphokines and not challenged with ND. G6: Negative control. The capital letters appearing on the averages of the same column denotea significant difference among the treatment means at ($p<0.05$). LSD: Less significant differences

Table 2: Effect of (Salmonella-immune lymphokines) on antibody titers (Means±SE) against a challenge with a local strain of Newcastle disease (100 ELD50 10^5) in broiler chickens at different periods by HI test

	ND antibody titer means±standard error			
	Periods			
Groups	7 days	14 days	21 days	28 days
G1	102.40±11.0^{AB}	70.4±11.0^{A}	115.2±9.0^{A}	179.2±22.0^{B}
G2	89.60±11.0^{B}	35.2±17.5^{B}	64.0±12.4^{B}	281.6±44.3^{AB}
G3	89.60±11.0^{B}	0±0^{C}	0±0^{C}	409.6±44.3^{A}
G4	153.60±18.1^{A}	0±0^{C}	0±0^{C}	409.6±44.3^{A}
G5	153.60±18.1^{A}	0±0^{C}	0±0^{C}	0±0^{C}
G6	153.60±18.1^{A}	0±0^{C}	0±0^{C}	0±0^{C}
LSD	61.95	20.88	25.86	134.71

Number of samples: 5 from each group, G1: Treated with salmonella-immune lymphokines and challenged with NDV, G2: Treated with non-immune lymphokines and challenged with NDV, G3: Non-treated and challenged with NDV, G4: Non-treated and naturally infected with NDV, G5: Treated with salmonella-immune lymphokines and not challenged with ND, G6: Negative control. The capital letters appearing on the averages of the same column denote a significant difference among the treatment means at ($p<0.05$). LSD: Less significant differences

Table 3: Distribution of viral load (RT-qPCR, means±SE) of the tracheal tissue of broiler chickens challenged with ND virulent local strains (100 ELD50 10^5) at one day of age

	Viral load means±standard error			
	Periods			
Groups	7 days	14 days	21 days	28 days
G1	215.2±8.13^{C}	848.4±38.0^{D}	1061.6±30.1^{D}	1892.8±42.0^{C}
G2	723.8±38.5^{B}	1641.2±54.9^{B}	2638.6±73.6^{C}	3887.6±142.4^{B}
G3	1149.4±36.5^{A}	2824.0±96.8^{A}	4481.2±254.8^{A}	8992.6±292.9^{A}
G4	132.6±14.3^{C}	1066.8±35.7^{C}	3540.0±141.7^{B}	8265.8±344.5^{A}
G5	0±0^{D}	0±0^{E}	0±0^{E}	0±0^{D}
G6	0±0^{D}	0±0^{E}	0±0^{E}	0±0^{D}
LSD	93.72	207.07	509.03	801.61

Number of samples: 5 from each group. G1: Treated with salmonella-immune lymphokines and challenged with NDV. G2: Treated with non-immune lymphokines and challenged with NDV. G3: Non-treated and challenged with NDV. G4: Non-treated and naturally infected with NDV. G5: Treated with salmonella-immune lymphokines and not challenged with ND. G6: Negative control. The capital letters appearing on the averages of the same column denote a significant difference among the treatment means at ($P < 0.05$). LSD: less significant differences

post-challenge) followed by the second group in which a slight increase in RNA copies was recorded among other groups, these differences were highly significant ($p<0.05$). The third and fourth groups showed a high replication rate at 21 and 28 days post-challenge.

Clinical signs and mortality: Table 4 shows the clinical signs that were recorded throughout the experimental period, including respiratory signs such as coughing, sneezing, rales and whistle, digestive signs including green whitish diarrhea, nervous signs including tremors, clonic spasms, ataxia,

Table 4: Clinical signs observed in treated groups during the experimental period

	Groups					
Signs	G1	G2	G3	G4	G5	G6
Respiratory	10/50 (20%)^{C}	40/50 (80%)^{B}	50/50 (100%)^{A}	40/50 (80%)^{B}	0/50 (0%)^{D}	0/50 (0%)^{D}
Digestive	0/50 (0%)^{C}	13/50 (66%)^{B}	50/50 (100%)^{A}	20/50 (40%)^{B}	0/50 (0%)^{C}	0/50 (0%)^{C}
Nervous	3/50 (6%)^{C}	32/50 (64%)^{B}	42/50 (84%)^{A}	38/50 (76%)^{B}	0/50 (0%)^{C}	0/50 (0%)^{C}
Others	2/50 (4%)^{C}	22/50 (44%)^{C}	50/50 (100%)^{A}	45/50 (90%)^{B}	0/50 (0%)^{C}	0/50 (0%)^{C}
Mortality	0/50 (0%)^{B}	50/50 (100%)^{A}	50/50 (100%)^{A}	50/50 (100%)^{A}	0/50 (0%)^{B}	0/50 (0%)^{B}

Respiratory signs include coughing, sneezing, rales and whistle. Digestive signs include green whitish diarrhea. Nervous signs include tremors, clonic spasms, ataxia, opisthotonos, or torticollis. Others include dehydration, depression, emaciation, lethargy, or prostration

Table 5: Gross lesions observed in treated groups during experimental period

	Groups					
Lesions	G1	G2	G3	G4	G5	G6
Respiratory	2/50 (4%)^{C}	20/50 (40%)^{A}	20/50 (40%)^{A}	10/50 (20%)^{B}	0/50 (0%)^{C}	0/50 (0%)^{C}
Digestive	0/50 (0%)^{D}	13/50 (26%)^{C}	23/50 (46%)^{A}	17/50 (34%)^{B}	0/50 (0%)^{D}	0/50 (0%)^{D}
Nervous	1/50 (2%)^{D}	5/50 (10%)^{C}	12/50 (24%)^{A}	8/50 (16%)^{B}	0/50 (0%)^{D}	0/50 (0%)^{D}
Others	1/50 (2%)^{C}	6/50 (12%)^{B}	10/50 (20%)^{A}	5/50 (10%)^{B}	0/50 (0%)^{C}	0/50 (0%)^{C}

Respiratory lesions include tracheal congestion and lung hemorrhage. Digestive lesions include hemorrhage of the gastroenteric mucosa. Nervous lesions include edema and hemorrhage of the brain. Others include moderate hemorrhage and enlargement of the spleen and slight swelling of the bursa of Fabricius

opisthotonos or torticollis and others including dehydration, depression, emaciation, lethargy or prostration. High morbidity and mortality were recorded in the third and fourth groups in comparison to the second group, revealing moderate clinical signs and a high mortality, the first group showed a low morbidity and no mortality, while the fifth and six groups did not indicate any morbidity or mortality since they were not exposed to the infections agent.

Table 5 shows the gross lesions that were recorded throughout the experimental period. These lesions included respiratory lesions including tracheal congestion and lung hemorrhage, digestive lesions including hemorrhage of the gastro enteric mucosa, nervous lesions including edema and hemorrhage of the brain, others including moderate hemorrhage and enlargement of the spleen and slight swelling of the bursa of Fabricius. A significant number of gross lesions were recorded in the third and fourth groups in comparison to the second group that had moderate gross lesions; the first group showed a low number of gross lesions, while the fifth and sixth groups did not have any gross lesions because they were not exposed to the infection.

DISCUSSION

The results of the present study showed that S-ILK had an effective role in providing absolute protection to the chicks by increasing their antibody titer against NDV at different periods after being challenged with a virulent local Newcastle isolate. These results agree with those of many researchers, confirming that a significant increase in antibody titers against ND infections occurs in broiler chickens after the use of S-ILK[19]. The results of the current study showed that the use of S-ILK did not hurt or harm the young chickens since no adverse impacts were noted throughout the experimental period. In addition, the use of S-ILK was able to reduce the occurrence of clinical signs, gross lesions and mortality caused by a virulent NDV as observed in the S-ILK treated group challenged with a virulent NDV and as established by Alfaro *et al.*[19] who reported that cytokines should be considered as natural mediators of the immune response process, whether innate or adaptive. Cytokines have an effective and decisive role in the function of the immune system by acting as mediators of immune functions, which include activation, differentiation of immune cells and enhancement of the immune response as well as the production of many other cytokines[20,21]. The results of the present study are consistent with a wide range of previous studies with regards to the increased occurrence of gross lesions in the respiratory, digestive and nervous systems during the experimental period in G2, G3 and G4 that were not treated with S-ILK and challenged with a virulent NDV. The presence of such lesions in the (G2) group that was treated with N-ILK is evidence that N-ILK does not include a biologically effective component capable of preventing the tissue damage caused by infection with a virulent NDV[19]. As a result of the properties of its components, which may be pleiotropic and redundant, S-ILK has the potential to induce leukocyte proliferation within lymphatic organs and other tissues[22]. The results of the present study show that the number of RNA copies in tracheal tissues in chickens treated with S-ILK in the G1 group were the lowest (low replication) at the different periods (7, 14, 21 and 28 days post-challenge), followed by the second group, which had a slight increase in RNA copies compared to the other groups, these differences were highly significant ($p<0.05$) compared to the third and

fourth groups, which recorded high replication rates at 21 and 28 days post-challenge. This proves the role of S-ILK in decreasing the number of RNA copies of NDV in tissues. These results agree with those of Alfaro *et al.*[19] and Hilton *et al.*[23], who reported that using non-specific immunity against *Salmonella entridis* in leghorn chickens reduced the viral load of RNA copies of NDV after challenge. The current study shows that understanding the biological functions of avian lymphokines may allow for the development of strong and efficient tools for the enhancement of immunity against viral infections. The current study shows that vaccination programs are not the only expedient available to support the humoral immune system. Certain cytokines may play an important role in humoral immune responses as confirmed by Durum and Oppenheim[24] Rahman *et al.*[25]. Additionally, Asif *et al.*[26] reported that the effects of using S-ILK against ND infections may involve the development and recruitment of leukocytes and the rapid aggregation of lymphocytes in many organs. These agents may modify or determine the nature of the humoral immune response prevalent after infection with ND and lead to the production of a rapid and high level of antibodies that may even prevent the replication of the virus. Despite the widespread use of vaccines against ND, whether attenuated or inactivated for the control of disease outbreaks, these strategies have not been able to fully prevent outbreaks against circulating virulent strains of NDV. This may be a result of the emergence of a highly virulent infectious patho type that shows antigen variations that can be responsible for recent NDV outbreaks[4]. As a result of the intensive breeding system currently practiced in poultry production, chickens are exposed to a wide variety of pathogens. As a result of the intensity of this antigen exposure, vaccination programs and antibiotic treatment are not always practical and effective. In such circumstances, the use of cytokines as non-specific substances that contribute to increased immune capacity may give a solution that has a greater protective effect against diseases caused by pathogens[8,9].

CONCLUSION

The results of the present study show the effective role of S-ILK in providing absolute protection to the chicks against ND by enhancing their immune response and reducing the number of virus isolates in living tissues following challenge with a virulent local Newcastle isolate.

SIGNIFICANCE STATEMENT

This study discovered the therapeutic role of poultry lymphokines against the negative impacts caused by a local strain of virulent Newcastle disease in chickens. This can be beneficial in enhancing the immune response and reducing the number of virus isolates in living tissues. This study will help researchers uncover the critical roles of salmonella immune lymphokines that many researchers were unable to previously explore. Thus, a new hypothesis may be formulated regarding the use of S-ILK against other respiratory diseases, such as influenza and infectious bronchitis. Finally, S-ILK can be dispensed in whole vaccines, whether attenuated or inactivated for the control of disease outbreaks, which have not proven fully effective against circulating virulent strains of NDV.

REFERENCES

1. Mayo, M.A., 2002. A summary of taxonomic changes recently approved by ICTV. Arch. Virol., 147: 1655-1663.
2. Kapczynski, D.R., C.L. Afonso and P.J. Miller, 2013. Immune responses of poultry to Newcastle disease virus. Dev. Compa. Immunol., 41: 447-453.
3. Cattoli, G., L. Susta, C. Terregino and C. Brown, 2011. Newcastle disease: A review of field recognition and current methods of laboratory detection. J. Vet. Diagn. Invest., 23: 637-656.
4. Wang, X., Q. Zhou, J. Shen, J. Yao and X. Yang, 2015. Effect of difference doses of Newcastle disease vaccine immunization on growth performance, plasma variables and immune response of broilers. J. Anim. Sci. Biotechnol., Vol. 6, No. 1. 10.1186/s40104-015-0019-y.
5. Kapczynski, D.R. and D.J. King, 2005. Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. Vaccine, 23: 3424-3433.
6. Miller, P.J., D.J. King, C.L. Afonso and D.L. Suarez, 2007. Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. Vaccine, 25: 7238-7246.
7. Kogut, M.H., R.B. Moyers and J.R. DeLoach, 1996. Las citocinas y sus interrelaciones funcionales: Potenciacion de la respuesta inflamatoriaen el control de infeccionessiste micas por Salmonella en las aves. Memorias del Curso de Actualizacion Avancesen Inmunologia Aviar, Mexico, DF. Asociacion Nacional de Especialistasen Ciencias Avicolas de Mexico, AC, Mexico, DF., March 15, 1996, pp: 8-12.
8. Stern, A.M. and H. Markel, 2005. The history of vaccines and immunization: Familiar patterns, new challenges. Health Affairs, 24: 611-621.

9. Liu, Q., L.C. Miller, F. Blecha and Y. Sang, 2017. Reduction of infection by inhibiting mTOR pathway is associated with reversed repression of type I interferon by porcine reproductive and respiratory syndrome virus. J. Gen. Virol., 98: 1316-1328.
10. Tellez, G.I., M.H. Kogut and B.M. Hargis, 1993. Immunoprophylaxis of *Salmonella enteritidis* infection by lymphokines in Leghorn chicks. Avian Dis., 34: 1062-1070.
11. Kogut, M.H. and T.S. Slajchert, 1992. T lymphocytes induce protection in chickens against *Eimeria tenella* by production of lymphokines. Immunol. Infect. Dis., 2: 69-80.
12. Reed, L.J. and H. Muench, 1938. A simple method of estimating fifty per cent endpoints. Am. J. Epidemiol., 27: 493-497.
13. Sun, J., Z. Han, Y. Shao, Z. Cao, X. Kong and S. Liu, 2014. Comparative proteome analysis of tracheal tissues in response to infectious bronchitis coronavirus, Newcastle disease virus and avian influenza virus H9 subtype virus infection. Proteomics, 14: 1403-1423.
14. Wise, M.G., D.L. Suarez, B.S. Seal, J.C. Pedersen and D.A. Senne *et al*., 2004. Development of a real-time reverse-transcription PCR for detection of Newcastle disease virus RNA in clinical samples. J. Clin. Microbiol., 42: 329-338.
15. Synbiotic® Corporation, 2005. Newcastle disease virus antibody test kit. Proflock R. Plus, Item No. 96-95 33, Frontera, San Diego, CA., USA.
16. OIE., 2004. Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals. 5th Edn., Office International Des Epizooties, Paris, France.
17. Grimes, S.E., 2002. A basic laboratory manual for the small scale production and testing of I-2 Newcastle disease vaccine. Food and Agricultural Organization (FAO), Animal Production and Health Commission for Asia and the Pacific (APHCA).
18. SAS., 2012. User's Guide: Statistical. Version 9, 1st Edn., SAS SAS Institute Inc., Cary, NC., USA.
19. Alfaro, J.C., V.M. Petrone, T. Fehervari, G. Nava, M. Kogut, D. Nisbet and G. Tellez, 2002. Resistance to velogenic Newcastle disease virus in leghorn chickens by use of prophylactic lymphokines. Avian Dis., 46: 525-534.
20. Arai, K.L., F. Lee, A. Miyajima, S. Miyatake, N. Arai and T. Yokota, 1990. Cytokines: Coordinators of immune and inflammatory responses. Annu. Rev. Biochem., 59: 783-836.
21. Kaiser, P., 2010. Advances in avian immunology-prospects for disease control: A review. Avian Pathol., 39: 309-324.
22. Kuby, J., 1997. Immunology. 3rd Edn., W. H. Freedman and Co., New York.
23. Hilton, L.S., A.G.D. Bean and J.W. Lowenthal, 2002. The emerging role of avian cytokines as immunotherapeutics and vaccine adjuvants. Vet. Immunol. Immunopathol., 85: 119-128.
24. Durum, S.K. and J.J. Oppenheim, 1993. Proinflammatory Cytokines and Immunity. In: Fundamental Immunology, William, E.P. (Ed.)., Raven Press, New York, pp: 801-835.
25. Rahman, M.M., E. Uyangaa, Y.W. Han, S.B. Kim, J.H. Kim, J.Y. Choi and S.K. Eo, 2012. Enhancement of Th1-biased protective immunity against avian influenza H9N2 virus via oral co-administration of attenuated *Salmonella enteric* serovar *Typhimurium* expressing chicken interferon-α and interleukin-18 along with an inactivated vaccine. BMC Vet. Res., Vol. 8, No. 1. 10.1186/1746-6148-8-105.
26. Asif, M., K.A. Jenkins, L.S. Hilton, W.G. Kimpton, A.G. Bean and J.W. Lowenthal, 2004. Cytokines as adjuvants for avian vaccines. Immunol. Cell Biol., 82: 638-643.