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IMMUNE STATUS OF IMPROVED BALADY CHICKEN AGAINST SOME ENDEMIC VIRUSES AND MYCOPLASMA

(With 4 Tables)

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الحاله المناعيه للدجاج البلدى المحسن ضد بعض مسببات الأمراض الفيروسيه المستوطنه والميكوبلازما

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تم أخذ عينات سيرم من دجاج بلدى محسن عند عمر خمس أشهر للكشف عن الحاله المناعيه لهم ضد أمراض فيروس النيوكاسل ، التهاب المفاصل ، تدنى البيض ، جدرى الطيور ، الجامبورو القصبه الهوائيه المعدى ، التهاب الحنجره ، الميكوبلازما ساينوفيا وجاليسيبتكم وقد تم إجراء الأختبارات السيرولوجيه المختلفه على عينات السيرم فيها. (اختبار الترسيب بالأجار - إختبار التلازن الدموى الغير مباشر - إختبار التلازن الدموى السريع وإختبار منع التلازن الدموى) وأظهرت النتائج الآتى :

كان معدل المتوسط الهندسى لمستوى الأجسام المناعيه ضد الفيروسات السابقه بإستخدام إختبار التلازن الدموى الغير مباشر بإستخدام مادة الكروميك كلورايد مع كرات الدم الحمراء للغنم أعلى من معدلها عند إستخدام مادة حمض التانيك وكان الإختلاف بينهم يتراوح من ١ر٦٤ ، ٢ر٠٥ ، ١ر٠٤٢ ، ٠ر٠٧٠ ، ٠ر١٢٠ على التوالى.

وبالمقارنه بين إستخدام أختبار التلازن الدموى الغير مباشر وإستخدام إختبار منع التلازن الدموى بالنسبه لقياس مستوى الأجسام المناعيه ضد مرض النيوكاسل ومرض تدنى البيض وجد أن معدل مستوى الأجسام المناعيه بإستخدام إختبار التلازن الدموى الغير مباشر أعلى من إستخدام الأختبار الآخر وكان الفرق بينهما هو ٢ر٦٣ ، ٥ر٦٠ على التوالى.

كان معدل إنتشار العدوى بالميكوبلازما ساينوفيا وميكوبلازما جاليسيبتكم وصل إلى حوالى ٨٥ر٩٢ ٪ ، ٦٤ر٨٧ ٪ على التوالى بإستخدام إختبار التلازن الدموى السريع وبإستخدام إختبار منع التلازن الدموى وجد أن معدل المتوسط الهندسى للإجسام المناعيه كان ٣ر٥ لوج .

SUMMARY

A screening of antibodies was done in the sera of 5 months old balady chickens against Newcastle disease virus, tenosynovitis virus, Egg drop syndrom, Fowl pox, Infectious bursal disease, Infectious bronchitis virus, Infectious larengeotrachitis virus, *Mycoplasma gallisepticum,* and *Mycoplasma synoviae,* using agar gel precipitation test, indirect haemagglutination test, rapid plate agglutination test, and haemagglutination inhibition test. The passive haemagglutination geometric mean antibody titers using the mentioned viruses adsorbed to chromic chloride treated sheep red blood cells were higher than that obtained using the same viruses adsorbed to tannic acid treated sheep red cells The differences were 1.64, 2.05, 0.1, 0.42, 0.3, 0.07, 0.12 respectively. The passive haemagglutination geometric mean antibody titers against NDV, EDS were higher than the analogous haemagglutination inhibition geometric mean antibody titers, the differences were 2.63, 0.5 respectively. The incidence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection was 8.7.14% and 92.85% respectively by rapid plate agglutination test while their haemagglutination inhibition geometric mean antibody titer was 5.3 Log2.

INTRODUCTION

Recently, many efforts have been made to establish poultry production as a source of human protein consumption in Egypt. Uncontrolled importation of day old parent stock, egg type chicks, and human carlessness, curiosity, ignorance and indifference constitute the greatest potential causes of introduction of diseases. Newcastle disease virus has beeen found to survive in the human respiratory tract and has been isolated from sputum *(UTTERBACK and SCHWARTZ, 1973). ALEXANDER* et al. *(1978)* isolated infectious bronchitis virus (IBV) from faeces of live birds 227 days after inoculation with the Hooand 52 strain. Infectious Laryngeotrachitis virus (ILT) has been

isolated from tracheal tissues of recovered chicken for as long as two years *(SEDDON, 1952)*. *BENTON* et al. *(1967)* found that hosues that had contaminated infected birds with infectious bursal disease virus (IBDV) were still infectious for other birds 122 days after their removal. Birds were found to excrete Egg Drop Syndrome Virus (EDS) from the pharynex and the faeces *(COOK and DARBYSHIRE, 1980)*; the main method of spread is vertically through the embryonated egg *(MCFERRAN* et al., *1978)*> Reovirus (Viral Arthritis VA) has been shown to persist in a bird for at least 289 days *(OLSON and KERR, 1966)* and its vertical transmission was shown by *MENENDEZ* et al. *(1975)*. Mosquitoes could remain infective with Poxviruses for several weeks and produce consecutive infections *(Da MASSA, 1966)*. Some avian pathogens have been isolated and identified from infected chicken in Egypt such as NDV, IB, ILT, IBDV, VA, EDS, Fowlpox, *Mycoplasma gallisepticum (MG)* and *Mycoplasma synoviae (MS)*. The response of chicken to microbial infection involves interaction of both innate and acquired immunity. Once having pentrated the skin or mucosal barrier, invading microorganisms immediately encounter the host's non-specific defence mechanisms which include inhibitors like lysozyme, interferon and complement and the phagocytic activity of heterophil, blood monocytes and tissue macrophages. Pre-existing specific antibody, if present can coat (opsonize) the micro-organisms and infection provokes a specific immune response to arrest and eliminate infection. The objective of the present study was to measure the incidence of infection with viruses, *Mycoplasma gallisepticum* and *Mycoplasma synovaie* by serological examination of improved balady chicken and to compare between different serological methods.

MATERIAL and METHODS

Serum Samples:

Two hundred twenty, one day old, improved balady chicken were purchased from Faculty of Agriculture Giza Governorate. The chicken were reared on the ground as a small flock, without sound management and vaccination. During rearing period, some chicken suffered from respiratory symptoms, lamness, retarded growth, diarrhoea and deaths. Seventy chicken were apperently healthy and continued to production period. Serum samples from these clinically healthy chicken (70) were taken, inactivated at 56°C for 30 min. Each 0.5 ml serum sample was then adsorbed

by adding 2.5 ml of a 20% suspension sheep red blood cells washed 3 times in buffered saline pH 7.2.

Prepration of Positive Serum:

These sera were prepared in rabbits as a routine work of immunology department as the method adopted by *HEASLIC and FRANC (1980)*. Briefly, adult rabbit received 4-5 doses of viral antigen. The first one consists of 1 ml of antigen mixed with 1 ml of complete Fraund's adjuvent s/c. A second dose consisted of 1 ml viral antigen with 1 ml of incomplete Fraund's adjuvent one month latter.. The rabbit received three doses of viral antigen i/m every two weeks post two weeks of second dose. The produced antiserum was adsorbed with a 20% chicken red cells after inactivation at 56°C for 30 min.

Prepration of Antigens:

Newcastle virus (NDV Lasota strain), Viral Arthritis (VA S1133), Egg Drop Syndrom (EDS76), Fowl Pox, Infectious Bursal disease (IBDV virulent IM strain), Infectious Bronchitis Virus (IB Baudette strain), Infectious Laryngeotrachitis (ILT vaccinal strain), were partially purified by fluorocarbon 113 method adopted by *TRIPATHY* et al. *(1970)*. These antigens were adsorbed on sheep erythrocytes treated with tannic acid as method adopted by *BOYDEN (1951)*. Another part of these antigens were adsorbed on sheep erythrocytes treated with chromic chloride as method adopted by *PHILIP* et al. *(1969)*. Sensitization of red cells was performed by mixing equal volumes (0.1 ml) of washed packed erythrocyte with 1/20,000 dil. of chromic chloride and antigen for 5 min. at 37°C. The cells, antigen and chromic chloride dilutions were made in phosphate free- saline. The antigensensitized erythrocytes were washed four times with a 30-40 volumes of saline using 30 sec. periods of centrifugation. 2% sensitized sheep red blood cells coated with chromic chloride were used in the proper test. The sensitized tannic acid and chromic chloride treated red cells were used in passive haemagglutination test against collected sera.

Prepration of Haemagglutination Antigens:

NDV antigen was used as a concentrated lypholized amnioallantoic fluids. EDS 76, supernatent lypholized and titrated for HA units with 0.8% chicken erythrocytes. IBV

antigen was prepared by the method adopted fby *MULDOON (1960)*. IBV containing allantoic fluid was treated with 1% trypsin for 30 min. at 56°C to release the non specific reaction of haemagglutinin on the virus. The treated virus was incubated for three hours at 37°C.

Agar Gel Precipitation Test:

The double diffusion method of *WOERNLE (1966)* was used for demonstrating precipitating antibodies. Partialy purified prepared antigens of mentioned viruses were reacted against tested sera. A control negative consists of a suspension of uninoculated chrioallantoic membranes and a control positive prepared serum were included.

Passive Haemagglutination Test:

The test was adopted by the method of *GEORGE and VAUGHAM (1962)* using twofold serial dilution of the tested sera. The dilutions were made by normal rabbit serum as a stabilizer to balance the agglutinating effect of tannic acid, and chromic chloride. A similar volume of prepared sensitized sheep coated erythrocyte treated with tannic acid or chromic chloride was added with dilution 2%. Examination was made at 3 and 12 hours. Controls in each test were sensitized cells with diluent alone, a known positive and a known negative serum samples as well as a control of unsensitized cells with diluent alone. The end point was determined by observing the highest dilution at which cells agglutinated. The geometric mean titer of tested sera against all antigens sensitizes sheep erythrocyte coated with tannic acid and chromic chloride were calculated and represented as geometric mean passive haemagglutination titers of tested sera (GM of PHA).

Haemagglutination Inhibition Test (HA and HI)):

The tests were performed as descrobed by *BEARD (1980)* and *ADAIR* et al. *(1986)*. They were performed in multi-well plastic plates using constant volumes of tested sera and NDV antigen IBV antigen or EDS antigen which contained four HA units. Phosphate buffered (0.05M PH 7.4) was used as diluent, red blood cells of chicken were 0.8-1% (packed cell v/v) cell suspension in diluent was used. Positive and negative control sera were incorporated in each test. Serial twofold dilutions were prepared starting with 1/2. The plates were held at 4°C

for 30-45 min. The back titrations were performed in duplicate. The cell controls should show no evidence of agglutination. The titre caculated from the antigen back titration should be four HA units. All test serum HI titers were expressed in term of Log2. Calculation of the geometric mean log2 HI titers of sera chicken against NDV, IBV, and EDS were done.

Rapid Plate Agglutination Test (RPA):

RPA tes: wad done as described by *ADLER (1954)*, coloured antigens for *Mycoplasma gallisepticum (MG)* and *Mycoplasma synoviae (MS)* were obtained from Intervet International B.V. Boxmeer-Holland. Haemagglutination inhibition test was performed as described by *FABRICANT (1969)*. The geometric mean titer of HI test against MG and MS were calculated.

RESULTS

The results of agar gel diffusion test were negative, using the collected tested sera aginst partial purified antigens of NDV, EDS, S1133, Fowl Pox, IBDV, IB, ILT, iruses. Positive prepared sera tested against control normal CA (chrioallantoic membrane) did not yield lines of precipitation. Distinct lines of precipitation appeared in gel after 24 to 48 hours of incubation of positive sera and its positive prepared viral antigens at room temperature.

Table 1 shows the results of passive HA test, the geometric mean titers of antibodies in the tested sera against NDV, S1133, EDS, Fowl pox, IBDV, IB, ILT, adsorbed to tannic acid treated sheep red blood cells were 2.26, 2.14, 3,3, 2.58, 0.9, 0.91, 0.68 Log2 and the highest PHA titres obtained were 16, 128, 64, 16, 16, 16. The results of PHA tests detected antibodies in the tested sera against the same viral antigens adsorbed to chromic chloride treated sheep red blood cells, and the geometric mean titers obtained as shown in table (1) were 4.9, 4.19, 3.4, 3, 1.2, 0.98, 0.8. The highest PNA titers obtained were 128, 128, 64, 128, 128, 64, 16.

The results of HI tests are shown in table 2, the geometric mean HI titers (GMT) obtained by using NDV, IB, EDS viral antigens with tested sera were 2.27, 2.87, 2.9 the highest HI titers 128, 128, 64 were recorded by 3,2 and 3 samples respectively.

Table 1: Passive haemagglutination tests of tested chicken sera against 7 viral antigens.

Viral antigen	GM	Using tannic acid highest titer	No. of positives	Using GM	chromic chloride highest titer	No. of positive
NDV	2.26	1/16	2	4.9	1/128	20
S1133	2.14	1/128	1	4.19	1/128	14
EDS	3.3	1/64	3	3.4	1/64	3
Fowl Pox	2.58	1/64	1	3	1/128	3
IBDV	0.9	1/16	1	1.2	1/128	2
IB	0.91	1/16	3	0.98	1/64	3
ILT	0.68	1/16	1	0.80	1/16	1

Table 2: Haemagglutination Inhibition (HI) titers of tested chicken sera against NDV, IB, EDS.

Viral antige	Geometric mean titer (Log2) of serum	Highest titer	No. of positive
NDV	2.27	1/128	3
IB	2.87	1/128	2
EDS	2.9	1/64	3

The results obtained in table (3) showed that from 70 serum samples antibodies were detected against MG, MS by RPA as (61) 67.14% and 65(92.85%) respectively. The geometric mean HI antibody titer (Log2) of serum against MG and MS was 5.3 and the highest HI antibody titer was 1/128 in 30 serum samples.

Table 3: Detection of antibodies against MG and MS by rapid plate agglutination and haemagglutination inhibition tests.

Examined number	Rapid plate agglutination test				Geometric mean HI titer (Log2) of	Highest titer
	MG		MS			
	No. of +ve	%	No. of +ve	%		
70	61	87.14	65	92.85	5.3	1/128

Table 4 shows the number of serum samples which contain antibodies against different agents. Samples number 6, 10, 24, contained antibody against NDV, S1133 and MG, MS. Sample number 40 contained antibody against S1133, EDS, IB, Pox and MG, MS. Sample number 59 contained antibody against S1133, IB, NDV Pox, and MG, Ms. Sample number 50 contained antibody against NDV, EDS, IBDV, and MG, MS. Sample number 63 contained antibody S1133, IBDV, and MG, MS. Sample number 6 contained antibody against NDV, S1133, and MG, MS. Sample number 55 contained antibody against Fowl pox, IB, and MG, MS.

Table 4: Antibody content of sera of improved balady chicken against natural infection with 9 different agents.

Serum No.	Gents producing the antibodies	Corresponding antibody titer
6,10,24	NDV,S1133,MG,MS	128,128,128,128
40	S1133,EDS,Ib,Fowl pox,MG,MS	128,64,128,128,128,128
56	NDV,ILT,MG,MS	128,16,128,128
59	NDV,S1133,IB,Fowl pox,MG,MS	128,128,64,128,128,128
63	S1133,IBDV,MG,MS	128,128,128,128
50	NDV,EDS,IBDV,MG,MS	128,64,128,128,128
55	Fowl pox,IB,MG,MS	128,64,128,128

DISCUSSION

The immune system of chicken has a discriminatory power to illustrate delineation of the humoral and cell mediated immune responses. Trials were made to aquaint with the humoral immune responses of improved balady chicken against different endemic pathogens, especially which result as synergistic action of viruses and *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. It is generally accepted that antibodies detected in series of virus infection, by the passive haemagglutination test are closely related or even identical with neutralizing antibodies *(FELTON and SCOTT, 1958)*.

In this study PHA test was chosen as a constant test for evaluation of antibodies against viral natural infection. AGP test was made as a primary serological test. The same serum samples were subjected to RPA and HI tests to detect antibodies against MG and MS. An adequately purified antigeic prepration is required for the specific antigen-antibody reactions.

Fluorocarbon treatment is useful to remove non-viral protein from crude viral prepration and was employed to purify antigens of ILT, IBDV, S1133 and Fowl Pox.

The result of AGP test using partially purified antigens with fluorocarbon indicated that all sera were free of precipitating antibodies against NDV, S1133, EDS, Fowlpox, IBDV, IB, ILT, while the positive prepared sera against the same viral antigen strains showed distinct lines of precipitation after 24 to 48 hours of incubation at room temprature. The negativity of AGP test does not always exclude the possibility that the flock under investigation had been exposed to infection.

The results of PHA showed that the birds have passive HA antibody titers against NDV, S1133, EDS, Fowl Pox IBDV, IB, ILT, sensitized sheep red blood cells either with tannic acid or chromic chloride.

The values of GM passive haemagglutinating titers obtained using sensitized chromic chloride treated sheep red blood cells antigens were higher than that obtained using sensitized tannic acid treated sheep red cells antigens, the differences were 1.64, 2.05, 0.1, 0.42, 0.33, 0.07, 0.12 Log2 respectively. This result indicated that chromic chloride is more valuable for treatment of sheep red blood cells than tannic acid due to the greater ability of virus sensitized chromic chloride treated sheep red cells to recognize and detect antibody particles in teted sera.

The results of AGP and PHA tests indicated that passive haemagglutination test is one of the most sensitive serologic tests. Detection of antibody by the PHA test rather than by AGP test is likely due to the difference in sensitivity of the two tests. The PHA test can detect as little as 0.005 mg antibody nitrogen per ml whereas the AGP is unable to detect less than 5 mg per ml *(CARPENTER, 1956)*. The former is therefore approximately 1000 times as sensitive as the latter. Besides the usual immune response is characterized by, first the appearance of 19s antibody, which is followed by 7s antibody. The 19s antibody has been found to be about 750 times as efficient at agglutinating red cell as the 7s antibody *(GREENBURG et al., 1963)*.

The HI titers of the tested sera against NDV, EDS, antigens were lower than PHA aantibodies (difference in GM titers Log2 were 1.63, 0.5 Log2), while it was increased when

IB trypsin treated antigen was used in HI test. These differences in results indicated that preparations of sensitized NDV, EDS and treated IB trypsin antigens are more sensitive in detecting HA antibody particles in tested sera.

The PHA test, in the forms applied in this study proved to a relatively simple and reliable test for the serological diagnosis of viral disease as reported by several workers *(BROWN et al., 1962 ; EDSON & SCHMITTLE, 1969; FRANKEL, 1958 and ALJEV et al., 1989).*

In our study a high incidence of *M.gallisepticum* (87.14%) and *M.synoviae* (92.85%) was detected by RPA test which would recognize immunoglobulin M (IgM) as suggested by *KLEVEN et al. (1975)*, while the incidence was 62.85% for *M.gallisepticum* and 60% for *M.synoviae* by HI test which would detect immunoglobulin G (IgG) as suggested by *ROBERTS and OLESIUK (1969).*

The immune system of the improved balady chicken was capable of responsing to more than one pathogenas manifested by nine serum samples, six of these sera (6,10,24,40,59,63) showed high antibody titers (1/128) against S1133 and MG, MS. These results show the dangerous role of avian reoviruses and mycoplasma infection in the balady chicken farms in Egypt.

The results obtained in this study showed the association between avian mycoplasmosis and respiratory viruses infection which was studied by many authors *(ADLER et al., 1962; SIMMONDS & LUKERT 1972; YODER, 1973 and AGHAKHAN et al., 1976).* The HI antibody titers against mycoplasma were higher (1/128) in serum samples showing high antibody content to the examined viruses. These results can explain the disturbances caused by the synergistic action of the viruses and its effect enhancing the multiplication of *M.gallisepticum* and *M.synoviae (SATO et al., 1970; KLEVEN et al., 1972 and VORLEY & JORDAN, 1978).*

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