Preparation And Characterization Of Canine Parvovirus Egg Yolk (IgY) Conjugated With FITC And Horse Radish Peroxidase (HRP)

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ABSTRACT

The present work could be considered a successful trail to prepare chicken egg yolk IgY against canine parvovirus (CPV) conjugated with horse radish peroxidase (HRP) and fluorescen isothiocyanate (FITC) as local products for diagnostic purposes of CPV infection. Indirect ELISA showed that the induced CPV antibodies in the sera and egg yolk of immunized hens were of high levels with OD (optical density) of 1.153 and 1.144 respectively. Direct ELISA revealed that the prepared CPV-IgY conjugated with HRP showed positive results up to a dilution of $1:10^5$ and the FITC conjugate showed strong positive apple green reaction up to a dilution of $1:10^4$ and less strong positive results up to $1:10^6$. So the obtained preparations are suitable of good quality and high specificity to detect CPV antigen.

INTRODUCTION

Dogs seem to be special animal species characterized by many favorable characters as intelligence and faithful making a strong relationship with peoples especially children. However, dogs represent a public health hazard where they act as a source of some zoonotic diseases especially those of viral nature as rabies, canine distemper and canine parvo *(1)*.

Canine parvovirus (CPV) is an autonomous member of the feline parvovirus subgroup and the smallest animal parvovirus with a single stranded DNA *(2)*. This virus causes a dangerous disease characterized by bloody diarrhea, pyrexia, vomiting, and emaciation and usually ends with death *(3,4)*.

After being detected in dogs in 1978, CPV was found to be globally distributed; mainly because the virus can survive in harsh environmental conditions for a long time and now endemic in populations of domestic and wild canines. Young puppies are highly susceptible to CPV infection, particularly because the natural immunity provided by maternal antibodies in the colostrums may wear off before the puppies own immune systems become mature enough to fight off infection *(5)*.

Regarding Egypt, CPV infection has been first reported to occur in police dogs as indicated from clinical and histopathological findings *(6)* and seroprevalnce *(7)*.

The most effective means for preventing CPV infection, is the vaccination either by live attenuated or inactivated vaccines *(8,9)*.

Usually there is a demand for local diagnostic kits which safe time and cost and help to reach accurate and rapid diagnosis especially of viral dangerous diseases. Nowadays, there is an increasing interest directed toward the use of chickens for production of antibodies in the egg yolk. This approach is relevant not only for production of antibodies to be used therapeutically in passive immunization programs but also to be used in immunochemical assays *(10,11)*. Yolk immunoglobulin G (IgG) is referred as IgY.

The present work was designed to prepare anti canine parvovirus egg yolk immunoglobulin (IgY) conjugated with horse radish peroxidase (HRP) and fluorescein isothiocyanate (FITC) to be used in ELISA and FAT as local products for detection of CPV antigens saving time and cost of imported kits.

MATERIAL AND METHODS

1.Canine Parvovirus vaccine

Live attenuated CPV vaccine was supplied by the Department of Pet Animal Vaccine Research, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo. It was used for immunization of experiment chickens and preparation of the viral antigen.

2.CPV antigen

CPV antigen was prepared from infected Vero cell culture *(12)* to be used for the evaluation of the prepared antiserum conjugated with horse radish peroxidase using ELISA.

3.Cell culture

Vero cell culture was supplied by the same department and used for preparation of CPV antigen.

4.Hens

Ten laying hens of about 14 weeks old were immunized with CPV vaccine using incomplete and complete Freund's adjuvant *(13)*. In addition to another three hens were kept without immunization as control.

5.Immunization of hens

Ten hens were immunized through the intramuscular injection in three sites of the breast muscles using 5 doses on week in travels . The injected dose; for each hen; was 0.5ml of CPV emulsified in complete Freund's adjuvant containing $10^{6.5}TCID_{50}$ of the virus/ml. 6 weeks later a booster dose was administrated using CPV antigen emulsified in incomplete Freund's adjuvant for another 6 weeks. Serum samples and egg from all hens were obtained at different intervals to estimate the induced serum and egg yolk CPV antibodies using indirect ELISA. Laid eggs of immunized hens were collected on 2 to 6 weeks intervals post the 2nd immunization and the yolks were separated and pooled.

6.Precipitation of anti CPV chicken egg yolk immunoglobulin (CPV-IgY)

It was carried out using ammonium sulphate *(14)*. The globulin content was estimated **(15)** and adjusted to be 20 µg/ml using 0.01M NaCO3 buffer.

7.Chemicals and reagents used for conjugation of the prepared CPV-IGY with horse radish peroxidase (HRP)

A) HRP product no. p-8375 type VI, lot 25C-9510 was supplied by Sigma Chemical Company. It had an activity of 365 purogallin units/mg.

B- Sodium borohydride ($NaBH_4$) was supplied by S.D. fine chemical LTD Company; Chemical Manufacturing Division Fair, Lawn, New Jersey. It had a molecular weight of 105.99.

C-Sodium periodate ($NaIO_4$).

8.Conjugation of the prepared CPV-IGY with HRP

It was carried out following up the method previously described *(16)*.

9. Direct and indirect ELISA

Indirect ELISA was carried out to estimate the CPV antibodies in the prepared immune egg yolk while direct ELISA was carried out for titration of the prepared CPV-IgY conjugated with HRP. The tow assays were applied according to *(17)*.

10.Chemicals used for conjugation of CPV-IgY with Fluorescein isothiocyanate ($C_4 H_{11} NO_5 S$) E

Was supplied by Merck, Darmstadt for Microscopy (M.Gew.389.39).

11.Conjugation of the prepared CPV-IgY with Fluorescein isothiocyanate:

It was done according to the method of described previously *(18)*.

12.Direct fluorescent antibody technique (FAT)

Direct FAT was carried out on CPV antigen to evaluate the prepared CPV-IgY conjugated with fluorescein isothiocyanate technique was carried out as cited previously *(19)*.

RESULTS AND DISCUSSION

The present study was conduct to prepare a specific chicken egg yolk immunoglobulin (IgY) against CPV conjugated with HRP and FITC as local kits available on request for diagnosis of CPV infection, of low cost and to save time to obtain similar imported products.

Chicken egg yolk is considered as an excellent source of inexpensive and highly specific immunoglobulin with a main advantage lies in the convenient acquisition of abundant amount without vene-puncture. The volume of one egg yolk is about 15ml equaled to those obtained from a rabbit with three months *(20)*.

The present prepared CPV-IgY showed detectable antibody titer by the 2nd week post the first immunization recording a peak titer by the 6th week post the 2nd immunization parallel to those estimated in the serum of immunized hens on the same periods Table 1. These results appear to be confirmed the study which showed that the IgG concentration in the yolk is the same as in the serum of immunized hens *(21)*.

Table 2 revealed that the optimal dilution of the HRP conjugated IgY is $1:10^5$ in order to evaluate the prepared egg yolk anti CPV-IgY using the indirect ELISA. In this respect, Table 3 showed that the obtained results agree with several studies *(22,23)*

Concerning the FITC conjugated yolk anti CPV-IgY, Table 4 showed strong positive results (Photo-1) up to a dilution of 10^4 with less positive reactions (Photo-2) up to a dilution of 10^6 . These results supported the previous study which demonstrated that FA test gave rapid and accurate results by using FITC conjugated IgY prepared against different viral anti gens *(24)*.

From the obtained results, it could be concluded that the prepared chicken egg yolk anti CPV-IgY conjugated with HRP and FITC is good local preparations provide specific diagnostic kits for CPV infection.

Table 1. Titer of CPV antibodies in vaccinated hen's serum and egg yolk using indirect ELISA.

Hen groups	CPV-ELISA antibody titer/ WPI*									
	1st I						2nd I			
	2WPI		4WPI		6WPI		4WPI		6WPI	
	S#	Y##	S	Y	S	Y	S	Y	S	Y
Immunized	0.751	0.748	0.912	0.895	1.035	1.029	1.092	1.089	1.153	1.147
Control	0.27	0.27	0.25	0.24	0.27	0.26	0.25	0.26	0.28	0.27

WPI= week post immunization **1st I= first immunization
2nd I= second immunization#S= serum ##Y= yolk

Table 2. Titer of the prepared anti CPV-egg yolk immunoglobulin (IgY) conjugated with HRP using OPD substrate.

Ten fold dilution of the conjugate	Optical Density (OD)		Two fold dilution of the conjugate	Optical Density (OD)	
	Positive results	Negative results		Positive results	Negative results
$1:10^2$	1.057	0.151	1:200	1.051	0.149
$1:10^3$	1.035	0.144	1:400	1.047	0.147
$1:10^4$	0.978	0.134	1:800	1.039	0.146
$1:10^5$	0.833	0.131	1:1600	1.030	0.142
$1:10^6$	0.752	0.111	1:3200	1.025	0.138
$1:10^7$	0.695	0.110	1:6400	1.018	0.133

NB. The ELISA reading was conducted at a wave length of 495nm representing the single OD.

Table 3. Detection of CPV antigen by direct ELISA using the prepared CPV-IgY conjugated with HRP

Ten fold dilution of the conjugate	OD of direct ELISA	Two fold dilution of the conjugate	OD of direct ELISA
1:10	1.034	1:2	1.057
1: 10^2	0.982	1:4	1.052
1: 10^3	0.915	1:8	1.046
1: 10^4	0.813	1:16	1.031
1: 10^5	0.665	1:32	1.027
1: 10^6	0.581	1:64	1.027
1: 10^7	0.423	1:128	0.977
1:10^8	0.372	1:256	0.953
Negative control	0.147	Negative control	0.152
Blank	0.006	Blank	0.007

NB. -The prepared conjugate was diluted to 1:104.
-The ELISA reading was conducted at a wave length of 495nm representing the single OD.

Table 4. Titer of the prepared CPV-IgY conjugated with FITC.

Tested dilution	1:10	1:10^2	1:10^3	1:10^4	1:10^5	1:10^6
Result	4+	4+	3+	3+	2+	+

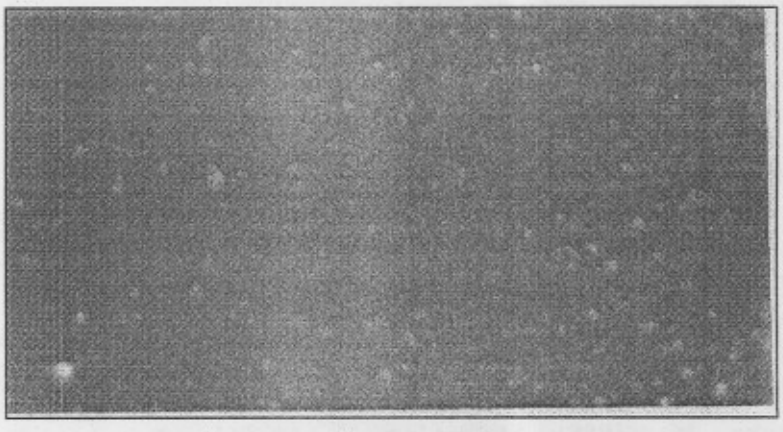

Photo 1. Strong positive (4+) FAT reaction.

Photo 2. Moderate positive (3+-2+) FAT reaction.

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الملخص العربى

تحضيروتقيم جلوبيولين بارفو الكلاب المناعى (IgY) فى مح البيض المحمل بالفلورسين أيزوثيوسينات والبيروكسيديز.

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* المعمل المركزى للرقابه على المستحضرات البيطريه الحيويه

**معهد بحوث الأمصال واللقاحات البيطريه بالعباسيه

تم خلال العمل الحالى تحضير جلوبيولين مناعى مضاد لفيروس بارفو الكلاب فى مح بيض الدجاج حيث تم إقران جزء منه بإنزيم الهورس راديش والآخر بمادة الفلوريسين أيزوثيوسينات. أوضحت نتائج أختبار الأليزا الغير مباشر أن معايير الأجسام المناعية المضادة للفيروس فى أمصال ومح بيض الدجاج المحصن هى ١٥٢ر١، ١٤٧ر١ دالة كثافة ضوئية على التوالى فى الأسبوع السادس بعد التحفيز المناعى الثانى. كذلك أعطى المقترن بإنزيم الهورس راديش نتائج إيجابية حتى تخفيف ١: ١٠٠٠٠٠ بينما أعطى المقترن بمادة الفلوريسين أيزوثيوسينات نتائج إيجابية قوية حتى تخفيف ١: ١٠٠٠٠ ونتائج أقل قوة حتى تخفيف ١: ١٠٠٠٠٠

مما سبق يمكن القول بأن المستحضرين المتحصل عليهما ذا كفاءة وحساسية عالية تكفى للكشف عن وجود فيروس البارفو وبذلك يكون قد توفر منتج محلي يوفر الوقت والمال بدلا من مثيله المستورد.