Detection of FMD virus antigens and antibodies using staph protein-a rapid agglutination test in comparison with the used traditional methods

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A B S T R A C T

Considering rapid accurate detection of FMD virus antigens and antibodies is an essential corner stone in the epidemiological studies; *Staphylococcal* protein-A *(SPA)* was successfully prepared and used for application of rapid agglutination test *(RAT)* on both of processed infected tongue epithelium and infected cell culture fluid for detection of *FMD* virus antigens using reference viruses and anti-sera. It was found that the traditional virus neutralization test (VNT) consuming long time (18-24 hours) and RAT with the shortest time (2-5 minutes) were able to detect the antigens of *FMDV type O; A and* SAT_2 with titer ranged from ≤ log10 up to 7log10 $TCID_{50}$/ml. In addition, RAT carried on serum samples obtained from vaccinated animals revealed positive results with titers similar to those obtained by serum neutralization test (SNT) ranged from ≤ 2 up to 256 for the three virus types with shorter time (2-5 minutes) than that consumed by SNT (18-24 hours). It was noticed that high antigen or antibody titers showed strong agglutination while weak agglutination was noticed with lower titers. So it could be concluded that the present work provides a rapid; sensitive and accurate test to detect FMDV antigens and antibodies as safe; easy to be used, highly sensitive, rapid field test, of low cost and having friendly environmental relationship.

Key word: Foot and mouth disease (FMD); Staph protein A (SPA); Rapid agglutination test (RAT).

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1. INTRODUCTION

Foot and mouth disease (FMD) is one of the most troubles viral diseases among livestock especially cloven footed animals of both wild and domestic animals like cattle, buffaloes, sheep, goats and pigs (OIE 1972 and Sharma 1981). It is caused by a filterable virus belongs to genus Aphthovirus, family Picornaviridae, as a non enveloped icosahedral RNA virus of positive sense, the virus has 7 serotypes that differ antigenically and immunologically with different subserotypes distributed world wide (Pereira 1977and Franki et al., 1991). The disease 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 world wide in extent where major epizootics have developed in many parts of the world every few years (Anon, 1978). Regarding Egypt different types of FMD virus like SAT_2, O and A were identified Type A and SAT_2 were the main causes of outbreaks of 1953, 1958 and 1960 (Moussa et al, 1974; Daoud et al, 1988; El-Nakashly et al, 1996; Farag et al 2004 and Abd El-Rahman et al, 2006).

Specific, rapid and sensitive serological tests are required for accurate diagnosis of FMD as an essential step in controlling the disease. Staphylococcal protein –A "SPA" is a protein bound to the cell wall of the pathogenic bacterium *Staphylococcus aureus (S. aureus)* binds to the Fc portion of most mammalian class G immunoglobulins. Therefore, this protein has a unique potential for making "universal" immunoassays (Aslam and Dent, 1998).

Some strains of S-aureus synthesize protein – A, a group specific ligand which binds the Fc region of IgG from many species. It binds to human, mouse, rabbit, cat, cow, dog, goat, Guinea pig, horse and sheep IgG but not bind to chicken (-) and hamster (±) IgG (Alan and Rabinthorpe, 1996). *SPA* has increasing importance as a tool in both quantitative and qualitative immunological techniques. *SPA* remains markedly stable on exposure to high temperatures, low pH and denaturizing agents (Montassier et al, 1994). *SPA* was used to develop a coagulation "COA" test for the detection and typing of *FMDV*. Its simplicity and rapidity of performance and its low cost, has a great potential for direct detection and identification of *FMDV* as screening strategy at FMDV outbreaks (Montassier et al, 1994 and Saad et al, 2000).

The present work aims to provide a rapid and accurate method for detection of FMD antigens and antibodies as a golden goal aid to reach more fast diagnosis than that carried out by traditional methods which are time consuming; require more complicated steps with higher cost. Such aim could be established through application of rapid agglutination test using *SPA* as non-specific anti-specie.

2. MATERIAL AND METHODS

2.1. FMDV serotypes:

Local foot and mouth disease (FMD) viruses type O, A and SAT2, were kindly provided by Veterinary Serum and Vaccine Research Institute (VSVRI) - Abasia Cairo. These viruses were propagated in BHK-21 cell culture to subject for detection by rapid agglutination test using SPA and virus neutralization test using specific reference anti-sera.

2.2. Samples from infected animals:

Tongue epithelium samples were obtained from experimentally infected cattle during the applied challenge test during the evaluation process *of trivalent FMD vaccine* by the Central Laboratory for Evaluation of Veterinary Biologics. These samples were processed and propagated 3 times in BHK21 cell culture and used in RAT.

2.3. Cell culture:

Baby hamster kidney (BHK_{21}) cell culture was supplied by VSVRI and used for virus propagation; virus neutralization and serum neutralization test.

2.4. Serum samples:

Fifty serum samples were obtained from vaccinated cattle with trivalent *FMD* vaccine in addition to reference *anti-FMD sera (type O; A and SAT2)* were supplied by the Department of Foot and Mouth Disease Research, VSVRI. These sera were subjected for detection of *FMD* antibodies by rapid agglutination test using *SPA* and *SNT* using specific *FMD type O, A and SAT2.*

2.5. Bacterial strain:

Cowan-1 strain of S.aureus was supplied by Prof. Dr. Saad, M.A.M (Animal Reproduction Research Institute; Al-haram Giza) for preparation of *SPA* according to Kessler (1975) and Sting et al (1990).

2.6. Preparation of SPA suspension according to Kessler (1975):

Cowan 1 strain of *S. aureus* was cultivated in one liter of 199 medium supplemented with 0.5% lacto-albumin hydrolysate and 0.5% yeast extract. The inoculated medium was dispended in bottle was incubated at $37C^0$ for 24hours with gentle shaking every 3 hrs. Growing bacteria were collected by centrifugation at 800xG for 10 minutes and washed twice with PBS at PH 7.2 containing0.05% (w/v) sodium azide (PBS azide). Bacteria were resuspended to approximately 10% (w/v) concentration in PBS- azide. Fixed bacteria were then washed once with PBS azide without formalin and re-suspended again into the same buffer to 10% concentration. The suspended bacteria were killed by heating at $80C^0$ with rapid swirling in water bath for 5 min followed by rapid cooling in PBS azide

and finally made to 10% suspension. The SPA suspension could be stored at 4^0C where it was stable for at least 4 months. Before use the SPA suspension should be treated with NP-40 (0.5%) in Net Buffer PH 8.0; for 20 min at room temperature. The treated SPA suspension was then washed once with 0.05% NP-40 in Net buffer and finally re-suspend to the original concentration (10% suspension) in the later buffer.

2.7. Virus neutralization test (VNT):

It was carried out according to OIE (2000) using the microtiter method to identify the obtained FMDV isolate.

2.8. Serum neutralization test (SNT):

It was carried out on serum samples obtained from the 100 cattle included in the present work before and post vaccination on different periods. The test was carried out according to Ferreira (1976).

2.9. Rapid agglutination test (RAT):

Rapid agglutination test was carried out on *FMD virus type O; A and SAT2* obtained from infected BHK21 cell culture for detection of the virus antigens using specific anti-sera and on vaccinated cattle sera and reference FMD anti-sera using the prepared SPA. The test was carried out according to Montassier et al (1994).

3. RESULTS:

SPA was prepared and used for application of *RAT* on processed infected tongue epithelium and *FMD virus* infected cell culture. It was found that both of *RAT and* traditional *VNT* were able to detect the antigens of *FMDV type O; A and SAT* with titer ranged from ≤ log10 up to 7log10 $TCID_{50}$/ml with faster results obtained by *RAT* within 2-5 minutes while VNT needs 18-24 hors (table-1). *RAT* carried out on serum samples from vaccinated animals revealed positive results with titers similar to those obtained by *SNT* [2≤ up to 256] for the three virus types as tabulated in table (2) (within 2-5 minutes for *RAT* and 18-24 hours for *SNT*). It was noticed that the strength of agglutination was strong with high titers of virus antigen or antibodies and weak with lower titers as demonstrated in photo (1&2).

Table (1): Detection of FMD viruses in infected cell culture and processed tongue epithelium fluids

Applied test	Detected FMD virus titer			
	Type O	Type A	Type SAT2	Required time
Virus neutralization	From log10≤ up to 7log10 $TCID_{50}$/ml			18-24 hours
Agglutination test				2-5 minutes

Virus neutralization test was carried out using specific anti-sera in BHK21 cell culture. Agglutination test was carried out using specific anti-sera and SPA.

Table (2): Detection of FMD antibodies

Applied test	Detected FMD antibody titer			
	Type O	Type A	Type SAT2	Required time
Serum neutralization test	From 2≤ up to 256			18-24 hours
Agglutination test				2-5 minutes

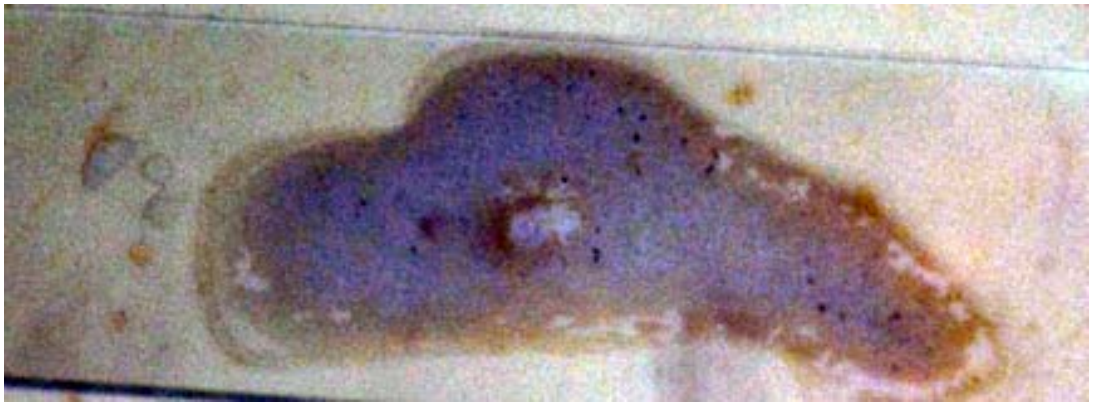

Photo (1): Strong agglutination reaction

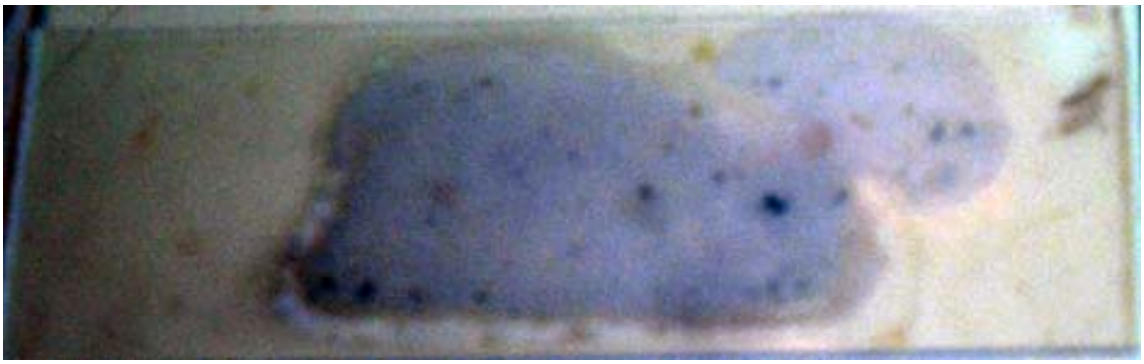

Photo (2): Weak agglutination reaction

4. DISCUSSION:

It is a golden goal to reach rapid; specific and accurate diagnosis of *FMD* through the detection of the virus antigens and antibodies. Successful preparation of *SPA* was confirmed through the present work and used for application of rapid agglutination test *(RAT)* on both of processed infected tongue epithelium and infected cell culture fluid for detection of *FMD* virus antigens in comparison with the traditional *VNT* showing that both tests were able to detect the antigens of *FMDV type O; A and SAT*$_2$ with titer ranged from ≤ log10 up to 7log10 $TCID_{50}$/ml but RAT showed faster results within 2-5 minutes while VNT needs 18-24 hors. In addition, RAT carried on serum samples obtained from vaccinated animals revealed positive results with titers similar to those obtained by serum neutralization test (SNT) ranged from ≤ 2 up to 256 for the three virus types as tabulated in table (2) with shorter time (2-5 minutes) than that consumed by SNT (18-24 hours). It was noticed that the strength of agglutination was strong with high titers of virus antigen or antibodies and weak with lower titers as demonstrated in photo (1&2). These findings could be explained depending on the fact that the reactivity of immunoglobulin-binding proteins for immunoglobulin proteins (Igs) of mammalian species is well known. These proteins are staphylococcal protein-A (*SPA*), streptococcal protein G *(SPG)*, staph protein AG-horse radish peroxidase (SPAG-HRP). These conjugates have a potential use in epidemiological surveys of zoonotic infections (Angel Alberto Justiz – Vaillant, 2013).

Hanaa et al (2011) demonstrated that due to the nature of SPA to bind with FC portion of the most mammalian immune-globulins class G. Therefore, this protein has a good ability to react with wide range of animal species mainly when anti-immunoglobulin not available as in case of camel IgG.

Similar applications showed typical macroscopic patterns of positive and negative coagulation reactions obtained by interaction of the homogenate supernatants of the skin biopsies (100%) with positive and negative sera of rabbits against lumpy skin disease virus. Staphylococcal protein – A Coagulation was rapid (Carried out in 5-10 min) accurate, sensitive, specific and economic and not require special equipment (Iman and Saad, 2007). Iman et al (2011) aggregated Rhabdoviral particles coated with the specific antibody were observed in addition to their attachment to the surface staphylococcus aurous protein A.

In addition, Sigma showed that protein – A is capable of binding to the Fc portion of immune globulins, especially IgGs, from large number of species. Protein- A is a highly stable cell surface receptor produced by several stations of *Staphylococcus aureus*. Protein A may be conjugated with various receptor molecules, including fluorescent isothiocyanate (FITC), enzyme marker, (peroxidase without affecting the antibody binding site on the molecule.

It was suggested that the discovery of protein A, and subsequently protein G, immune- affinity Chromatography has ground in popularity and is now the standard methodology for the purification of antibodies which many be implemented for selection of different application such as immunodiagnostics (Darcy et al., 2011)

It was concluded that Staphylococcal protein–A *"SPA"* has increasing importance as a tool in both quantitative and qualitative immunological techniques. Montassier et al, (1994) developed a coagulation "COA" test for the detection and typing of foot and mouth disease virus *FMDV* depending on its simplicity; rapidity of performance and its low cost, having a great potential for direct detection and identification of *FMDV* as screening strategy at *FMDV* outbreaks in agreement with Montassier et al (1994) and Saad et al (2000). In addition, Omnia et al (2015) concluded that SPA is safe; easy to be used, high stable, highly sensitive, can be used as rapid field test, of low cost having friendly environmental relationship.

Depending on the obtained results and in agreement with Nielsen et al. (2004)**;** it could be concluded that *Staphylocoocus aureus Cowan strain* is among a group of bacteria that produce protein with capacity attach to immune globulin molecules of various species aiding to rapid detection of bacterial and viral antigens and antibodies in order to reach rapid control of FMD facing different live stocks.

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