Detection Of Rabies Virus And Its Antibodies Using Staphylococcus Aureus Protein A

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ABSTRACT

Staph protein-A conjugated with fluorescein isothiocyanate and horseradish peroxidase were prepared and used in indirect fluorescent antibody technique (IFAT) and enzyme linked immunosorbent assay (ELISA), respectively. In addition, serum neutralization test (SNT) was used to detect post-vaccination rabies antibodies in sera of 30 vaccinated dogs and 20 vaccinated cats. Unconjugated staph protein -A was used of agglutination test (AT). All the form mentioned and applied methods for detection of the incidence of anti-rabies antibodies in animal sera even with the use of non- specific anti-species (staph protein-A). FAT, AT and ELISA were found to be specific; sensitive and rapid tests required for rapid detection of rabies antibodies and virus in infected cell culture and mice brain. Regarding the sensitivity and rapidity of these tests, FAT comes first followed by ELISA and SNT then AT. It concluded that staph protein-A either unconjugated or conjugated as non-specific anti-species can be used for rapid detection of rabies antibodies in the sera of vaccinated dogs and cats.

INTRODUCTION

Rabies is a zoonotic viral infection affects the central nervous system (CNS) provokes acute and fatal encephalitis in the mammalian hosts. The rabies virus (RABV) is a neurotropic RNA virus classified within the Order Monoegavirales, Family Rhabdoviridae; Genus Lyssa-viruses *(1)*. Transmission mediated via infected saliva deposited in a bite wound or abradede skin scratches, and breached mucous membranes. Bites on head and neck are associated with shortest incubation period "IP" *(2,3)*.

Staphylococcus aureus protein A is a protein bound to the cell wall of the pathogenic bacterium. This protein has the ability to bind Fc portion of most mammalian immune globulins class G, this portion has unique potential for making "universal" enzyme conjugates which can be used in various enzyme immunoassays *(4)*. Enzyme linked immunosorbent assay (ELISA) has been proposed by several authors as a possible alternative test to neutralization test for determination of rabies antibodies *(5-9)*.

The present work aimed to determine rabies virus and antibodies using agglutination (AT); fluorescent antibody(FAT) and enzyme linked immunosorbent assay (ELISA) using staph protein A either or none conjugated with fluorescein isothiocyanate and horseradish peroxidase in comparison with serum neutralization test (SNT).

MATERIAL AND METHODDS

Rabies virus

Baby Hamster kidney (BHK-21) cell culture adapted Evelyn Rokitincki Abelesth (ERA) strain of rabies virus of a titer 10^7 $TCID_{50}$/ml was supplied by the Department of Pet Animal Vaccine Research (DPAVR); Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo and used in application of serological tests.

BHK-21

BHK-21 cell culture was used for preparation of rabies virus antigen; detection of rabies virus by FAT and SNT.

Rabies virus antigen

It was a whole virus preparation of the ERA strain of rabies, grown in BHK-21 cells *(10)*. It was inactivated with Binary Ethylene mine at 37°C to viral suspension as 3% concentration of 0.01M. The mixture was stirred continuously at 37°C for 3.5 h. Inactivation process was stopped by addition of cold sodium thiosulphate with a final 2% conc. The rabies antigen used for the agglutination test, indirect ELISA and FAT to determine rabies antibodies in sera of vaccinated dogs and cats.

Challenge Rabies virus strain

It is a fixed virus strain derived from the original Pasteur strain. It was propagated and fixed in mice brain. It was supplied kindly by Pasteur Institute, Paris, in a lyophilized form with a titer of 10^5 MILD50/ml and used for experimental infection of mice.

Serum samples

(30) dog sera and (20) cat serum samples were obtained from vaccinated animals through the government veterinary hospital, Abbasia, Cairo.

Mice

Thirty Swiss albino mice (3-5 weeks old) were supplied by DPAVR, VSVRI, Abbasia, Cairo. They were injected intra-cerebral (IC) with a dose of 0.03 ml CVS and used for detection of rabies virus in their brains through experimental infection.

Preparation of Staphylococcus aureus Protein A (SPA)

Staph protein-A was prepared *(11)*, and supplied kindly by Animal Reproduction Research Institute, Giza

Chemicals and reagents used for conjugation of the prepared antiserum with horseradish peroxidase (HRP)

HRP product number p-8375 type VI, lot 25C-9510 was supplied by Sigma Chemical Company. It had an activity of 365 purogallin units/mg.

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.

Sodium periodate ($NaIO_4$).

Conjugation of staph protein-A with horseradish peroxidase

It was carried out following up the method via Staphylococcal protein-A conjugated with peroxidase ELISA KPL " Sigma" was used in the tested sera for detection of rabies virus antibodies *(12)*.

Chemicals used for conjugation of staph protein-A with Fluorescein isothiocyanate (C_4 H_{11} NO_5 S)E

(C_4 H_{11} NO_5 S) E was supplied by Merck, Darmstadt for Microscopy (M.Gew.389.39).

Conjugation of the prepared staph protein-A with Fluorescein isothiocyanate

It was done according to the method of *(13)*.

Reference anti-rabies serum

Reference anti-rabies serum was supplied by Copenhagen, Denmark, as 30 IU / ampoule. It was used as positive control in the applied serological tests and used for detection of rabies virus in mice brain and infected cell culture through application of virus neutralization test (VNT); indirect ELISA; agglutination test and indirect FAT using SPA.

Serum neutralization test (SNT)

It was carried out *(14)*, using the micro titer technique for estimation of rabies antibodies in vaccinated dog and cat sera. The antibody titer was calculated as the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ /ml of rabies virus.

Slide Agglutination test (AT)

It was carried out as the follow: 5ml of 10% SPA suspension were mixed with 5 µl of tested serum sample , then 5 µl of specific viral antigen were added to the SPA serum

mixture and the slide aggregate have been found for 5-10 min at room temperature. The agglutination pattern was read macroscopically *(15)*.

Indirect Enzyme Linked-Immunosorbent Assay (ELISA)

To measure the rabies antibodies using ELISA kit (Zoetis In., USA) according to the manufacturers instructions. Positive and negative control sera were provided in the kit. Serum samples (1:100) were incubated in plates pre-coated with the rabies virus antigen at 37°C for 1 h. After four washes , horseradish peroxidese-conjugated protein-A was added and incubated at 37°□ for another 1 h. The plate was washed again followed by the addition of TMB substrate. The optical density of the plate was recorded at 450 nm. Sera titers were expressed as equivalent units per ml (EU/ml) corresponding to international units by using the values obtained by the WHO .reference serum *(16)*.

Indirect Fluorescent antibody technique (FAT)

The FAT was performed according to the procedure described by the Office International des Épizooties (OIE) and World Health Organization (WHO) *(17,18)*. Impression smears slides were prepared from each brain of dead mice or mice showing rabies signs on slides were fixed in cold acetone (20°C) for 20 min. After three successive wash with phosphate buffer saline (PBS, pH 7.2), the slides were incubated with hyper immune serum against rabies for 45 min at 37°C, and then stained with fluorescein isothiocyanate (FITC)- conjugated protein-A. After rinsing with PBS, the slides were air-dried and mounting buffered glycerin (Southern Biotechnology Associate, USA) was applied. The slides were examined under cover slips at magnification of 400 using a fluorescent microscope (Nikon, Japan). Positive and negative controls were run together with the test samples. The slide showing specific fluorescence was confirmed as positive.

RESULTS AND DISCUSSION

Table 1. Detection of rabies antibodies (IgG) in vaccinated dog and cat sera using different serological tests

Tested Animal Sera	Number of tested samples	Percentage of positive results obtained by			
		Serum neutralization test	Slide SPA-agglutination test	ELISA using conjugated SPA	FAT using conjugated SPA
Dog sera	30	90% (27/30)	76.6% (23/30)	93.3% (28/30)	83.3% (25/30)
Cat sera	20	90%(18/20)	80% (16/20)	95% (19/20)	90% (18/20)
Unvaccinated dog sera	5	-ve	-ve	-ve	-ve
Unvaccinated cat sera	5	-ve	-ve	-ve	-ve

N.B: Sera from unvaccinated control dogs and cats were negative (-ve)

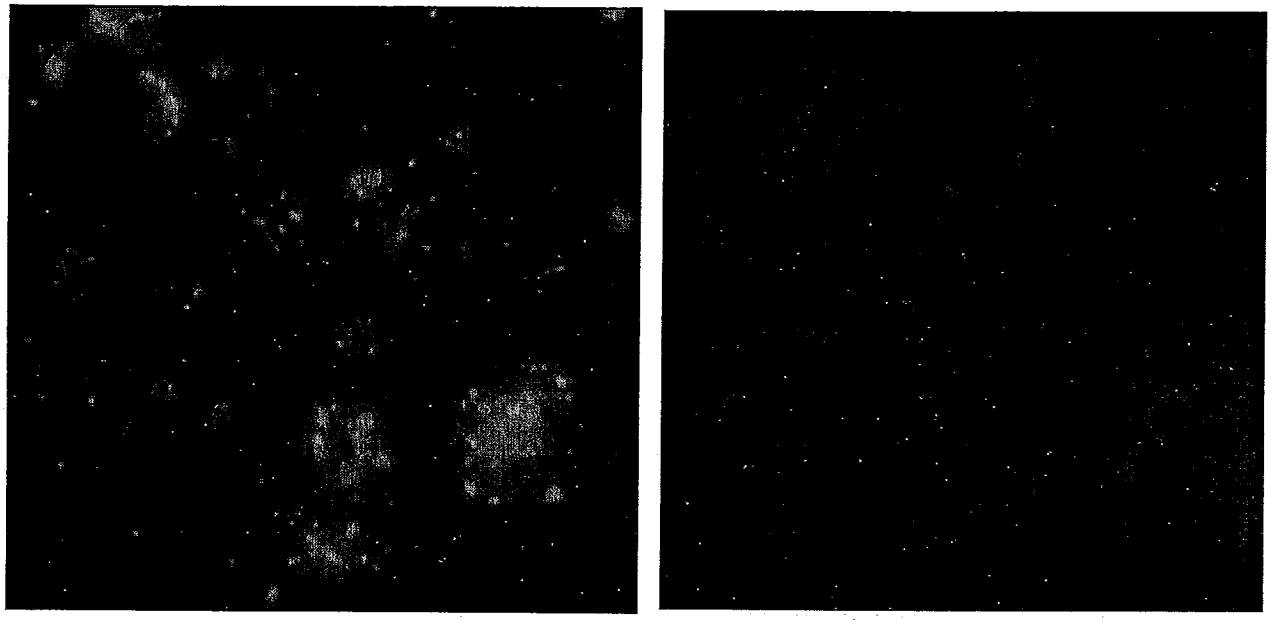

Photo 1. Right photo showed positive apple green fluorescent antibody reaction in infected mice brain impression smears (400×). The left photo showed negative FAT reactio

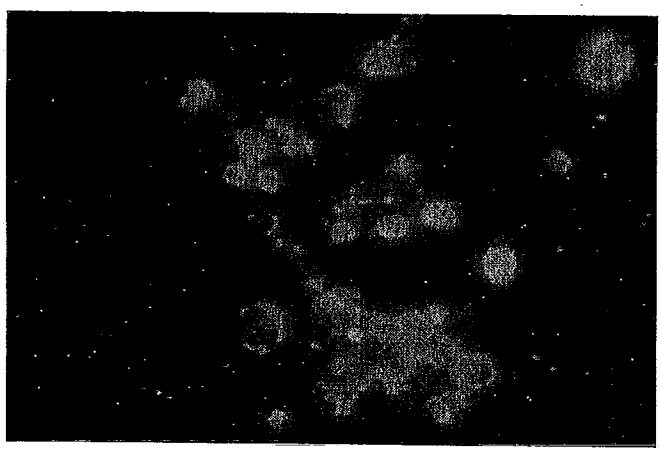

Photo 2. Infected BHK-21 cells (400×) showing positive apple green fluorescent antibody test(FAT) reaction

Table 2. Detection of rabies virus in mice brain and infected BHK-21 cells

Tested material	The applied tests			
	VNT	Slide agglutination test	Indirect ELISA	Indirect FAT
Mice brain Infected BHK Cell culture	Confirmed Virus detection	+++ (75%) ++++ (100%)	Confirmed Virus detection	++++ ++++

FAT: Fluorescent antibody test VNT: Virus neutralization test
ELISA: Enzyme Linked immunosorbent assay +++: Positive result(Presence of virus).

Rabies is caused by a virus of the genus Lyssa virus in the family Rhabdoviridae which is an RNA single stranded negative-sense virus *(19)*. It was recommended that a minimum value of 0.5 IU/ml of neutralizing antibodies should be protective level *(20)*. IgG anti-rabies antibody detected by ELISA may be of greater importance than the early (IgM) antibody response to infection or immunization that is measured by neutralization tests *(8)*. Thus the use of protein A which binds to IgG of most animals and only partially to canine and feline IgM and IgA *(21)*, indicates the promising use of staph protein A in ELISA for the sero-diagnosis in monitoring the success of canine rabies vaccination. The ELISA described here offers an alternative test which unlike neutralization assays can be easily performed routinely. The protein A from Staphylococcus aureus is able to detect antibodies of IgG class. Production of IgM antibodies is the initial humoral response to infection or immunization lead to a high number of false negative results in the ELISA test, indicated a lower sensitivity *(22)*.

The results of the present work illustrated in Table (1), the immune response of SNT recorded that 27 out of 30 serum samples of vaccinated dogs and 18 out of 20 serum samples vaccinated cats were positive. The positively of SNT(90% ,90%) was nearly as those obtained by using SPA-ELISA. The slide SPA-agglutination test revealed that 23 out of 30 serum samples of vaccinated dogs and 16 out of 20 serum samples of vaccinated cats were positive. It was clear that the positivity of slide SPA- agglutination test (76.6%, 80%) was nearly as those obtained by using SPA-FAT(83.3% ,90%), in dog and cat, respectively. The results of SPA-agglutination test showed different positive reactions of aggregates ranged from typical macroscopic patterns, which can be read by naked eye to sandy-like ground as reported by *(23)*. The agglutination test (AT) was used as a rapid screening test. On the other hand, ELISA assay revealed that 28 out of 30 serum samples of vaccinated dogs and 19 out of 20 serum samples of vaccinated cats were positive The results of FAT were less than the results obtained by SPA-ELISA (93.3%, 95%), in dog and cat, respectively. The ELISA described here is simple, rapid and limit cost. It is possible to conclude that this test can be used in the rabies laboratory no matter what kind of vaccine has been used for immunization. The results of the present study illustrated as in Table (2), revealed that all infected mouse experimentally were showed clinical signs and some others dead, thus showed positive with direct non specific anti-species conjugated SPA with horseradish peroxides and fluorescein isothiocyanate of indirect ELISA and indirect fluorescent antibody technique (FAT) as shown in Photos (1&2), respectively, and slide agglutination test for detection of rabies virus in mice brain and infected BHK-21 cell culture were found to be valuable products. These obtained results showed that slide agglutination test has a unique advantage as used rapid test for detection of rabies antibodies in all mammalian sera with high stability, sensitivity, specificity and easily application with economic consideration. Therefore, we could recommend the use of SPA agglutination test for rapid screening of rabies virus.

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AKNOLEDGMENT

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الملخص العربى

تحديد وجود فيروس السعار وأجسامه المناعية بأستخدام بروتين "A " للمكور العنقودي الذهبي

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معهد بحوث الامصال واللقاحات البيطرية- العباسية- القاهرة- ص.ب: ١٣١

أملا في التوصل لطريقة سريعة ودقيقة للكشف عن فيروس السعار وأجسامه المناعية فى أمصال الكلاب والقطط المحصنة وفى مزارع نسيجية وأمخاخ فئران سويسرية مصابة تجريبيا بفيروس السعار الضاري المؤقلم في الفئران، باستخدام بروتين" أ " للمكور العنقودي الذهبي (كمضاد غير نوعى للفصائل) بأجراء الاختبارات السيرولوجية المختلفة مثل اختبارا لتلزن واختبار المصل المتعادل ، اختبار الاليزا، اختبار الوميض الفلورسينتى المناعي الغير مباشر وذلك بإقران البروتين بالبيروأوكسيديز للاختبار الثالث و بالفلورسينس أيزوسيوسينت للاختبار الرابع ، وجد ان كل هذه الاختبارات السيرولوجية لها القدرة على استبيان وجود الأجسام المناعية للسعار في أمصال القطط والكلاب المحصنة بلقاح السعار. وقد أكدت النتائج ان الاختبار الوميض الفلورسينتى المناعي أعطى نسبة ايجابية عالية وصلت الى ١٠٠% بدرجات متفاوتة وهذا يدل على مدى دقة وحساسية هذا الاختبار يليه اختبار الاليزا واختبار المصل المتعادل ويأتى اختبار التلزن بنتائج ايجابية سريعة بدرجات متفاوتة. يمكن القول باستخدام بروتين" أ " للمكور العنقودي الذهبي كمركب فريد للكشف عن الاجسام المناعية في أمصال الحيوانات المحصنة دون الحاجة إلى مصل نوعى مضاد للفصيلة والذي يتطلب وقت طويل لتوفيره مع ارتفاع ثمنه.