Vet. Med. J., Giza. Vol. 59, No. 3 (2011): 109 - 116

Preparation of monospecific antisera against fowl cholera, fowl coryza and Newcastle disease reference strains in laboratory animals

Ghada M. El-Sadek; Amal M. El-Sawah; Hanaa A. Mostafa; Hanan I. Mahmoud; El-Safty, M.M.; Elham A. El-Ebiary and Abdel Baky, M.H.

Central Laboratory for Evaluation of Veterinary biologics, Abbasia, Cairo

Received: 15/03/2011
Accepted: 08/05/2011

SUMMARY

Monospecific antisera against *Pasteurella multocida* strains that belonged to capsular and somatic serogroups A:1, A:3 and A:4, *Avibacterium paragallinarum* serovars A and C and Newcastle disease virus, were prepared in three separate rabbit groups for each using reference bacterial and viral cultures emulsified in Freund's adjuvant. Antisera of *P. multocida* were qualitatively and quantitatively evaluated by serological tests using their homologous and heterologous antigens (cross matching). Sera of immunized rabbits showed a reasonable titre of *P. multocida* capsular group (A) antibodies by passive haemagglutination test and to somatic serotype (A) 1, 3 and 4, antibodies by tube agglutination and agar gel diffusion tests. Serovars A and C antibodies of *Avibacterium paragallinarum* were detected in sera of the immunized rabbits by plate agglutination test. The main titre of 8.5 log2 haemagglutination inhibitory antibodies against Newcastle disease virus was achieved in sera of immunized rabbit which received 3 successive inoculation of ND LaSota strain. Conclusively, the attained results proved that the prepared mono-specific antisera could be used as a highly reliable guide in the serological identity tests of the challenge and vaccinal strains of fowl cholera, infectious coryza and Newcastle disease.

INTRODUCTION

Fowl cholera (FC) is a contagious bacterial disease affecting domestic and wild birds caused by *P. multocida* species. FC is usually appeared as septicaemic disease associated with high morbidity and mortality rates (Rimler *et al.,* 1997).

Serogrouping of *P. multocida* is determined by capsular and somatic antigens. Five capsular groups A, B, D, E and F are currently recognized by using passive haemagglutination test. Capsular groups A

and D are commonly isolated from fowl. Sixteen somatic serogroups have been determined as determined by tube agglutination test and agar gel precipitation test (Saif, 2008 and Dwight *et al.,* 2004).

The most important and pathogenic serogroups of *P. multocida* strains is a capsular serogroup A that combined with Heddleston somatic serogroups 1, 3 and 4 (Heddleston *et al.*, 1972).

Avibacterium paragallinarum is the causative bacteria of an acute respiratory disease affecting the poultry that known as infectious coryza (IC). Plate agglutination test using cultures of whole bacterial cells classified H. paragallinarum strains into 3 serovars (A, B and C). Bivalent inactivated vaccine of serovars A and C is commonly used for controlling such disease (Aly, 2000, Dwight *et al.,* 2004 and Saif, 2008,).

Newcastle disease (ND) is a highly contagious viral disease caused by avian paramyxovirus type (1) of the genus Avulvavirus belonging to the family Paramyxoviridae. Cross-reaction in haemagglutination inhibition test between ND virus and some of other avian Paramyxoviruses (types 2 to 9) specially of types 3 and 7 may cause some problems that can be resolved by the use of suitable antigen and antiserum control (Tumova *et al.,* 1979 and Alexander, 1991).

The present trial was done for preparation and evaluation of monospecific antisera against a variety of reference strains of *P. multocida*, H. paragallinarum and ND virus out in order to save the necessary laboratory needs for requirement of such antisera in application of the vaccine quality control tests.

MATERIALS AND METHODS

1. Experimental rabbits

Twenty adult Boskat rabbits of approximately 2.5 Kg body weight were used for preparation of monospecific antisera against reference strains of *Pasteurella multocida, Avibacterium paragallinarum* and Newcastle disease (3 rabbits/strain). Group of rabbits was kept as a negative control.

2. Bacterial and viral strains

* Reference strains A:1, A:3, A:4 of *Pasteurella multocida* representing capsular group A accompanied with somatic groups 1, 3 and 4.
* Reference strains of *avibacterium paragallinarum* serovars A and C.
* Newcastle disease virus, LaSota strain.
* Inoculums and antigens were prepared from these reference strains and used in immunization of rabbit as well as conduction of the corresponding serological tests.
* All strains were kindly obtained from Reference Bank of Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo.

3. Antigens

Soluble capsular antigens prepared from *P. multocida* strains (Carter, 1955), soluble somatic antigens of *P. multocida* strains (Heddleston *et al.,* 1972) and agglutination antigens of *H. paragallinarum* strains were prepared (Sawata *et al.,* 1978).

Allantoic fluid harvested of specific pathogen free-embryonated chicken eggs (SPF-ECE) inoculated with ND virus was titrated by haemagglutination test and used as antigen in application of haemagglutination inhibition test (OIE, 2008).

4. Immunization of rabbits

Nine rabbits were divided into 3 groups and were immunized according to the following table

Rabbit group	Type and route of inoculation	
	Type of inoculation	Dose and route of inoculation
1st group	Rabbits were immunized with an initial and booster doses of formalized suspension of *P. multocida* emulsified in equal volume of Freund's adjuvant 2 weeks apart **(Borkowsk et al., 1995)**	Rabbits were inoculated with 1ml I/M route of inoculation
2nd group	Rabbits were inoculated with a first and a booster doses of a suspension of H. paragallinarum emulsified in an equal volume of Freund's adjuvant with 2 weeks apart **(Nasser, 2000)**	
3rd group	Rabbits were inoculated with three successive doses of 1ml of LaSota strain of ND virus fluid of titres $\geq 8.5 \log_{10}$ EID_{50} emulsified in equal volume of Freund's adjuvant 2 weeks apart	

Sera were collected 2 weeks after last dose of inoculation of all groups (3 rabbits/group/strain) and kept in refrigerator.

Complete Freund's adjuvant was mixed with the 1st dose and the incomplete Freund's adjuvant was incorporated with the booster dose.

5. Serological tests:

Sera of immunized rabbit were tested as follows:

1. *P. multocida* capsular group (A) antibodies by passive haemagglutination test using capsular antigens (Carter, 1955) and somatic groups 1, 2 and 4 antibodies by tube agglutination test and agar precipitation test using soluble somatic antigens (Hjeddleston *et al.,* 1972 and Namioka and Murata, 1961).
2. H. paragallinarum serovars A and C antibodies were tested by plate agglutination test using agglutination antigens (Carter, 1955).

3. Newcastle disease virus antibodies tested by haemagglutination inhibition (HI) test (OIE, 2008).

RESULTS

Pasteurella multocida capsular group A antibodies

Sera of immunized rabbits showed a high titre of *P. multocida* capsular group (A) antibodies as determined by passive haemagglutination test. Its mean titres ranged between 160 and 320, and between 80 and 240 with homologous and heterologous antigens, respectively (Table 1). The difference was no more than two folds log_2.

Pasteurella multocida, somatic groups 1, 2 and 3 antibodies

As shown in Table 2, tube agglutination test was capable to differentiate quantitatively and qualitatively the antibodies against the used somatic groups in sera of immunized rabbits. The obtained mean titres ranged between 120 and 160. There were cross reactions between some serum samples and their heterologous antigen only at dilution of 1:10. Sera of immunized rabbits reacted positively only with their homologous antigens by agar gel precipitation test.

Avibacterium paragallinarum, serovars A and C

Sera of immunized rabbits reacted positively only with their homologous antigens by plate agglutination test (Table 3).

Newcastle disease virus antibodies

The mean geometric titre of HI-antibodies in sera of rabbits immunized by ND virus was 8.5 log_2.

Table 1: *P. multocida*, capsular group A antibody titers in sera of rabbits immunized and tested by passive haemagglutination test

Antisera type	Antigen type		
	A:1	A:3	A:4
A:1	320 *	160	240
A:3	80	160	80
A:4	80	120	240
Non-immunized control	-ve	-ve	-ve

* Mean titre = Reciprocal of serum dilution

Table 2: *P. multocida*, somatic groups 1, 3 and 4 antibodies in sera of rabbits immunized using tube agglutination and agar gel precipitation tests

Antisera	Antigens					
	A:1		A:3		A:4	
	TAT	AGPT	TAT	AGPT	TAT	AGPT
A:1	160*	+	10	-	10	-
A:3	< 10	-	120	+	10	-
A:4	< 10	-	< 10	-	160	+
Non-immunized control	-ve	-	-ve	-	-ve	-

* Mean titre = Reciprocal of serum dilution
AGPT: Agar Gel Precipitation Test
TAT: Tube Agglutination Test

Table 3: *H. paragallinarum*, serovars A and C antibodies in sera of immunized rabbits with *A. paragallinarum* strains of serovars A and C using plate agglutination test

Antisera type	Antigen type	
	Serovar A	Serovar C
Serovar A	+ve	-ve
Serovar C	-ve	+ve
Non-immunized	-ve	-ve

+ve Positive result
-ve Negative result

DISUSSIOIN

Avian pathogens *Pasteurella multocida* capsular and somatic serogroups A:1, A:3 and A:4, *Avibacterium paragallinarum* serovars A, C and Newcastle diseases virus are the causative agent of the important acute respiratory diseases causing significant economic impact on poultry industry. Reference or standard monospecific antisera against these pathogens are necessary to accomplish the identity of challenge strains which currently used to check the potency of such commercial vaccines against fowl cholera and infectious coryza as well as detecting identity of the Newcastle disease virus. So, the present trial for preparation of monospecific antisera against the previously mentioned pathogens in rabbits was taken to justify the method of preparation and evaluation of these antisera.

The obtained results proved that two successive inoculations of *P. multocida* and *A. paragallinarum* were sufficient to induce considerable high titres of capsular and somatic antibodies in immunized rabbits. Similar observations were previously reported (Davison *et al.,* 2008 and Saif, 2008). Antibodies against such diseases in sera of control non-immunized rabbits did not detected completely.

Passive haemagglutination test was able to differentiate between different capsular groups of *P. multocida* species A (Rimler and Rhoades, 1987). The difference between the antibody titres given using homologous and heterologous antigens of *P. multocida* strains in this study through the passive haemagglutination test was not more than two fold by passive haemagglutination test.

The obtained high titres of *P. multocida* capsular group A antibodies in sera of tested rabbits were expected because rabbit are naturally and highly susceptible host to the virulent avian strains of fowl cholera (Dwight *et al.,* 2004). Rabbits are immunologically responsive to formalin inactivated *P. multocida* strains of fowl cholera (Heddleston and Watko, 1963 and Davison *et al.,* 2008).

Tube agglutination and agar gel precipitation tests are quantitative and qualitatively *P. multocida* somatic serotyping specific test. Some sera of rabbit were only react positive at serum dilution of 1:10 with heterologous antigens by tube agglutination

test. These results could explain the incomplete specificity of the prepared antigens compared to the high titer (1:160) that recorded with homologous specific antigen (Dwight *et al.*, 2004).

A. paragallinarum testing by plate agglutination test was different between antisera of *A. paragallinarum* serovars A and C as there was no cross reaction between the two strains (Davison *et al.,* 2008).

Newcastle disease virus antibodies in sera of rabbit which had received 3 inoculum of the virus at 2 weeks intervals confirmed that this method was sufficient to induce a sufficient high NDV haemagglutinin as the mean haemagglutinating antibody titre reached 8.5 $\log_2$ by HI test. These results are in close agreement with that recorded previously by Nasser (2000).

In conclusion, the present study outcome results has proved that the prepared mono-specific antisera could be used as a highly reliable guide in the serological identity tests of the challenged and vaccinal strains of fowl cholera, infectious coryza and Newcastle disease.

REFERANCES

Alexander, D.J.; Manvell, R.J.; Collins, M.S. and Brochman, S.J. (1991): Evaluation of relationship between avian paramyxoviruses isolated from birds of Family Columbidae. Arch. Virol., 116: 267-276.

Aly, M. (2000): Characteristics and pathogenicity of *Avibacterium paragallinarum* isolates from Upper Egypt. Assiut Vet. Med. J., 43 (85): 319-338.

Borkowsk-Opacka, B.; Rutkowska, J.I. and Truzezynski, M. (1995): An attempt to evaluate the efficacy of vaccines against Pasteurellosis in rabbits. Bull. Vet. Ins. Pulway, 39: 9-13.

Carter, G.R. (1955): Studies on *Pasteurella multocida.* Identification of serological types. Am. J. Vet. Res., 16: 481-484.

Davison, F; Kaspers, B. and Schat, K.A. (2008): Avian Immunology, Vol. (11), 10th ed.

Dwight, C.H.; Mclachlan, N.J. and Walker, R.L. (2004): Veterinary Microbiology.

Heddleston, K.L. and Watko, L.P. (1963): Fowl cholera: Susceptibility of various animals and their potential as disseminators of disease. Proc. 67th Ann. Meet. US Livest. Sanit. Assoc., 247-251.

Heddleston, K.L.; Gallagher, J.E. and Rebers, P.A. (1972): Fowl cholera diffusion precipitation test for serotyping of *Pasteurella multocida*. Avian Dis., 16: 925-936.

Namioka, S. and Murata, M. (1961): Serological studies on *Pasteurella multocida.* II. Characteristics of somatic O antigen of the organism. Cornell Vet., 51: 507-521.

Nasser A.Y. Sherif (2000): Laboratory studies on the effect of some extraneous contaminants present in imported and locally produced poultry vaccines. Ph.D. Thesis, Clinical Pathology, Cairo Univ.

OIE (2008): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Sixth Edition, Volume 1.

Rimler, R.B. and Glisson, J.R. (1997): Fowl cholera. In: Diseases of Poultry, Tenth Edition, Calnek, B.W., Barnes, H.J., Beard, C.W., McDougald, L.R. and Saif, Y.M., eds. Iowa State University Press, Ames, Iowa.

Rimler, R.B. and Rhoades, K.R. (1987): Serogroup F, a new capsule serogroup of

Pasteurella multocida. J. Clin. Microbial., 25: 615-618.

Saif, M. (2008): Diseases of Poultry. 12th Ed., Chapter 19, Chapter 20.

Sawata, A.; Kume, K. and Nakase, Y. (1978): Avibacterium infection in chickens. 2. Types of *Avibacterium paragallinarum* isolates from chicken with infectious coryza in relation to Avibacterium gallinarum strain No. 221. Jpn J. Vet. Sci., 40: 645-652.

Tumova, Stumysa, A.; Jonout, V. and Chamela, J. (1979): A further member of Yocaipa group isolated from the common wren. Acta Virol., 23: 504.

تحضير أمصال مناعية لعترات كوليرا الطيور وزكام الطيور ومرض النيوكاسل فى حيوانات التجارب

غـادة محمد صـادق، أمـل مـصطفى السواح، هناء عبد العـزيز، حنان إبراهيم محمود، منير محمد الصفتى، الهام عطا الإبيارى، منصور هاشم عبد الباقى

المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية – العباسية – القاهرة

تم تحضير أمصال مناعية أحادية لعترات الباستريلا مالتوسيدا عترات أ١، أ٣، أ٤ وعترات الكوريزا أ، د وكذلك عترة النيوكاسل وذلك باستخدام العترات القياسية فى أرانب البوسكات.

تـم قياس مستوى الأجسـام المناعيـة بالاختبـارات النوعيـة والكميـة باسـتخدام الاختبـارات السيرولوجية المتخصصة بكل ميكروب على حدة. حيث تم استخدام اختبار تلازن الدم الغير مباشر واختبار الأجار جل لتحديد مدى التوافق والاختلاف بين الاجسام المناعية بعضها البعض لعترات الباستريلا مالتوسيدا أنواع أ١، أ٣، أ٤. تم استخدام اختبار انابيب التلازن لعترات الكوريزا أ، د لمثل هذا الغرض ايضاً. وقد تم كذلك قياس مستوى الاجسام المناعية للنيوكاسل باستخدام تلازن الدم مباشر.

وقد أثبتت نتائج هذا البحث إمكانية الأعتماد على تلك الأمصـال المتخصصـة المذكورة والمحضرة معملياً فى الأرانب للاستخدام فـى اختبـارات تقييم الكفـاءة المناعيـة للقاحات الخاصـة بتلك الأمـراض (كوليرا الطيور، كوريزا الطيور، النيوكاسل).