Preparation Of Anti-Canine And Antifeline Sera Conjugated With Fluorescin Isothiocyanate And Peroxidase As Antispecies For Diagnosis Of Dog And Cat Diseases

***Attyat M Kotb , *Naglaa I Ali, *Zeinab T Salem , *Hemmat S El Emam and **Magda M Sayed**

*Department of Pet animal Vaccine Research
Veterinary Serum and Vaccine Research Institute, Abbassia
**The Central laboratory Quality Control of Veterinary Biologics

ABSTRACT

The present work includes preparation of anticanine and antifeline sera conjugated with Fluorescin Isothiocyanate (FITC) and Horse Radish Peroxidase (HRP) as specific local kits. Such sera were prepared in Boscat rabbits where the immunoglobulione was adjusted to be 18 mg/ml, then conjugated with FITC and HRP. The prepared kits were evaluated through the application of indirect Fluorescent Antibody Technique (FAT) and indirect ELISA on sera of dogs and cats delivered to the Department of Pet Animal Vaccine Research. The results of the two techniques revealed that anti-canine conjugate induced positive results up to a dilution of 1: 10^5 while antifeline conjugates induced positive results up to dilution of 1: 10^4 for both FITC and HRP. The results of FAT and ELISA using the prepared kits were in agreement with the results of Serum Neutralization Test (SNT) carried out on dog and cat sera for detection of rabies, canine distemper, canine parvo and feline pan-leukopenia antibodies. So, the prepared kits could be considered good specific local anti-canine and anti-feline kits, available on request saving time and cost.

INTRODUCTION

Nowadays, flurochromes and enzymes are widely used in the conjugation process to obtain an effective degree of labeled antibodies *(2)*.

With the world rapid changes towards General Agreement on Tarssif and Trade (GATT) and intellectual property rights: developing countries; like Egypt; must have their own tools and skills for rapid and accurate diagnosis of dangerous viral diseases which could distruct their national wealth like rabies..

The immunofluorescence technique has the ability to simultaneous evaluation of two or more antigens depending on the availability of the conjugation particularly if the antigens are situated very close to each other even on the same cell and the ability of antibodies to be reached individually *(2)*.

Also labeled reagent assays have come to play a major role in the diagnostic laboratory purposes. The enzyme linked immunosorbent assay (ELISA) always served as rapid turn around time and possibility lower costs for both detection of the virus and antibody *(3)*.

Recently, all countries applied restriction rules about the entrance of pet animals, recommended that such animals should have the protective levels of immunity against infectious zoonotic diseases, like rabies.

There are four viral infectious disease (Rabies, Canine distemper, canine parvo and feline panleukopenia) which could be considered the most important infections causing great losses among dog and cat populations in addition to their public health Hazard *(4,5)*.

Rabies is an acute viral (encephalomyelitis caused by etiological agent in the family Rhabdoviridae, genus lyssa virus *(6)*. It's mainly transmitted by biting of a rabid host to a healthy one and usually end dramatically *(7,8)*. The highest incidence of dog and cat rabies occurs in area where wild life is epidemic and no measures help to reduce it. The development of efficient and wide spread vaccination of domestic dogs could reduce animal and human rabies *(9)*.

Canine distemper is an infectious disease of canines especially puppies of generalized

infection *(10)*. It is caused by a virus of the family paramyxovirdae, genus morbillivirus. It has one serotype only but it could be present in different degree of virulence. Within canine distemper infection, specific IGM, IGA and IGG antibodies can be detected *(11)*.

Canine parvovirus (CPV) is a highly contagious disease, frequently fatal enteric disease that cause sudden death due to myocarditis in dogs of eight weeks age or older. The disease is characterized by sudden vomiting, haemorrhagic diarrhea, Leukopenia and death within 24-72 hours *(12)*.

Feline panleukopenia (FPL) is a highly contagious acute viral infection of cats and all members of felidae family. It is characterized by high fever, anorexia, vomiting and Leukopenia. Other frequent clinical signs could be observed as diarrhea leading to death within a few days *(9)*.

Dog and cat serum samples are arrived to the department of Pet Animal Vaccine Research, Vet. Ser. Vac. Res. Inst. In order to determine their immune status to such viral diseases.

So the main goal of the present work is to prepare anticanine and antifeline sera conjugated with flourescin isothiocynate and horse radish peroxidase as antispecies for detection of antibodies against viral diseases of dogs and cats, as local products saving time and high cost for importation of a native ones.

MATERIAL AND METHODS

1. Animals

1.1 Rabbits

Fifteen Boscat rabbits each of about 3.5 kg body weight were divided into three equal groups, group 1 was used for the preparation of anticanine serum and group 2 was used for preparation of antifeline serum while the last group was kept without inoculation as test control. All rabbits were kept under hygienic measures receiving balanced diet and adequate water.

1.2 Dogs and cats

Local breed 5 puppies and 5 Kitten of three months of age were screened using S.N.T. and found to be free from antibodies against Rabies, Canine distemper. Canine parvo and Feline panleukopenia. These animals were used to obtain canine and feline sera and blood and to prepare the antispecies sera in rabbits.

2. Serum Samples

One hundred dog serum samples and 75 cat serum samples were delivered to the Department of Pet Animal Vaccine Research by the animal owners to determine their immune status. These sera were subjected to the indirect FAT and indirect ELISA using the prepared conjugates.

3. Preparation of anticanine and antifeline sera

Normal dog and cat sera were obtained from the screened animals. Each rabbit was inoculated S/C with 2 ml of either dog or cat serum 5 times with a week intervals. Then, 2 ml of the whole blood were inoculated on the 6^{th} week. After another week, inoculated rabbits were slaughtered and their blood was collected and the sera were separated aseptically *(13)*.

4. Precipitation of the immune globulines

It was carried out by using a saturated ammonium sulphate solution *(14)*.

5. Conjugation of the obtained immune globulines

Conjugation of the prepared antisera with FITC *(15)* and horse radish peroxidase *(3)* was carried out.

6. Viruses

Evelyn Rokitniki Abelseth, (ERA) strain of Rabies virus; Rock-Born canine Distemper virus strain, Canine parvo virus strain (CPV_{39}) and Feline panleukopenia virus strain (CU_3) viruses were supplied by Dep. of Pet Animal vacc. Res., and used to prepare the viral antigens which were used in serum neutralization test.

7. Antigens

Rabies, CD, CP and FPL antigens were prepared in vero cell culture *(16)*. These antigens were used to evaluate the prepared conjugate and to determine the immune status of the obtained samples using IFAT and ELIZA.

8. Serum neutralization test (SNT)

SNT was carried out according to for estimation of antibodies in the delivered samples *(17)*.

9- Fluorescent antibody technique

The indirect FAT was done to evaluate the prepared conjugates *(18)*.

10- Enzyme linked immunosorbant assay

The indirect ELISA was carried out *(19)* to evaluate the prepared conjugates and determine the immune status of delivered canine and feline sera.

RESULTS AND DISCUSSION

Specific rapid and sensitive serological tests like fluorescent antibody technique (FAT) and enzyme linked immune sorbent assay (ELISA) are required for accurate diagnosis of infectious agents as well as for determination the immune status of vaccinated animals.

Pets as lovely animals represents closely relationship between them and their owners, the thing which make the life without them is impossible and accordingly they should travel together from country to country.

Such traveling requires that pets must be immunized against infectious diseases specially the zoonotic ones, requiring rapid accurate determination of their immune status against such diseases. So, the present work was designed to prepare anti-canine and anti-feline sera conjugated with flourescin isothicyanate and horse radish peroxidase to be available as local products on request.

During the present work, the obtained results in Table 1 revealed that rabies; canine distemper and canine parvo neutralizing antibodies were detectable in the obtained dog sera with titers of 8 (20 samples), 16 (50 samples), 32(10 samples) and 64 (20 samples) for rabies antibodies ; 8 (15 samples), 16 (30 samples), 32 (50 samples) and 64 (5samples) for canine distemper antibodies ; 8 (18 samples), 16 (32 samples), 32 (40 samples) and 64 (10 samples) for canine parvo antibodies. These titers could be considered of protective levels for rabies *(20,21)* for canine distemper *(22-24)* and for canine parvo *(22,25-28)*. Protective antibody titers in Cat sera showed titer of 8 (15 samples); 16 (20 samples); 32 (40 samples) and 64 (no sample) for rabies and 8 (23 sample); 16 (17 sample); 32 (30 samples) and 64 (5 samples) for FPL antibodies respectively (Table 2). Several studies *(20,21,29,30-32)*.

The prepared anti-canine serum conjugated with FITC was evaluated by the application of indirect fluorescent antibody technique on the received dog serum samples against rabies ; canine distemper and canine parvo viruses. It was found that such conjugate was able to induce strong positive apple green reactions with the tested serum samples up to dilution of $1 : 10^5$ while the anti-feline serum conjugated with FITC showed strong positive reaction against rabies and FPL viruses in cat sera up to dilution of $1:10^4$ (Table 4).

Among the evaluation results of the prepared anti-canine and anti-feline sera conjugated with horse radish peroxidase using indirect ELISA, it was found that positive results were obtained with conjugate dilution of $1:10^5$ and $1:10^4$ respectively against the tested antigens. These results showed that the prepared conjugates are of good quality, sensitivity and specificity and could be used for detection of rabies ; CD ; CP and FPL antibodies coming in agreement with and parallel to those obtained by serum neutralization test. Similar results were previously reported *(3,16,33,34)*.

FAT and ELISA were used for detection of rabies; CD; CP and FPL viruses and antibodies.

From the represented results in the present work, it could be concluded that the prepared anti-canine and anti-feline sera conjugated with FITC and horse radish peroxidase are of good quality, high sensitivity and high specificity.

Table 1. Detected serum neutralizing antibodies in dog sera

Number of tested samples	Titer*of detected neutralizing antibodies											
	Rabies				CD				CP			
	8	16	32	64	8	16	32	64	8	16	32	64
100	20	50	10	20	15	30	50	5	18	32	40	10

- **Antibody titer=** The reciprocal of serum dilution which neutralize and inhibit the cytopathic effect of 100 $TCID_{50}$ of the used virus.

Table 2. Detected serum neutralizing antibodies in cat sera

Number of tested samples	Titer*of detected neutralizing antibodies							
	Rabies				FPL			
	8	16	32	64	8	16	32	64
75	15	20	40	0	23	17	30	5

- **Antibody titer** = The reciprocal of serum dilution which neutralize and inhibit the cytopathic effect of 100 $TCID_{50}$ of the used virus.

Table 3. Serum proteins in the prepared rabbit anticanine and antifeline sera.

Tested serum	total protein (gm %)	Albumin (gm %)	Globulin (gm %)
Anticanine	8.3	1.0	7.3
Antifeline	7.5	2.3	5.4
Control	5.1	2.07	3.07

Table 4. Estimation of the prepared FITC conjugated anti sera.

Tested conjugate	Positive reacted dilution against antigens			
	Rabies	CD	CP	FPL
Anticanine	$1:10^5$	$1:10^5$	$1:10^5$	-
Antifeline	$1: 10^4$	-	-	$1: 10^4$

Table 5. Titers of anticanine and antifeline sera conjugated with radish peroxidase.

Tested conjugate	Positive reacted dilution against antigens			
	Rabies	CD	CP	FPL
Anticanine	$1:10^5$	$1:10^5$	$1:10^4$	-
Antifeline	$1: 10^4$	-	-	$1: 10^4$

REFERENCE

1.AlanS and Robin Thrope (1996): Coupling antibodies to fluorochromes enzymes and biotin. Immuno chemistry in practice 3rdedition, 77-729.

2.Lefkovtis I (1997): Immunology methods manual vol.4, PP 2243-2249. Academic press, Harcourt Brace & company publishers, San Diego, London, Boston, New york, Sydney, Tokyo, Tronto

3.Tijseen P and Kurstak E (1984): Highly efficient and simple method for the preparation of peroxidase and active peroxidase antibody conjugates for enzyme immune assays. Analytical biochemistry Journal, 136 : 451-457.

4.WHO (1992): World survey of rabies. No. 25 PP. 92-203. Geneva, world health organization

5.Gaskell R M and Bennet M (1996): Feline and canine infectious diseases. Blackwell Sa: Inc., U.S.A, 111-117

6.Hummeler K, Tomassini N , Sokol K , Kuwert F and Koprowski H (1968): Morphology of mucleo protein component of rabies virus J. Viral. 2: 1191-1194.

7.Smith- Cors J F (1958): Purification of rabies virus grown in tissue culture.

8.Bear,GM (1975): The natural history of rabies. Acad. Press New York, 3rd Ed: 290.

9.Green C E (1998): Canine distemper infectious diseases of dog and cat 2nd Ed: 9-22

10.Frank J, Fenner E , Paul J , Gibbes frederic A , Murphy Rodolf Rotti, Michael J Studdert, David O White (1993): General virology. Second edition. Academic press San Diego, New York, Boston

11.Karkowka S and Wallace A L (1979): Lymphocyte associated immune response to canine distemper and measles. Viras in distemper infected gnotobiotic dogs An. J. Vet. Res., 40:669-672

*12.Appel M J , Scott F W and Carmichael L E (1989):*Isolation and immunization studies of a canine parvo-like virus dogs with haemorrhagic enteritis. Vet. Rec. 105:156-159.

13.Benedict A A (1967): Methods in immunology and immuno chemistry" Ed.. Williams and M.R. Chase, P.229, Academic press, London and New York.

14.Narin R C Marrack J K (1969): Fluorescent protein tracing 2nd Ed., Edinburgh and London living stone.

15.Narin R C (1969): Fluorescent protein tracing . 3rd ed., Edinburgh and London Livingstone.

16.Brian W J Mahy and Hillor O Kangro (1996): Virology methods manual P. 318 Academic press, Harcourt Brace and company publishers London, San Diego, New York, Boston, Sydney, Tokyo, Toronto.

17.Rossiter P B , Jessete D M and Taylor W P (1985): Micro neutralization system for use with different strains of pest des petits ruminants and rinder pest virus.Trop. Anim. Heal. Prod. 17 (2) : PP. 75-81.

18.Soliman A K , Botros B A U, Rsiazek T G , Hoogstreal H , Helmy I and Morin J C (1989): Sero-prevalence of rickettsia typhas and Rickettsia cornil infection among rudent and dogs in Egypt. J. trop, Med. Hyg 92 :345-349

19.Voller A , Bidwell D E and Bartlett A (1982): ELISA techniques in virology. In C.R. Howard (ED), New Development in practical virology, New York :Alan R. Liss, PP. 59-81.

20.Arnold and Salvatierra J (1974): Immunity against paralytic rabies in cattle following vaccination with ERA vaccine under ranch conditions in Bolivia. Part IV- five years post vaccination studies. Trop. Anim. Hlth. Prod., 6 (4) : 199-201.

21.Sikes R K , Peacock G V , Acha P N , Arko R J and Dierks R (1971): Rabies vaccines duration of immunity study in dogs JAVMA 159: 1491-1499.

22.Sprino P J and Harris L L (1983): Serological interference study of a canine virus, distemper, hepatitis, parainfluenza and L. canicola-intero haemorrhagiaea. Vet. Med. Small Anim. Clinican, 78 (3) :337-339.

23.Guirguis W I (1991): Trials for preparation of a vaccine against distemper. Ph. D. Thesis. (Microbiology) Fac. Vet. Med. Cairo university.

24.Miyamoto Taura Y, Une S, Yoshitaka M Nakama and watanobe S (1995): "Immunological response to polyvalent

canine vaccines to dogs. J. Vet. Med. Sci. 57 (2): 347.

25.Bass E P , Gill M A and Beckenhaner W H (1982): Development of a modified live canine origin Parvo virus. J. Amer. Vet. Med. Ass., 181 (9) : 909-913

26.Cooper P E , Chappuis G , Saint Gerand A L and Duret (1991): Comparison de L'efficacite des different vaccines du eveluation les responses serologiques et apres epruves. Virulentes 12, 72 et 26 moispres vaccination. Bulletin Mensuei dela Societe veterimaire pratique de France, 75: 131-152

27.Ackerman O, Stegman H and Jaeger O (1983):Simultaneous immunization of dogs against parvo virus, distemper, rabies, contagious hepatitis and leptospirosis. Blauen Hefte furdentierzt. 67:302-308.

28.Castro A and Heuschele W P (1992):Veterinary Diagnostic virology. Mosby year book.

29.Johnson R H (1971): Serological procedures for the study of feline panleukopenia JAVMA 189(6): 876-887

30.Davis E L Gregary G C and Beckenheur W H (1970): Infections feline panleuko penia (Development of a tissue culture origin formalin inactivated vaccine) V.M.S.A clinical March: 237-242

31.Scott F W (1971): Comments on feline panleuko penia Biologics JAVMA 158(6): 910-915.

32.Khodier M H (1999): Studies on vaccination of farm animals (cattle, horse, sheep) in addition to dogs and cats with inactivated tissue culture rabies vaccine. Beni Suef Vet. Med. J., 9 (3-A): 111-120

33.Durojajye O A (1984): Detection of the antigen of pest des petit ruminants virus in tissue by the indirect fluorescent antibody technique. Nig. Vet. J. 13: 77-80 Dzhmukhadze, VA. And Gribencha, S.V. (1972): Properties of a field strain of rabies virus (strain 71 isolated in Georgia in 1968). Vet. Moscow J. 12: 45-50.

34.Avrameas S (1969): Coupling of enzymes to proteins with glatraldehyde. Use of the conjugates for detection of antigens and antibodies. Immuno chemistry , 6: 43-52 .

الملخص العربى

تحضير أمصال مضادة للفصيلة الكلبية و القطية محملة بالفلوريسين أيزوسينات والبيروكسيديزلإستخدامها في تشخيص أمراض الكلاب و القطط

عطيات محمد قطب*، نجلاء إبراهيم علي*، زينب طه سالم*، همت سليمان الإمام*، ماجدة محمد سيد**

*قسم بحوث لقاحات الحيوانات المنزلية الأليفة

معهد بحوث الأمصال و اللقاحات البيطرية – العباسية – القاهرة – ج.م.ع.

** المعمل المركزي للرقابة على المستحضرات الحيوية البيطرية العباسية – القاهرة – ج.م.ع

تضمنت الدراسة الحالية تحضير أمصال مضادة لفصيلة الكلاب و القطط مقترنة بالفلورسين أيزوثيوسيينات و الهورس راديش بيروكسيديز حيث تم تحضير هذه الأمصال في ارانب بوسكات وتم ترسيب الجلوبيولين المناعي وضبط تركيزه ليكون ١٨مجم/مللي, و قد بينت نتائج التجارب العملية أن كلا الكاشفين ذو جودة عالية حيث أعطت الأمصال المضادة لفصيلة الكلاب نتائج إيجابية حتي تخفيف ١ : ١٠ ° و أعطت الأمصال المضادة الفصيلة القطية نتائج إيجابية حتي تخفيف ١ : ١٠ ٤ مع كل من إختباري الوميض الفلوريسنتي المناعي الغير المباشر و إختبار الأنزيم المناعي المرتبط المدمص الغير المباشر على التوالى.

و قد اجري الاختبارين علي أمصال كلاب و قطط وردت إلي قسم بحوث لقاحات الحيوانات المنزلية الاليفة لإستبيان المستويات المناعية بها لكل من السعار و حصبة الكلاب و بارفو الكلاب و الليكوبينيا التي تصيب القطط و كانت نتائج الإختبارين موازية لنتائج إختبار المصل المتعادل الذي أجري علي نفس عينات الأمصال الامر الذي يؤكد أن المستحضرات الحالية هي كواشف نوعية و ذات جودة عالية توفر الوقت و المال عند الطلب.