TRIALS FOR VACCINATION OF GUINEA FOWLS WITH FOWL CHOLERA; NEWCASTLE (ND) AND INFECTIOUS BRONCHITIS (IB) VACCINES

By
Hanan, M.Ibrahim;* Elham, A.El-Ebiary*and Khodier, M.H. **
*Central Laboratory for Evaluation of Veterinary Biologics, Abbassia, Cairo
**Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo

SUMMARY

The present work as an interesting job included the vaccination of Guinea fowls with an inactivated bivalent ND and IB vaccine simultaneously with FC inactivated vaccine. The results of SNT and HI test showed that these birds responded immunologically well where they exhibited high levels of specific ND, IB and FC antibodies showing no signs of illness revealing the safety of the used vaccines. The induced immunity enable vaccinated fowls to withstand the challenge with the virulent strains of ND virus; IB virus and Pasteurella multocida type A:1 while unvaccinated birds were unable to overcome such challenge indicating that these birds are susceptible for both of ND; IB and FC and may play a role in the epidemiology of these diseases. So it could be recommended that Guinea fowls must be vaccinated with these vaccines to protect them and other poultry population which may be found in contact with them.

INTRODUCTION

There are about 515 species and sub-species of birds in Egypt either they are domestic or wild. These birds belong to 71 families and 12 orders (Tharwat, 1997). Wild birds are used as in captive birds in zoo gardens of which there are several ones in Egypt.

Generally, it is well known that infectious diseases either they are bacterial or viral represent a dangerous enemy which could destruct a bird population completely.

One of the oldest known viral diseases is Newcastle disease (ND) which is a highly contagious fatal disease affecting all gallinaceous birds as fowls; turkeys; Guinea fowls; quails and pheasants. Once ND is occurred in an area, it spreads away with a high percentage among poultry populations. It had created a serious economic challenge to the rising poultry industry allover the world.

Regarding Guinea fowls, Ballarini (1964) recorded an outbreak of ND while Sheble (1961) and Elham (1979) isolated the virus from 15.5% of 180 birds in Giza zoo garden. Signs of ND in Guinea fowls were described by

Bennejean et.al. (1974) as nervous and respiratory symptoms and showed that the mortality rate among theses birds was 50% in experimental infection. These findings indicate that zoo gardens may constitute one of the most serious sources of disease ND spread.

Incrimination of wild birds in the spread of ND when supported by virus isolation, demonstration of natural and experimental humeral antibodies and cohabitation of infection confirm the active role of such birds in transmission and spread of the disease (Levine, 1962).

Infectious bronchitis (IB) is an acute highly contagious viral respiratory disease of chickens characterized by tracheal rales; coughing and sneezing and associated with high mortality; so it is of economical importance (Broadfoot et.al., 1957 and King and David, 1991). Usually vaccination is considered the corner stone in the control of IB as other infectious diseases (Wakenell and Sharma, 1986 and Wakenell et.al., 1995).

On the other side, fowl cholera (FC) represents another respiratory disease of bacterial causing agent facing avian population in dramatic forms which may lead to 100% mortalities. It is caused by Pasteurella multocida and characterized by septicemia with high morbidity and mortality rates causing great economic losses (Birggs and Skeels, 1984 and Schlink and Olson, 1987).

Nowadays there is an increase attention toward the diseases of wild birds and their role in transmission of poultry infectious diseases and their immune response to some poultry vaccines. So, it is a golden goal to investigate the immune response of Guinea fowls to ND; IB and FC vaccines and their ability to infection with the virulent strains.

MATERIAL AND METHODS

1-Fowl cholera vaccine:

Inactivated fowl cholera (FC) vaccine was supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. It was used for vaccination of experimental Guinea fowls at the dose of 0.5ml/bird inoculated S/C and a 2nd dose was administrated 2 weeks later.

2-Virulent fowl cholera strain:

A local strain of virulent Pasteurella multocida serotype A:1 was supplied kindly by the Central laboratory for Evaluation of Veterinary biologics; Abassia; Cairo. It was used for challenge of vaccinated birds at the dose of 0.1ml of 1/10 dilution of 24 hours culture of the organism.

3-Bivalent Newcastle (ND) and infectious bronchitis vaccine (IB):

An inactivated bivalent ND and IB vaccine was supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. It was used for

vaccination of experimental fowls at the dose of 0.3ml/bird inoculated S/C and a 2nd dose was administrated 2 weeks later.

4-Virulent virus strains:

Local virulent strain of velogenic visrotropic ND virus of a titer of 10^8 EID_{50}/ml and a local isolate of virulent IB viruses of a titer of 10^5 EID_{50}/ml were kindly supplied by the Central Laboratory for Evaluation of Veterinary Biologics, Abbassia, Cairo. They were used for challenge of vaccinated birds.

5-Cell culture adapted viruses:

VERO cell culture adapted ND virus (Afaf et.al., 2000) of a titer 10^7 $TCID_{50}$/ml and IB virus (Elham et.al.,1996) of a titer of 10^8 $TCID_{50}$/ml were used in serum neutralization test.

6-African green monkey kidney cell culture (VERO):

VERO cell culture established by Yasumara and Kawatika (1963) supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo was used in serum neutralization test.

7-Guinea fowls:

60 Guinea fowls of about 3 months of age were obtained from Giza Zoo where they were screened using serum neutralization test and indirect haemagglutinaton test and found to be free from ND; IB and FC antibodies. These birds were divided into 2 major groups as follow:

*The first group consisting of 30 birds was vaccinated simultaneously with the bivalent ND and IB vaccine with FC vaccine receiving 2 doses with 2 weeks in between. Three weeks post the second vaccination, this group was subdivided into 3 subgroups (10 birds/subgroup) as follow:

-Subgroup-1 challenged with the virulent ND virus at the dose of 10^6 EID_{50} /bird inoculated intramuscular.

-Subgroup-2 challenged with the virulent IB virus at the dose of 10^6 EID_{50} /bird inoculated intramuscular.

-Subgroup-3 challenged with the virulent P.multocida type A:1 with a dose of 0.1ml of 1/10 dilution of a 24 hours culture of the virulent Pasteurella multocida type A: 1 inoculated I/M.

*The second group of 30 birds was kept unvaccinated and on the time of challenge of vaccinated fowls; this group was subdivided into 3 subgroups (10 birds/subgroup) managed as the first subgroups.

Each fowl group was kept separately under hygienic measures and serum samples were obtained from all groups on week intervals post vaccination.

8-Serum neutralization test (SNT):

It was carried out using the microtiter technique according to Villagays (1990) to estimate ND and IB neutralizing antibodies in vaccinated fowls. The antibody titer was calculated as the reciprocal of the final serum dilution which neutralized and inhibited the cytopathic effect (CPE) of 100-200 TCID50 of the used virus according to Singh et.al. (1967).

9- Haemagglutination test (HI) and indirect haemagglutination Test (IHA):

HI test was carried out to Majuiabe and Hitchner (1977) to estimate ND and IB antibodies while IHA test was carried out according to Carter and Rappy (1962) to estimate fowl cholera antibodies in vaccinated fowls.

RESULTS AND DISCUSSION

Guinea fowls represent beauty specie of zoo birds attracting the visitor attention and as they are one of the wild bird populations an attention was directed to study their immune response to some of the most important avian vaccines which used routinely in vaccination programs of chickens and for such purpose, the present work was designed and carried out on 45 Guinea fowls where they were vaccinated simultaneously with the locally produced bivalent inactivated Newcastle (ND) and infectious bronchitis (IB) viral vaccine and fowl cholera (FC) vaccine.

Clinical observation of vaccinated fowls did not revealed any signs of illness or post vaccinal reactions confirming the safety of the used vaccines. The experimental results of serum neutralization test (Table-1) showed that vaccinated Guinea fowls responded well for ND and IB vaccine where they exhibited good detectable neutralizing antibodies against both of ND and IB from the 1st week post vaccination recording their peaks by the 3rd week post boostering. The HI test (Table-2) revealed parallel findings to those of SNT demonstrating that the induced antibodies against ND and IB in vaccinated Guinea fowls; were of good protective levels as confirmed by the results of the challenge test (Table-4) which showed protection ratios of 90% and 100% for ND and IB respectively. Although there were no available data that discuss vaccination and immune response of Guinea fowls to the used vaccines, and depending on the fact that these birds are related to avian species; it could be concluded that the obtained results come in agreement with those obtained by Allan et.al. (1973); Majuiabe and Hitchner (1977); Madkour (1995); Hala (1996); Afaf et.al. (2000) and Ab0-Zaid et.al.(2001) who discussed the immune response of poultry to the inactivated ND vaccine either in single form or in combination with other viral or bacterial vaccines. Among the immune response of vaccinated fowls; similar results were recorded by Blore and Skeeles (1981); King and David (1991) Philip et.al. (1997) and Susan et.a. (2000).

The challenge of the experimental fowls showed that vaccinated birds were able to withstand the virulent ND virus while unvaccinated ones were unable showing the characteristic signs of ND as reported by Ballarini (1964); Sheble (1961) and Elham (1979). Also vaccinated fowls withstood the challenge with the virulent IB virus while unvaccinated birds showed respiratory signs including snacking and rales; coughing and sneezing and end with death as described by Sevoian and Levine (1957); King and David (1991) and Susan et.a. (2000).

Regarding the immune response of vaccinated Guinea fowls to fowl cholera vaccine, the results of HI test showed that they exhibited good levels of antibodies recorded beak titer by the 4th week post boostering without any antagonizing effect between the bacterial and viral vaccine immunogeneicity (Table-3). These findings appear to be confirmed by those obtained by Bieror and Derieux (1974); Kelly et.al. (1982); Madkour (1995); Khodier and Amina (1999); Zeinab (1999) and Zeinab et.al. (2004) when they vaccinated poultry against ND and FC singly and simultaneously. The challenge of Guinea fowls against the virulent strain of Pasteurella multocida (type A:1) showed a protection percentage of 80 in vaccinated birds while it was 0 in unvaccinated birds which showed signs of illness characterized by rough feather, depression, loss of appetite; nasal and ocular secretions and death as mentioned by Bhasin (1982); Abd El-Dayem (1990); Zeinab (1999) and Zeinab et.al. (2004).

From all the obtained results; it could be concluded that Guinea fowls must be vaccinated against both of ND; IB and FC where the unvaccinated birds showed typical symptoms of such diseases when they were subjected to the challenge with the virulent stains and died within 18-72 hours. In addition the present work confirms that the locally produced inactivated bivalent ND and IB and FC vaccines are safe and immunogenic vaccines for Guinea fowls.

REFERENCES

Abd El-Dayem, N.S. (1990): Pasteurella infection in Kalubia Province. M.Vet.Sci.Thesis (Microbiology) Fac.Vet.Med.Zagazig Univ.

Abo-Zaid, A.A.; Khodier, M.H.; Nadia, M. Hassan; Fekria El-Bourdini and Awad, M.H. (2001): Preliminary trials for preparation of a bivalent inactivated vaccine against Newcastle disease and egg drop syndrome. Beni-Suef Vet.Med.J.Vol.XI.,N0.2: 193-203.

Afaf, H.Abdel Hadi; Khodier, M.H. and Mervat, A. El-KOffy (2000): Trials for preparation of a combined vaccine against Newcastle and gumboro diseases. SCVMJ, III (2): 453-464.

Allan, W.H.; Lancaster, L.E. and Toth, B. (1973): The production and use of Newcastle disease vaccines.FAO publication (1973).

Ballarini, Q. (1964): Newcastle disease in Guinea fowls Novaret 40: 299-411

Bennejean, G.; Guitte, M.; Picatt, J.P. and Meurier, C. (1974): Prevention de la maladie de Newcastle chezla pintade.Avicul.de ploufrangon, 14: 29-38.

Bhasin, J.L. (1982): Serological types of Paseurella multocida isolates from turkeys in Canada.Cand.J.Microbiol.28 (4): 1078-1080.

Bieror, B.W. and Derieux, W.T. (1974): Exposing turkeys by various routs to aviruleny starins of Pasteurella multocida.Poult.Sci.53: 2290-2298.

Birggs, D. and Skeels, J.K. (1984): An enzyme linked immunosorbant assay for detecting antibodies to Pasteurella multocida in chickens. Avian Dis.,28 (1): 208-215.

Blore, P.J. and Skeeles, J.K. (1981): Use of a constant virus diluting serum microneutralization technique for the detection and quantification of infectious bronchitis virus antibodies.Avian Dis. 25 (4): 801-809.

Broadfoot, D.L.; Pomeroy, B.S. and Smith, W.H. (1957): Effects of nfectious bronchitis on egg production.J.Am.Vet.Med.Ass.,124: 128-130.

Carter, G.R. and Rappy, D.E. (1962) Formalized erythrocytes in the haemagglutination test for typing of Pasteurella multocida.Brit.Vet.J.118: 289-292.

Elham, A. El-Ebiary (1979): The role of free flying birds in transmission of Newcastle disease virus.M.Vet.Sci.Thesis (Microbiology), Fac.Vet.Med.Cairo Univ.

Elham, A. El-Ebiary; Susan, S.El-Mahdy and Khodier, M.H. (1996) Studies on cell cultures as a method used for adaptation of infectious bronchitis virus.Egypt.J.Med.Microbiol., 5 (2): 205-209.

Hala, M.E.El-Makaky (1996): Trials for preparation of bivalent inactivated Newcastle and infectious bursal disease vaccine.M.Vet.Sci.Thesis (Poultry Diseases), Fac.Vet.Med., Cairo Univ.

Kelly, B.J.; Dungan, W.M. and Yar Ghazihamian, G. (1982): Immunization of turkey breeder hens against fowl cholera with combined oral and web dministration of attenuated CU Pasteurella multocida. Avian Dis.27 (1): 133-140.

Khodeir, M.H. and Amina, A. El-Bayomy (1999): Preparation and evaluation of a combined vaccine against fowl cholera and egg drrop syndrome. Egypt.Vet.Med.Ass., 59 (2&3): 444-470.

King, D.J. and David, M.C. (1991): Infectious bronchitis.Dis.Poult.9th Ed.: 471-484.

Levine, P.P. (1962): The place of vaccination in the control of poultry diseases.Vet.Rec. 74: 1394-1398.

Madkour, M.S. (1995): Some trials for preparation of a bivalent inactivated owl cholera and Newcastle disease vaccine. Ph.D.Thesis (Microbiology) Fac.Vet.Med., Cairo Univ.

Majuiabe, K.A. and Hitchner, S.B. (1977) Antibody response to strain combination of Newcastle Disease virus as measured by haemagglutination inhibition test.Avian Dis.,21: 576-584.

Philip, H.C.; Wakenell, P.S. and Farver, T.B. (1997): Pathogenesis of attenuated infectious bronchitis viruses for oviducts of chickens exposed in ovo.Avian.Dis.41: 598-603.

Schlink, G.T. and Olson, L.D. (1987): Fowl cholera vaccination of growing turkeys with CU strain via routes other than oral.Avian Dis.,31(1): 22-28.

Sevoian, M. and Levine, P.P. (1957): Effect of infectious bronchitis on the reproductive tract; egg production and egg quality of laying chickens.Avian Dis.,1: 136-164.

Sheble, A. (1961): Newcastle disease in Pea fowls (Pavo cristatus): 2nd An.Vet.Cong.Cairo : 187-190.

Singh,K.V.; Osman,O.A.; Ivon,F.E. and Thanaa,I.(1967): Colostral transfer of rinderpest neutralizing antibody to offspring of vaccinated dams. J.Comp.Med.Vet.Sci.,31:295-298

Susan, S.El-Mahdy; Khodier, M.H. and Elham, A. El-Ebiary (2000): Comparative evaluation of egg and tissue culture attenuated infectious bronchitis vaccines. Vet.Med.J.Giza, Vol.48 (4): 609-616.

Tharwat, M. (1997): Birds known to occur in Egypt.Publication of National Bio-diversity Unit No.8.

Villagays, P. (1990): Laboratory manual of Avian viral diseases. College of Vet. Med. Athens, Georgia,

Wakenell, P.S. and Sharma, J.M. (1986): Chicken embryo vaccination with avian infectious bronchitis virus.Am.J.Vet.Res.,47: 933-938.

Wakenell, P.S. ; Sharma, J.M. and Slocmbe, R.F. (1995): Embryo vaccination of chickens with infectious bronchitis virus: Histological and ultra-structure lesion response and immunological response to vaccination.Avian Dis.,39: 752-765.

Yasumara, Y. and Kawatika, L.W. (1963): Studies on SV40 virus in tissue cultures.Nihon Rinsho, 21: 1201-1215.

Zeinab, M. Soror (1999): Evaluation of some living and inactivated vaccines in profection of turkeys against fowl cholera.Ph.D.Thesis (Bacteriology; Immunology and Mycology) Fac.Vet.Mrd.Cairo Univ.

Zeinab, M. Soror; Hana,M. Ibrahim and Khodier, M.H. (2004): Evaluation of the immune response of turkey to the mutual and simultaneous vaccination with the local fowl cholera and infectious bronchitis vaccines. Proc. The 1st Intern.Conf.Vet.Res.Div.NRC, Egypt: 231-237.

Table (1): Mean neutralizing ND and IB antibody titers in vaccinated Guinea Fowls

Estimated antibodies	Neutralizing antibody titer*/WPV**							
	0	1WPV	2WPV	1WPB***	2WPB	3WPB	4WPB	Control
ND	0	8	16	64	64	128	128	0
IB	0	4	8	32	64	64	64	0

*Antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100-200 $TCID_{50}$ of the used virus.
**WPV=week post vaccination ***WPB= Week post boostering

Table (2): ND and IB mean HI antibody titers in vaccinated Guinea Fowls

Estimated antibodies	Log2 of HI antibody titer/WPV*							
	0	1WPV	2WPV	1WPB***	2WPB	3WPB	4WPB	Control
ND	0	6.8	7.0	8.0	9.0	9.0	10.0	0
IB	0	6.6	6.4	7.6	8.0	9.0	8.6	0

*WPV=week post vaccination **WPB= Week post boostering

Table (3): Fowl cholera mean IHA antibody titers in vaccinated Guinea Fowls

Bird group	Fowl cholera IHA antibody titer/WPV*							
	0	1WPV	2WPV	1WPB***	2WPB	3WPB	4WPB	5WPB
Vaccinated	>2	184	243	485	579	844	1040	1040
Control	0	2	4	8	2	11	4	4

*WPV=week post vaccination **WPB= Week post boostering

Table (4): Results of Challenge test of vaccinated Guinea fowls against the virulent ND; IB and FC strains

Bird groups	Challenge strain	Number of challenged fowls	Number of survived fowls	Protection percentage
Group-1:				
Subgroup-1	ND virus	10	9	90
Subgroup-2	IB virus	10	10	100
Subgroup-3	FC	10	8	80
Group-2:				
Sugroup-1	ND virus	10	0	0
Subgroup-2	IB virus	10	2	20
Subgroup-3	FC	10	0	0

الملخص العربى

محاولات لتحصين دجاج الوادى بلقاحات كوليرا الطيور والنيوكاسل والألتهاب الشعبى

حنان محمد إبراهيم*-إلهام عطا الإبيارى*- محمد حسن خضير**

*المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية

**معهد بحوث الأمصال واللقاحات البيطرية

====

صمم هذا العمل كخطوة نحو توجيه الأهتمام بالطيور البرية والتى تمثل جزءا ليس بالهين من الثروة القومية إضافة إلى ما قد تلعبه هذه الطيور من دور هام فى نقل الأمراض المعدية للطيور الأخرى خاصة الداجنة منها0 وعلى ذلك تم أختيار مجموعة من دجاج الوادى من حدائق الحيوان بالجيزة فى سن حوالى ثلاثة أشهر تم تحصين البعض منها تلازميا بلقاح النيوكاسل والالتهاب الشعبى الثنائى المثبط مع لقاح كوليرا الطيور المثبط وكلاها ينتج محليا مع ترك البعض الآخر دون تحصين كضوابط للتجربةهذا ولم تظهر أية أعراض مرضية أو رد فعل عكسى للتحصين على هذه الطيور وقد تم جمع عينات من أمصال هذه الطيور على فترات أسبوعية بعد التحصين (بجرعتين بفترة بينية أسبوعين) حيث أجرى كل من أختبار المصل المتعادل ومنع التلازن الدموى لقياس مستويات المناعة المتكونة وقد أظهرت الطيور المحصنة استجابة مناعية عالية لهذه اللقاحات تمكنت معها من مقاومة التحدى بالعترات الضارية لكل من النيوكاسل والالتهاب الشعبى و كوليرا الطيور فى حين أظهرت الطيور الغير محصنة أعراضا تشابه تماما الأعراض المرضية لهذه الأمراض الثلاثة ونسبة نفوق عالية الأمر الذى يدل على قابلية دجاج الوادى للإصابة بهذه الأمراض مما يستوجب معه ضرورة تحصينها باللقاحات المناسبة حفاظا عليها ومنعا لاحتمالية نقلها إلى الطيور الداجنة بطريقة أو بأخرى

==========