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CORRELATION OF 146 S ANTIGEN DOSE WITH THE SERUM NEUTRALIZING ANTIBODY RESPONSE AND THE LEVEL OF PROTECTION INDUCED IN CATTLE BY FOOT AND MOUTH DISEASE VACCINES

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SUMMARY

The main goal of the present work is a comparison of methods for evaluating the potency of Foot and mouth disease (FMD) vaccine in laboratory . FMD virus (O_1/3/93Aga) , (A/1/ Egypt /2006) and SAT2 /2012) were concentrated by using polyethylene glycol 6000. The146 S antigen of different dilutions of concentrated virus for each viral fluids were estimated by using sucrose density gradient ultracentrifugation. The vaccine potency was evaluated in calves susceptible to FMDV free cattle aged 6-8 months . Three vaccines were prepared from each strain with different concentration of 146S (3.2 µg , 2.1 µg and 1.8 µg) , each vaccine were injected in three calves 2ml/ dose .The anti-FMDV antibody titer detected by SNT at 4th Week post vaccination were (1.47 , 2.07 , 2.28 log_{10}) against type (O_1/3/93Aga), (1.47, 2.19 ,2.4 log_{10}) against type (A/1/ Egypt /2006) and (1.44 , 2.04 , 2.31 log_{10}) against type (SAT2 /2012) in relation of 146S in different concentration (1.8, 2.1 and 3.2 µg) per dose of FMD (O) (A) and(SAT 2) respectively . The vaccine dose contain 3.2 µg and 2.1 µg of 146 S can protect animal against challenge with 100 % while the dose have 1.8 µg of 146 S the protection were 60% . The results demonstrated the relation of 146 S antigen in vaccine dose and the protective responses elicited in cattle . From the previous results we concluded that the dose of vaccine which contain 2.1 µg (146S) or more can protect animal against challenge test . So the measuring of 146S during the process of FMD vaccine product facilitate the insurance of efficiency of vaccine quality with SNT the benefits of yielding rapid and applicable results from an inexpensive methods.

INTRODACTION

Foot and mouth disease (FMD) is highly contagious viral disease affecting cloven hoofed animals (camels,pigs,sheep and goat). It causes production loss and constraint imposed on international trade in live animals and their

products (1, 2) . The causative agent is foot and mouth disease virus (FMDV), aphthovirus of the Picornaviridae family . The virus exists in the form of seven major serotypes (O, A, C, SAT 1,SAT2 , SAT3 and ASIA **(3).** The economic consequences of an FMD outbreak include high levels of mortality in young stock , fever, inappetance, , lamness and vesicles on the feet, teats and in the mouth reduced production of milk and meat **(4)** . FMD is classified as a reportable disease by the office international des epizootics (OIE) **(5).**

The FMDV has a positive-sense RNA of 7.5-8.5 kb. The RNA is translated after entery into the cellular cytoplasm with synthesis of proteins required for RNA replication. The input viral RNA also acts as the template for the synthesize positive-sense genomes **(6).** It is generally accepted that the component of FMDV responsible for the stimulation of protective antibody in vaccinated animals is an icosahedra ribonucleoprotien structure with sedimentation coefficient of (146S) antigen **(7,8)** . Certain strains of FMDV produce a second structure which lacks RNA and has a sedimentation coefficient of 75S (**9,10)**. Particles of the 75S component, often referred to as "natural empties", appear to have the same antigenic properties as particles of 146S component **(9) .** A third virus component, usually referred to as 12 S subunit antigen, is a common constituent of virus preparations and can be produced from 146 S particles by heat or mild acid treatment **(11)** . The 12 S particles don't stimulate significant levels of neutralizing antibody against 146 S particles **(7,12)** although both contain VP1 , the protein generally considered to be responsible for stimulation of protective antibody **(13,14)** Thus, the integrity of 146 S particles is almost certainly one of the most important of various factor which influence the potency of vaccine preparations **(15).**

The concentration of 146 S particles in purified preparation was measured by a quantitative sucrose density gradient procedure **(15).** In Egypt , where the disease is endemic , prophylactic vaccination is the only means for control **(16 -18)** . The recommendation of the conference of the permanent

commission of the office international des epizooties (OIE) on foot and mouth disease form the basis for the testing of foot and mouth disease (FMD) vaccine potency measured by quantitative method , a level of 80% protection in cattle after primary vaccination with a single dose following the observation by **(8,19)** .

As the economic impact of an FMD outbreak can be large ,the quality control of vaccines in most counties in strictly regulated , and in Egypt animal challenge tests are prescribed to show vaccine efficacy . As a result of such challenge test animal are either considered protected against clinical sings or not **(20).** This procedure required long- distance transportation of animals and their maintenance in isolation conditions, with consequence of viral escape. In addition, for economic reasons , only one viral strain cold be evaluated at a time **(21).**

Serological testing is an important component of any program to control FMD. So this study was designed and aimed to : i) get safe, efficient and good quality FMD vaccine by application of restricted evaluation of 146 S antigen / dose . ii) study the relation between serum neutralizing antibody titer and 146S antigen per dose of FMD vaccine . In addition to study the relationship between 146S/dose for types (O_1/3/93Aga) , (A/1/ Egypt /2006) and SAT2 /2012) and potency against virus challenged .

MATERIALS AND METHODS

1-Tissue culture

Baby hamster kidney (BHK) cells clones 13 were obtained from the world Reference lab (WRL), Pirbright, surrey, U.K. and the cell were serially passaged and maintained in the FMD research department,Veterinary Serum andVaccineResearchInstitute (VSVRI), Abbasia, Cairo. It used in vaccine preparation and serum neutralization test **(22)** .

2- FMD Virus strains

Locally isolated Foot and Mouth Disease Virus type (O_1/3/93Aga), (A/1/ Egypt /2006) and(SAT2/2012)were propagated in BHK-21 monolayer cell

line as described by **(23).** The seed virus was prepared by adapting virulent cattle tongue virus to BHK21 monolayer cells (six passages) ,according to **(24) .**

3- Infectivity titration

The virus infectivity titer was calculated after 10 fold serial dilution according to **(25).**

4- Virus concentration

The cell culture supernatant of FMD virus of the 7^{th} passage on BHK monolayer with titer 10^8 $TCID_{50}$ of three serotypes (O_1/3/93Aga), (A/1/ Egypt /2006) and (SAT2/2012) were centrifuged at 7000 rpm for 30 minute then concentrated to 1/10 of the original volume using polyethylene glycol (PEG-6000) according to **(19).**

5-Estimation of 146 S to determine cattle dose

The146 S antigen of different dilution of concentrated virus for each viral fluids were estimated by using sucrose density gradient ultracentrifugation (45000 rpm for one hour) by determination the absorbance at 254 nm using ISCO 520 C Density Gradient system as described by **(26,27).**

6- Vaccine preparation

According to **(28),** FMD virus serotypes were inactivated with 1% of 0.1 M of binaryetheleneamine (BEA ; Sigma) in 0.2 N of NaOH and the pH adjusted to 8.0 by sodium bicarbonate . The virus and BEI mixture was mixed well and inocubated at 37ºC for 24 hours with continuous steering for inactivation of virus . At the end of inactivation period, residual BEA was neutralized by 2% sodium thiosulphate .

Inactivated FMD oil adjuvenated Vaccines were formulated according to **(29)** , each inactivated FMDV strain was mixed with oil adjuvant Mantonide ISA 206 (kindly supplied from Seppic, France). The ratio of the aqueous antigen to the oil adjuvant was 50:50 The emulsions were produced by recycling the aqueous antigen - oil mixture several times . Sterility and safety of the prepared vaccines were done according to **(30).** FMD virus concentration in the final vaccine formula was adjusted equal (3.2 µg , 2.1 µg and 1.8 µg) 146S viral particles /dose/ serotype , pH brought to 8.2 with glycol buffer according to **(31).**The vaccine were stored at 4 ºC until used .

7-Animals

Twelve groups (each group contain 3 animals) were clinically healthy and free from antibodies against FMD virus as proved by using SNT .

a-The first three group were vaccinated S/C in the dewlap with recommended dose 2 ml of vaccine batches contains (3.2 µg , 2.1 µg and 1.8 µg) 146 S viral particles / dose / serotype (O_1/3/93Aga).

b-Second three group were vaccinated S/C in the dewlap with recommended dose 2 ml of vaccine batches contains (3.2 µg , 2.1 µg and 1.8 µg) 146 S viral particles / dose / serotype (A/1/ Egypt /2006) .

c-Third three group were vaccinated S/C in the dewlap with recommended dose 2 ml of vaccine batches contains (3.2 µg , 2.1 µg and 1.8 µg) 146 S viral particles / dose / serotype (SAT2 /2012).

d - Fourth group (6 animals) kept as non- vaccinated controls

Sterility test

Sterility of vaccines were tested according to **(32).**

Safety test

The vaccine was tested to be safe as cited by **(33)** .

Challenge test

Four week post vaccination according to **(34),** the first three groups were inoculated by 10^4 cattle infective $dose_{50}$ of the homologous virus serotype (O_1/3/93Aga) , second three group were inoculated with 10^4 of (A/1/ Egypt /2006) and the third three group were inoculated with 10^4 of (SAT2 / 2012) by intradermolingual route and also control unvaccinated calves for each strain . Animal were observed for 7 days post inoculation of challenge virus.

Serological test

Blood samples were collected from all animals pre and weekly for four weeks post vaccination the time of challenge. Serum neutralization test (SNT) was performed by the micro technique as described by **(30)** using BHK cells. Neutralizing titer were determined against three FMDV serotypes strains (O,A & SAT 2) and The neutralization titer of the tested sera was calculated expressed as the $\log_{10}$ of the inverse dilution which protected 50% of wells according to the **(35).**

RESULTS AND DISCUSSION

Foot and mouth disease virus (FMDV) exists as seven different serotypes one serotype does not protect against the others **(36 , 37**) . In addition , many antigenic strains have been recognized within serotypes **(38)** and some of these differences may be important in relation to cross protection , therefore , serological tests are routinely used as part of the process for selecting the most appropriate vaccine strain for protection against a given field isolate **(39) .**

The humoral response in sera against foot-and mouth disease virus (FMDV) is well documented **(40),** and the contribution of antibodies to the major immune defense against the virus is clear **(41 - 43)** , during which they have been applied to the determination of vaccine efficacy **(44-46).** It has even been possible to relate particular titers of virus-specific antibody measured in the SNT to protection against infection **(42 ,47)**. Similar analyses have been carried out using immunoassay technology, SNT has found successful application in FMDV vaccinology **(42 ,47).**

Serum neutralization test has been shown to be a golden tool for assessing specific neutralizing antibodies **(48)** It has been used to predict their degree of protection against virulent challenge in cattle **(46-49).**
The concentration of payload antigen used in these study was maintained (3.2 µg , 2.1 µg and 1.8 µg) 146 S viral particles / dose / serotype.
(Tables 1 , 2 and 3) showed that serum neutralizing antibody titers of calves vaccinated with different amount of 146 S/ dose in foot and mouth disease vaccine strain O , A and SAT 2 . The potency of FMD vaccine shown in table (1) illiustrated SNT titers of cattle vaccinated with different 146S of FMD antigen type O_1. The mean SNT antibody titers of cattle in the first week (0.84 , 0.9, 1.08 log_{10}) , 2^{nd} week post vaccination (1.11, 1.35 , 1.62 log_{10}), 3^{rd} WPV (1.27, 1.71 , 1.92 log_{10}) and 4^{th}WPV (1.47, 2.07, 2.28 log_{10}) for 146S of FMD antigen type O_1 (1.8 , 2.1 and 3.2 µg) per dose of FMD vaccine respectively .

Challenged FMD virus serotype (O_1) was inoculated after 4 WPV, the animals 1 and 4 of first group vaccinated with FMD 1.8 µg 146S per dose have local lesion on the tounge while animals number 2 ,3 &5 of the same group were protected , groups 2 and 3 which received 3.2 µg and 2.1 µg were protected. Table 2 illiustrated SNT titers of cattle vaccinated with different 146S of FMD antigen type A .The mean SNT antibody titers of cattle in the first week
(0.78 ,0.81,0.93 log_{10} , 2nd week post vaccination (1.11, 1.32 , 1.5 log_{10}) , 3rd WPV (1.29, 1.74 , 1.89 log_{10}) and 4th WPV (1.5, 2.19, 2.4 log_{10}) for animals received 1.8, 2.1 and 3.2 µg of 146S of FMD antigen type A vaccinal dose respectively .

Challenged FMD virus serotype (A/1 /Egypt) was inoculated after 4 WPV ,the animals 1 and 2 of second group vaccinated with FMD 1.8 µg 146S per dose have local lesion on the tounge while animals number 3 ,4 &5 of the same group were protected. While groups 2 and 3 were protected.Table 3 illiustrated SNT titers of cattle vaccinated with different 146S of FMD antigen type A . The mean SNT antibody titers of cattle in the first week (0.6 , 0.81, 1.11 log_{10}),2nd WPV(1.08, 1.38, 1.71 log_{10}) , 3rd WPV (1.26, 1.83, 2.1 log_{10}) and 4th WPV (1.44 , 2.04 , 2.31 log_{10}) 146S of FMD antigen type SAT 2 (1.8, 2.1 and 3.2 µg) per dose of FMD vaccine respectively .Challenged FMD virus serotype (SAT2 / 2012) was inoculated at 4th WPV, the animals 1 and 5 of second group vaccinated with FMD 1.8 µg 146S per dose have local lesion on the tounge while animals number 2, 3 and 4 of the same group were protected. While groups 2 and 3 were protected. Control animals showed severe generalized lesions in mouth, fore and hind limbs .

From the previous results we recorded the vaccinated cattle with SN titers greater than 1.5 log_{10} were protected from generalized FMD, while cattle with SN titers less than 1.5 were not protected and developed generalized infection **(46-48)** . All animal vaccinated with vaccine contain 2.1 or 3.2 µg /dose of any FMD antigen strain were protected against challenge virus and have SN titers more than protective level 1.5 log These results were

consistent with the results reported by **(31**) who said that the dose of FMD vaccine should contain not less than 2 µg /dose / serotype . and also with **(19)** who recorded that the antibody titre is one of referenced criteria to evaluate vaccine potency as it is positively linked with protection rate , but it influenced by factors such as vaccines antigen content (14 S) , animal individual status .These results also were in agreement with **(51)** who recorded that then high potency vaccines induce protection against heterologous challenge with FMDV .All results and observation are reported by **(20,50,52**) who stated that the relation between serum neutralizing antibody titers and protection from challenge following a single dose of primary vaccination .Serological examination of the animals by SNT revealed that the profile of response were similar to of the results of challenge test .

Evaluation of the potency of FMD vaccines was performed in cattle and was based on the protection of vaccinated animals against challenge by live virus . This procedure required long- distance transportation of animals and their maintenance in isolation conditions , with consequence of viral escape .In addition , for economic reasons , only one viral strain cold be evaluated at a time .In conclosion the previous results we assess the possibility of using the 146 S as a method for evaluating the efficacy of FMD vaccines with SNT the benefits of yielding rapid and applicable results from an inexpensive methodology .

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