6th Sci. Congr. of Egypt. Soc. For Anim. Manag. 27-31 August.,2013: 14 -23 .

COMPARATIVE STUDIES ON FOOT AND MOUTH DISEASE SAT 2 HYPER IMMUNE SERA PREPARED IN DIFFERENT HOSTS CONJUGATED WITH FLOURESCEIN ISOTHIOCYANATE AND HORSE RADISH PEROXIDASE

Hiam, M. Fakhry*, khoudeir M.* and ., Hanaa A. Mostafa**

*Veterinary Serum and Vaccine Research Institute; Abassia, Cairo, P.B.13l
**Central Laboratory for evaluation of Veterinary Biologics, Abassia, Cairo

SUMMARY

Immunofluorescence and enzymes labeled reagent assays play a major role in diagnostic laboratory purposes. So, the main goal of the present work was designed to prepare anti-FMD (local type SAT 2) antisera conjugated with fluorescein isothiocyanate and horse radish peroxidase in different hosts (guinea pigs , Bosket rabbits and goat) for detection of FMD virus . This antisera was found to have neutralizing antibody index of (2.55 , 2.7 ,2.7) for GP, Bosket rabbits and goat respectively . Antisera were divided into two portions where the first one was conjugated with the fluorescein isothiocyanate (FITC) for fluorescent antibody technique while second portion was conjugated with horse radish peroxidase (HRP) to be used in ELISA purposes. The application of direct FAT on infected tongue epithelial and BHK cells showed that the FITC conjugate showed clear apple green colure (strong positive reactions) up to a dilution of 1/500 (GP)1/1000 (Rabbits and Goat) ,The reactions were obtained up to dilution of 1/8000(GP) , 1/10000 (Rabbits and Goat) . It was found that the HRP conjugate induced positive ELISA results with dilution of 1/450 , 1/500 and 1/500 for Guinea pigs, Rabbit and goat respectively with the infected tongue epithelium and with dilution of 1 / 300 , 1/350 and 1/350 with the cell culture antigen for GP, Rabbit and goat respectively .The prepared hyper immune serum against FMD virus type SAT 2 conjugated with FITC and horse radish peroxidase in three laboratory animals have good quantity, high sensitivity and high specificity . Hyper immune serum conjugated with FITC and horse radish peroxidase considered a diagnostic benefit in detection of FMD type-SAT2 antigen helping in rapid accurate diagnosis. Although rabbit and goat give the same good quality and quantity antisera than guinea pigs. but in the final conclusion : rabbit is consider the cheaper and better host used in preparation of hyper immune serum conjugated with fluorescein isothiocyanate and horse radish peroxidase for rapid accurate diagnosis .

INTRODACTION

Foot and mouth disease (FMD) is still one of the most important infectious diseases that could not be neglected where it causes great economic losses among cloven hoofed animals. The disease became worldwide in extent where major epizootics have developed in many parts of the world every few years ***(Anon, 1978).***Several outbreaks of FMD were recorded in Egypt as reported by ***Moussa et al. (1974) ; Daoud et al (1988); EI-Nakashly et al. (1996); Farag et al (2004 and 2005)*** ,where the causative agent was FMD virus type O while type A ***(Abd EI-Rahman et al., 2006) and*** *type SAT 2* ***(Shawkey et al., 2013)***

Antibodies are useful for specifically recognizing antigens and the detection of antigen antibody complex could be easy when precipitation occurs. Labeling of antibodies makes antigen identification easier. Specific, rapid and sensitive serological tests like fluorescent antibody technique (FAT) and Enzyme linked Immunosorbent assay (ELISA) are required for accurate diagnosis of infectious agents to reach a well applicable control measures. FAT depends mainly on antigen- antibody reaction in the presence of fluorescent dye ***(fluorescin isothiocyanate*** **"FITC**") which irradiates with ultraviolet light emitting apple green flourescence ***(Tizard, 1996).***

The technique depends on the presence of specific antiserum conjugated with FITC. FAT has gained wide acceptance in virology serving for many purposes such as the detection of cellular localization of viral antigens **(*Watson and Coons, 1954 and Spendlove at aI., 1963*** **E**stablishment of temporal sequence after infection appearance ***(Breitenfeld and Schafer, 1957)***; and detection of virus infected cells which fail to produce viral progeny and still synthesize virus coded antigens which continue to harbor viral genetic material **(*Pope and Rowe, 1964 and Tevethia et aI., 1965).*** Due to the great importance of FMD, accurate and rapid identification of the causative agent is an essential step in controlling the disease. Such purpose needs the availability of specific diagnostic antiserum and kits for rapid, sensitive and specific virus identification as fluorescent and ELISA kits.

So, the main goal of the present work was designed to prepare anti-FMD (local type SAT 2) antisera conjugated with fluorescein isothiocyanate and horse radish peroxidase in different hosts (guinea pigs , Bosket rabbits and goat) for detection of FMD virus, as local products saving time and high cost for importation .

MATERIALS AND METHODS

1- **FMD virus**

BHK-21 cell culture adapted local strain of FMD virus type SAT 2 of a titer $I0^{8}TClD_{so}$ /ml was used in vaccine preparation, serum neutralization test and preparation of FMD virus antigen for ELISA.

2-Inacivation of FMD virus by binary Ethyleneimine (BEI)

3- Concentrated FMD virus

A local inactivated FMDV (type SAT 2) was concentrated by 10 % poly ethylene glycol-6000

4- FMD vaccine

A local inactivated concentrated FMD virus (Type-SAT 2) adjuvanted with Mantanaid oil-50 was prepared. It was used for preparation of FMD type SAT2 antiserum .

5-lnfected tongue epithelial and BHK cells

FMDV type-SAT2 infected bovine tongue epithelium and SAT2 Tissue culture cells were supplied by FMD - department and used to evaluate the prepared antiserum conjugated with fluorescein isothiocynate in the direct fluorescent antibody technique.

6-FMDV type-SAT 2 antigen

It was prepared in BHK cell culture according to ***Lefevre and Diallo (1990)*** to be used for the evaluation of the prepared antiserum conjugated with horse radish peroxidase using ELISA.

7 -Guinea pigs , Rabbits and goats

five healthy guinea pigs each of about 500 gram body weight , five healthy Bosket rabbits each of about 3kg body weight and Three healthy goats age were used for preparation of FMD virus type-SAT2 antiserum . In addition two from each lab. Animal species were kept as negative control . These animals were housed under hygienic conditions in separate isolated boxes receiving balanced ration and adequate water.

8-Preparation of FMD SAT 2 antiserum

FMD type-SAT2 antiserum was prepared in Guinea pigs , Bosket rabbits and goats using the inactivated FMD oil vaccine type-SAT2 according to the method described by ***(Ihemelanadu et al. (1985).***

9-Serum neutralization test (SNT)

Serum neutralization test was carried out to estimate the antibody titer in the prepared antiserum. The test was performed according to **Ferreira (1976).**

10-Precipitation of the immunoglobulin of the prepared antiserum

It was carried out using a saturated ammonium solution according to ***Narin and Marrack (1964).*** The globulin content was estimated according to ***Weichselbaum (1946*)** and adjusted to 20 mg/ml using phosphate buffer saline.

11-**Chemicals and reagents used for conjugation of the prepared antiserum with horse radish peroxidase (HRP):**

A-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.

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 ($NaI0_4$).

12-Conjugation of the prepared FMD type-SAT2 antiserum with HRP:

It was carried out following the method described by ***Tijssen and Kurstak (1984).***

13- Check board ELISA:

It was carried out according to ***Rose et al. (1986)*** , to detect the end point of prepared HRP conjugate.

14- Direct ELISA:

It was applied according to ***Memeny (1991)*** and modified by ***Hamblin et al. (1986),*** **Chendar et al. (2003)** and ***OIE (2009)*** for titration of the prepared FMD type-SAT2 antiserum conjugated with HRP.

15- Chemicals used for conjugation of the prepared antiserum with Fluorescein isothiocyanate (C_4 $H_{11}NO_5$ S):

(C_4 H_{11} N_5 S) was supplied by Merck, Darmstadt for Microscopy (M.Gew.389.39).

16- Conjugation of the prepared FMD type-SAT2 antiserum with Fluorescein isothiocyanate:
It was done according to the method of ***Narin (1969).***

17-Imported standard anti -FMDV sera conjugated with horse radish peroxidase and fluorescein isothiocyanate
These conjugates were supplied by the Department of Foot and Mouth Disease Research; Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

18- Direct fluorescent antibody technique (FAT):
Direct FAT was carried out on infected tongue epithelial and BHK cells to evaluate the prepared FMD type-SAT2 antiserum conjugated with fluorescein isothiocyanate. The technique was carried out according to ***Soliman et al. (1989).***

RESULTS AND DISCUSSION

During the present work, the prepared antiserum against FMD virus type-SAT 2 had a serum neutralizing index of 2.7. This level of FMD antibody titer appears to be of high level where it was concluded that Mantonid oil ISA50 has the ability to improve the immune response of vaccinated animals with FMD vaccine resulted in higher levels of immunity recorded by(***Barnett et al. 1996 and, Abd EI-Karim 2007) .*** The immunoglobulin of the prepared FMD virus type -SAT 2 was successfully precipitated and it was it was adjusted to be 18 mg /ml of phosphate buffer saline as recommended by ***Tijssen and Kurstak (1984)*** to be conjugated with FITC and horse radish peroxidase.

Rabbits were widely used for preparation of viral hyper-immune sera (**Dzhmukhadze, V.A. and Gribencha, S.V 1972).** TableI showed the hyperimmune serum conjugated with FlTC were strong positive reaction with tested infected tongue epithelium and infected BHK cells with dilution 1/500 in GP and 1/1000 for rabbit and goat and were moderate reaction with dilution 1/8000 for GP and 1/10000 for rabbit and goat and no reaction with dilution 1/20000

Table (1): Evaluation of the prepared anti FMD virus hyper immune serum conjugated with FlTC

Used conjugated dilution			Apple green fluorescent reaction in	
GP	Rabbit	Goat	Infected tongue	Infected BHK cells
500	1000	1000	++++	++++
8000	10000	10000	++	++
20000	20000	20000	-	-

++++ = Strong positive reactions.
+++ = Moderate positive reaclions.
- = Negative reactions.

The prepared conjugated FMD type - SAT2 antiserum conjugated with FITC was evaluated by the application of direct fluorescent antibody technique on infected tongue epithelium and BHK cells . It was found that such conjugate was able to induce positive reaction (apple green coloure) (Photo-I & 2) with the tested specimens up to a dilution of 1/10000 (Rabbits and Goat) and 1/8000(GP) and no reactions (Photo-3) were recorded with a dilution more than 1: 10000 .These results showed that the direct FAT is a rapid, sensitive and accurate technique confirming the presence of FMD virus antigen in infected tongue epithelium and BHK cells in agreement with what reported by **Durojajye (1984); Brain and Hillor (1996) and Abd EI-Aty et al. (1999) .**

Among the evaluation results of the prepared anti FMD virus hyper immune serum conjugated with horse radish peroxidase using direct ELISA, it was found that positive results were obtained with conjugate dilution of 1/450 for Guinea pigs and 1/500 (Rabbit and goat) in tongue epithelium and 1 / 300 , 1/350 in BHK cells . These results showed that the prepared conjugate is of good quality, sensitivity and specificity and could be used for detection of FMD virus antigen type SAT 2 coming in agreement with and parallel to those obtained by **Avrameas (1969); Voller et al. (1979) and Abd EI-Aty et al. (1999) Manal, M‹and Mervat,M. (2008) .**

From the represented results in the present work. it could be concluded that the prepared hyper immune serum against FMD virus type SAT 2 conjugated with FITC and horse radish peroxidase in guinea pigs , Bosket rabbits and goat are of good quality, high sensitivity and high specificity, but Rabbit and goat give more quantity antisera than guinea pigs .The final conclusion. Rabbit is consider the cheaper and better host used in preparation of hyper immune serum conjugated with fluorescein isothiocyanate and horse radish peroxidase for rapid accurate diagnosis .

Photo 1: Immunofluorescent positive reaction to FMDV type (SAT 2) carried out showing Apple green reaction) in infected tongue epithelium

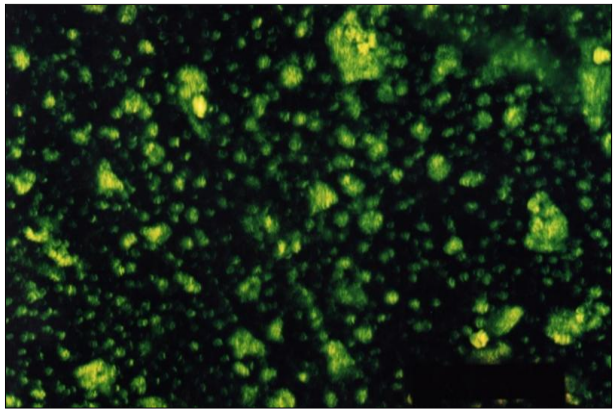

Photo.(2) Immunofluorescent positive reaction to FMDV type (SAT 2) showing Apple green reaction in infected cell culture

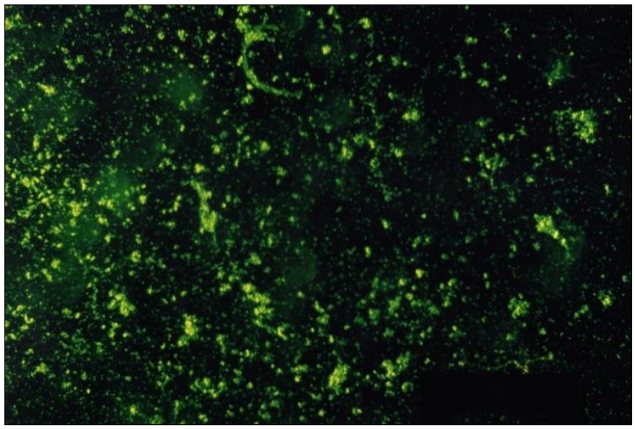

Photo 3. Immunofluorescent negative reaction

EFERENCES

Abd El Aty , M Samira El-Kilany ; Shawky, M and Halema El Watany (1999): Preparation and evaluation of immune fluorescent conjugate against FMD virus. Vet Med. J Giza, Vol. 47 (2) :239-246.

Abd El-Karim , A.S. (2007) : Comparative studies on foot and mouth disease vaccine containing different adjuvants in sheep. M. Vet. Sci. Thesis (Infectious disease) Fac. Vet. Med. Cairo Univ.

Abd El-Rahman, A.O.; Farag , MA.; Samira El-Kilany ; Ali S.M. and Manal, Abo El Yazeid and Zeidan,S.M.(2006): isolation and identification of foot and mouth disease virus during an outbreak of 2006 in Egypt. J Kafr El-Sheikh Vet.Med.4 (1) :451 -464.

Anon (1978): History of foot and mouth disease Vet. Rec., 102 :184-185.

Avrameas, S. (1969) : coupling of enzymes to proteins with glutradehyde. Use of the conjugated for detection of antigens and antibodies.Immunochemistry, 6: 43-52.

Barnett. P.V.; Pullen. L.; Williams. L. and Docl, T.R. (1996*):* International Bank for Foot and Mouth Disease vaccine: Assessment of Mantonide ISA 25 and 206, two commercial available adjuvants. Vaccine.14(13): 1187-1198.

Brain. \V . .I. and Hillor, O. *(1996):* Virology Methods Manual. pp: 31 H. Acd. Press. London. San Diego. New York, Sydney and Tokyo.

Breitenfeld, P.M. and Schafer, W. (1957): The formation of fowl plague virus antigens in infected cells as studied with fluorescent antibodies. Virology. 4: 328-345.

Chendar, G.; Middemak, Moonen, P.; Schrijver, R.S and Aldo Dekker (2003) : A solid phase blocking ELISA for detection of type O foot and mouth disease virus. J virol. Meth 107 (1) 89-98.

Daoud , A.M. ; Abd El Rahman, A.O.; El-Bakry, M.; Metwaly, N.; El-Mekkawi, M..and Samira El-Kilany (1988) : Strains of Foot and Mouth disease virus recovered from 1987 outbreak in Egypt.J. Egypt. Vet. Med. Ass. 48 (1) :63 -71.

Durojajye, O.A. (1984) : Detection of the antigen of peste des petits ruminants virus in tissue by the indirect fluorescent antibody technique. Nig. Vet . J. 13 : 77-8.

Dzhmukhadze, V.A. and Gribencha, S.V (1972) : Properties of field strain of rabies virus (strain 71 isolated in Georgia in 1968).vet Moscow J. 12 : 49-50

El-Nakashly, S.; Abou Zaid , A.A. ; Samira El-Kilany and Abd El Aty, M.M. (1996): Isolation and identification of foot and mouth diseases virus during outbreak in 1993 in Egypt 7th Sci Conf. Fac.vet. Med Assiut Univ.: 679-687.

Farag, M.A. Aggour , A.M and Daoud, A.M. (2005) : ELISA as a rapid method for detecting the correlation between the field isolates of foot and mouth disease and the current used vaccine strain in Egypt. J.Vet. Med. Giza, 53 (4) : 949-955.

Farag, M.A.; Halima, M. El-Watany and Abeer , A. Talaat (2004): Detection of FMD virus using a dot immunobolot and RT-PCR from field samples.1st.Sci. Conf. Fac. Vet. Med. Moshtohor, ¼ : 89 - 99.

Ferreira, M.E. (1976) : M.E. (1976) : Prubade microneutralization poraestudios. Cent. Paname. Fiebre. Aflosa, 2 Land. 22: 17-24

Hamblin , C.; Barnett, I.T. and Hedger, T. (1986) : A new enzyme linked immunosorbent assay (ELISA) for detection of antibodies against FMDV. Development method of ELISA. J. Immunol.Meth., I: 115-121.

Ihemelandue, E.C.; Nduaka,O and Ojukwa, E.M. (1985) : Hyper-immune serum in the control of peste des petits ruminants.Trop. Anim. Heal. Prod.17 (2): 83-88.

Lefevre, P.C. and Diallo, A. (1990) : Peste des petits ruminants Rev. Sci Tech. Int. Epiz. 9:951-965.

Manal, Aboel-yazed Mustafa and Mervat,M.Ali (2008):preparation of anti-FMD virus type-A immuno globuline conjugated with horse radish peroxidase and fluorescein isothiocy ana te for serological use . J.Egypt.Vet.Med.Assoc. 68(3) 2008, Arab vet.. Medical congress. (15-17) December 2008 , pp.(129-139) .

Memeny, D.M. (1991) : A practical guide to ELISA. Bergman press Oxford 1st Ed.

Moussa, A.A. ; Boulaus , S.M.; El-Sayed, T.E.; Fahmy, F.S. and Bohm, H.O. (1974): typing and subtyping of a strain of FMD virus isolated from Sharquia Province, 1970. J. Egypt . Vet. Med . Ass. 34 (3-4) : 413-419.

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

Narin , R.C. and Marrack. J.R. (1964) Fluorescent protein tracing. 2nd Ed Livingstone Edinburgh and London.

OIE (2009) : Terrestrial Manual 2009 (5), Foot and mouth disease (Chapter 2.1.5.) (1-29 .

OIE (2010): Manual of diagnostic tests and vaccine terrestrial animals.5th Edition 2010 part 2 Chapter2.11.

Pope, J.H. and Rowe, W.P. (1964) : Detection of specific antigen in SV40 transformed cells by immunoflurescence . J.Exp. Med. 120 :121-128.

Rose , N.R.; viodman , H. and Fany, G.L. (1986) : manual Clinical laboratory

Immunology. 3rd Ed.Ed.Am. Soc. Microbiol. Washington, D.C.; 107-109.

Shawky M., Abd El-Aty M., Hiam M.Fakhry, Hind M. D., El-Sayed E., Mossad W., Sonia A.Rizk., Abu-Elnaga H., Abubakr A. M., Abd El-kreem A. and Farouk E.M. (2013): Isolation and Molecular characterization of Foot and Mouth Disease SAT-2 Virus Isolate During Outbreak 2012 in Egypt . *J Vet Adv* 2013, 3(2): p.1-9

Soliman, A.K.; Botros, B.A.M. ; Rsiazek, T.G.; Hoogstreal, H.Helmy, I. and Morin, A. (1989): Sero-prevalence of Rickettsia typhosa and Rickettsia cornil infection among rodent and dogs in Egypt. Egypt .J Trop. Med. Hyg. 92 :345-349. J. Egypt. V cl.Med.Assoc. 1!J!,3,200H 138

Spendlove, R.S.; Lennette, E.H. and John, A.C. (1963):The role of miotic apparatus in the intracellular locations of Reo virus antigen. J. Immunol.90:554-560.

Tevethia, S.S.; Katz, M. And Rapp, F. (1965) : New surface antigen in cells transformed by Simian PapovirusSV40. Proc. Soc. Exp. Biol. (NY), 119 : 896-901.

Tijssen, P. and Kurstak, E. (1984) : Highly efficient and simple method for the preparation of peroxidase and active peroxidase - antibody conjugates for enzyme immunoassays. Analytical Biochem.136 : 451-457.

Tizard, I.R. (1996) : Veterinary Immunology, 5th Ed. Saunders Comp., Philadelphia.

Watson, B.K. and Coons, A.I. (1954) : Studies of Influenza virus infection in the chick embryo using fluorescent antibodies .J. Exp. Med. 99 : 419-428.

Weichselbaum, T.E. (1946) : An accurate and rapid method for the detection of protein in small amounts of blood serum and plasma. Am. J. Clin. Path. 16:40-49.