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Emergency evaluation for existing vaccine against recently isolated Foot and mouth disease virus type SAT2 in Egypt 2018

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Foot and mouth disease virus is a highly infectious and contagious pathogen. Recently the topotype VII, Lib-12 lineage of serotype SAT2 was reported through outbreaks in Egypt during 2018. Vaccination is an effective way to control and combat the foot and mouth disease virus outbreaks especially in endemic areas like Egypt. The present study was aimed to evaluate the efficacy of the current produced foot and mouth disease vaccine, against the recently isolated field strain foot and mouth disease virus SAT2 topotype VII, Lib-12 lineage (SAT2 Libya), by applying in vitro and in vivo studies. Two batches of the current foot and mouth disease virus vaccine were inoculated in calves. At the 28th day post-vaccination serum samples were collected and tested against tissue culture adapted foot and mouth disease virus SAT2 Libya and SAT2/EGY/2/2012 using virus neutralization test to determine serological relationship (r1-value). The challenge test for vaccinated calves was carried out against the virulent foot and mouth disease virus SAT2 Libya. It was found that neutralizing antibody titers induced by the two vaccine batches (1 and 2) and those in unvaccinated animals were 0.48, 0.39 and 0.15 log₁₀ TCID₅₀/mL, respectively, while the challenge revealed protection values of 20%, 0% and 0%, respectively. Furthermore, the r1 values were 0.195 and 0.186 for vaccine batches (1 and 2), respectively. It was concluded that the available local commercial inactivated foot and mouth disease virus vaccine batches (SAT2 SAT2/EGY/2/2012) are unable to protect calves against the current circulating foot and mouth disease virus field isolate SAT2 topotype VII, Lib-12 lineage, thus it is highly recommended to update the existing vaccines with the present isolated strain.

Keywords: Foot-and-Mouth Disease; vaccine potency; livestock; serogroup.

Introduction

Foot and mouth disease virus (FMDV) is a highly infectious and contagious pathogen that infects cloven-hooved livestock and wildlife species and influences economic and animal welfare grounds.⁽¹⁾

FMDV belongs to the *Aphthovirus* genus, *Picornaviridae* family.⁽²⁾ It spreads by direct or indirect contact with infected animals and their secretions or by contaminated feed. Airborne transmission can occur over extensive distances by infectious aerosols (droplets).^(3,4)

Egypt is endemic for FMDV from 1950 onward. Three FMDV serotypes: O, A and South-Africa territories-2 (SAT2) have been reported and circulated in the field till now. O serotype has a long history of causing regular outbreaks in Egypt and it is the dominant strain. In 2006, an East African type A strain was introduced and reported in Egypt.⁽⁵⁾ In 2012-2013, the A serotype

of Asian topotype related to Iran genotype was reported,⁽⁶⁾ also in 2012,⁽⁷⁾ the FMDV SAT2 topotype VII was recorded in Egypt. Recently, topotype East Africa 3 (EA-3), genotype IV (African topotype) and Lib-12 lineage (topotypes VII) strains of serotypes O, A and SAT2 were reported, respectively. The topotype VII, Lib-12 lineage of serotype SAT2 was reported in outbreaks during 2018.^(8,9)

Vaccination is the effective tool to control and combat the FMDV outbreaks, especially in endemic areas like Egypt. The available trivalent inactivated FMDV vaccine (serotype O, topotype Middle East-South Africa, Panasia2 lineage; serotype A, Asian topotype, Iran05 lineage; and serotype SAT2, topotype VII, Gharbia12 lineage) is produced locally and used in vaccination campaigns in Egypt.⁽¹⁰⁾

The efficacy of available FMDV vaccines against the newly isolated field strain FMDV SAT2 topotype VII,

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Lib-12 lineage (SAT2 Libya) needs to be determined because recent circulating field isolates exhibit genomic variation in relation to the vaccinal strain (SAT2) and FMDV serotypes don't confer complete cross protection against sub-serotypes and newly isolated field strains;^(8,9) therefore, in the present work, *in vitro* and *in vivo* studies are carried out, to know to what extent current locally produced vaccines can protect calves against the recently isolated strain.

Materials and Methods

Virus

FMDV serotype SAT2, topotype VII, Lib-12 lineage (SAT2 Libya) was isolated and identified at the Animal Health Research Institute by Real time polymerase chain reaction (RT-PCR)⁽⁹⁾ and it was officially supplied to the Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) to be used in the evaluation of the available inactivated FMDV vaccine. This virus was adapted on BHK21 cell line having a titer of 10^4 TCID₅₀/mL to be used for virus neutralization test (VNT) and as a virulent virus for the challenge test of experimentally vaccinated calves. FMDV SAT2, topotype VII, Gharbia12 lineage (SAT2/EGY/2/2012) was supplied by the Strain Bank Department at CLEVB to be used for FMDV vaccine evaluation according to OIE.⁽¹¹⁾

Vaccine

Local commercial trivalent oil inactivated FMDV vaccine batches (n=2) produced in Egypt and prepared from local isolate serotypes O/EGY/4/2012, A/EGY/1/2012 and SAT2/EGY/2/2012 were used in this study. The available vaccine batches were previously evaluated with satisfactory results by CLEVB, these data are available and compared with current results.

Calves and experimental design

Fourteen calves (local breed) 6 to 8 months old, about 200-300 kg body weight, were allotted into 4 groups and kept in separate breeding stables where the animals were maintained under veterinary care conditions and free access to regular concentrated ration and water. These calves were previously screened for the presence of specific antibodies against FMDV type SAT2 using VNT and did not reveal any specific antibodies (seronegative). The four groups are presented below:

Group (1): two calves were used for virus titration.

Group (2): five calves were vaccinated subcutaneously (S/C) with one field dose according to the manufacture insert of the previously evaluated local commercial FMDV vaccine batch (1).

Group (3): five calves were vaccinated S/C) with one field dose according to the manufacture insert of the previously evaluated local commercial FMDV vaccine batch (2).

Group (4): two calves were kept as non-vaccinated group (control positive for challenge test).

Virus neutralization test

The test was performed in BHK-21 cells by using the microtiter neutralization technique as described by Ferriera⁽¹²⁾ and OIE.⁽¹¹⁾

Virus titration in calves' tongue

Infectivity titration of FMDV strain SAT2 topotype VII, Lib-12 lineage (SAT2 Libya) to be used in the challenge test, was carried out. Serial tenfold dilutions of the virus in Hank's balanced salt solution were prepared for viral titration in the bovine tongue. Dilutions were inoculated in the tongue of calves (two calves) whom were hypnotized with a tranquilizer (Xylazine 20 mg/mL) with a dose of 0.2 mg/kg body weight before virus inoculation. The tongue of calves was divided into rows by using Indian ink; each dilution was inoculated intradermolingually in a row, at five sites, using 0.1 mL for each. The inoculated tongue sites were examined carefully and the induced lesions were recorded daily during 3 days post-inoculation to assess vesicles formation as reported by Dekker et al;⁽¹³⁾ to avoid rupture of these vesicles, a tranquilizer was applied to cattle before tongue examination. The virus titer was calculated and expressed as Log₁₀ Bovine infective dose BID₅₀/mL according to Karber.⁽¹⁴⁾

Challenge test

At 28th day post vaccination, both vaccinated calves' groups (2 and 3) and control group (4) were moved to challenge room at animal house facility where were challenged (after bleeding). The challenge virus serotype SAT2, topotype VII, Lib-12 lineage (SAT2 Libya) was adjusted to a titer of 10^4 BID₅₀/0.3mL and inoculated intradermolingual into 3 to 5 sites for each animal. The challenged calves were observed daily for significant clinical signs (tongue and feet ulcers) of FMDV during 7 days. Animals that showed clinical signs were subjected

to virus re-isolation. Positive control animals must show at least three feet ulcers for the test to be considered valid. The protection level against generalized foot infection should be not less than 75% (at least 3.75 animals out of 5 vaccinated animals); the mean value for expectancy of protection (EPP) of 75% indicates the vaccine strain is suitable to be used together with appropriate field measures to control outbreaks with the field strain under test.^(11,15)

The animals that were used for the challenge test were hypnotized before the challenge and at one time of examination. The infected animals received veterinary care and medical treatment until complete recovery and moved to a designated room for ex-experimental animals.

The serological relationship (r1-value) determination

At 28th day post-vaccination, sera from animal immunized with vaccine batches 1 and 2 (groups 2 and 3) were collected (before the challenge) and tested against the vaccines strain (SAT2/EGY/2/2012) and the field isolated strain (SAT2 toptotype VII, Lib-12 lineage) using VNT.^(11,16) The serological relationships (r1-value) were calculated according to the following equation:

$$r1 - value = \frac{\text{serum titer against heterologous field strain}}{\text{serum titer against homologous vaccine strain}}$$

Interpretation of r1-value:^(11,17)

$R < 0.3$ indicated highly significant antigenic variation from the vaccine strains and another vaccine strain should be chosen.

$R > 0.3$ demonstrated that the vaccine and field strains are sufficiently similar and the vaccine could provide good protection.

Ethical approval

Institutional Animal Care and Use Committee at Central Laboratory for Evaluation of Veterinary Biologics hereby acknowledges the research manuscript and it has been reviewed under our research authority and is deemed compliance with bioethical standards in good faith.

Results

Local commercial trivalent oil inactivated FMDV vaccine batches (n=2) were produced in Egypt and prepared from local isolate serotypes O/EGY/4/2012, A/EGY/1/2012, and SAT2/EGY/2/2012 which were previously evaluated at CLEVB for their potency against FMDV serotype SAT2/EGY/2/2012 using VNT and challenge test. The FMDV vaccine batches indicated satisfactory results as shown in Table 1.

Infectivity titration of FMDV strain SAT2 toptotype VII, Lib-12 lineage (SAT2 Libya) was assessed in calves' tongue (group 1). The titer was 10^6 BID₅₀/mL.

Both vaccinated groups (2 and 3) with inactivated FMDV vaccine batches (1 and 2) and control calves group (4 non-vaccinated) that were bled before the challenge for screening of antibody titer at 28th day post-vaccination using VNT, showed neutralizing antibody titers 0.48, 0.39 and 0.15 log₁₀, respectively; regarding the challenge test, the protection level indicated 20%, 0% and 0% against SAT2 Libya, respectively as shown in Table 1, in comparison to the results obtained against FMDV serotype SAT2/EGY/2/2012.

Characteristic lesions in tongue and feet were recorded at 7-day post challenge for vaccinated calf groups (2 and 3) and control group (4); severe lesions in the four feet

Table 1. Evaluation of humoral immune response and protection level of vaccinated calves with inactivated FMDV vaccine batches using VNT and challenge test.

Vaccine batches No.	SAT2/EGY/2/2012		SAT2, toptotype VII, Lib-12 lineage (SAT2 Libya)		
	1	2	1 (Group 2)	2 (Group 3)	Positive Control (Group 4)
* VNT antibody titer (Log ₁₀ TCID ₅₀)	2.46	2.1	0.48 ▼	0.39 ▼	0.15
** Protection level (Percentage %)	100	100	20 ▼	0 ▼	0

* The protective virus neutralizing antibody titer ≥ 1.65 log₁₀

** The protection level (%) of challenge test $\geq 75\%$

▼ Unsatisfactory results

Table 2. r1-value of FMDV type SAT2 topotype VII, Lib-12 lineage (SAT2 Libya) using VNT.

Vaccine Batches No.	1 (Group 2)	2 (Group 3)
r1 value	0.195	0.186

R < 0.3 indicated highly significant antigenic variation from the vaccine strains and another vaccine strain should be chosen.

R > 0.3 demonstrated that the vaccine and field strains are sufficiently similar and the vaccine would provide good protection.

and mild to moderate lesions at the tongue were shown for all recorded positive animals (challenge test).

The serological relationship (r1-value) of the recently isolated FMDV type SAT2 topotype VII, Lib-12 lineage (SAT2 Libya) was determined by VNT, using sera of vaccinated calves (group 2 and group 3) with vaccine batches 1 and 2. The r1 values were 0.195 and 0.186, respectively as shown in Table 2.

Discussion

In the 2018 FMDV outbreak, the Egyptian authorities declared the emergency and triggered highly control and precautionary measures for tackling the outbreak. Extensive surveillance promoted early isolation and identification of FMDV and rapid response to combat the FMDV outbreak efficiently. The genomic sequencing and variation analysis studies indicated the newly emerged FMDV was closely related to strains isolated from Libya (topotype VII, lineage3) and clearly differed from endemic strains SAT2/GVII/Gharbia/Egy/2012 and SAT2/GVII/Alex/Egy/2012 (topotype VII, lineage 2).^(8,9)

The control of FMDV disease mainly depends on the availability of effective vaccines that can be selected based on genome alignment, epidemiological information, and serological cross-reactivity of bovine post-vaccinal serum with circulating viruses. In addition, the availability of sufficient doses of vaccines of good quality and potency is also equally considered.^(18,19,20) Polyvalent inactivated vaccines are currently used in Egypt for FMDV prevention, but the recent circulating field isolates indicated genomic variation in relation to the vaccinal strain (SAT2),^(8,9) a likelihood of impotence of existing vaccines; therefore we investigate the efficacy of the available local commercial vaccine against the recently isolated strain FMDV strain SAT2 topotype VII, Lib-12 lineage (SAT2 Libya) using VNT and challenge test.

The local commercial trivalent oil inactivated FMDV vaccine batches (n=2) were produced in Egypt,

prepared from local isolate serotypes O/EGY/4/2012, A/EGY/1/2012 and SAT2/EGY/2/2012 and previously evaluated with satisfactory results. The FMDV vaccine batches were inoculated (S/C) in calves from groups 2 and 3. In calves immunized with these two vaccine batches, the humoral immune response against the FMDV field strain SAT2, topotype VII, Lib-12 lineage, was determined by VNT and exhibited titers of 0.48 and 0.39 log₁₀, respectively; while the humoral immune response against the FMDV vaccinal strain SAT2/EGY/2/2012 using VNT indicated 2.46 and 2.1 log₁₀, respectively, regarding the minimum protective virus-neutralizing antibody titer (1/45) 1.65 log₁₀.⁽¹¹⁾

In a similar study it was found that the immunogenicity of SAT2 strain in cattle recorded high and uniform neutralizing antibodies levels after 2 weeks of vaccination $\geq 1:45$.⁽²¹⁾ The serological relationship (r1-value) of the recently isolated FMDV type SAT2 topotype VII, Lib-12 lineage (SAT2 Libya) was assessed and calculated, the r1-values were 0.195 and 0.186 for vaccine batches 1 and 2, respectively. According to OIE,⁽¹¹⁾ for neutralization, r1-values greater than 0.3 indicate a close antigenic relationship between the vaccine strain and the field isolate, a likelihood that the vaccine strain confers cross-protection against the field strain; whereas r1-values less than 0.3 indicate a lack of such cross-protection. Interestingly, similar studies demonstrated that a system for FMDV strain differentiation based on the use of the virus neutralization reaction is recommended, taking into account the statistical and biological significance of observed r values.^(22,23)

Another recent study compared the antigenic relationship (r1 values) of the outbreak viruses with reference antisera and indicated a good vaccine match with 90% of r1 values > 0.3. The r1 values for the 2013/2014 outbreak viruses were 0.4 and above for the three South African vaccine/reference strains. These results confirmed the presence of genetic and antigenic variability in SAT2 viruses and suggest the emergence

of new variants at the wildlife–livestock interface in South Africa.⁽²⁴⁾

The results for protection level of vaccine batches (1 and 2) against FMDV strain SAT2 topotype VII, Lib-12 lineage indicated 20% and 0%, respectively, compared to challenge test results against FMDV SAT2/EGY/2/2012, which indicated 100% for both batches. The OIE⁽¹¹⁾ recommended a 75% cut off of vaccine potency acceptance for vaccines intended for use in regular vaccination regimens, and the expectancy protection method using a 75% cut off for fitness of a vaccine to be used against field isolates, which has been used with great success in South America to control outbreaks.⁽²⁵⁾

Previous studies^(8,9) indicated the clear genetic variation between the FMDV virus SAT2 Libya (topotype VII, lineage 3) and the endemic strains SAT2/GVII/Gharbia/Egy/2012 and SAT2/GVII/Alex/Egy/2012 (topotype VII, lineage 2), which is consistent with the relevant results of the current study that demonstrated the insufficient antigenic relationship of the current circulating VFA SAT2 topotype VII, lineage Lib-12 with the VFA vaccine strain SAT2/EGY/2/2012, and that existing vaccines against FMDV cannot induce cross-protection against circulating field isolation neither *in vitro* nor *in vivo* studies.

Conclusions

The available local commercial inactivated FMDV vaccine batches (SAT2 SAT2/EGY/2/2012) are impotent and not effective against the current circulating FMDV filed isolate SAT2 topotype VII, Lib-12 lineage; it is highly recommended to update the existing vaccines with the isolated strain.

Conflict of interest

The author certifies that he has no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Evaluación de emergencia de una vacuna existente contra el virus de la fiebre aftosa tipo SAT2, aislado recientemente en Egipto en el 2018

Resumen

El virus de la fiebre aftosa es un patógeno altamente infeccioso y contagioso. Recientemente, el topotipo VII, linaje Lib-12 del serotipo SAT2 se describió en brotes en Egipto durante 2018. La vacunación es una forma eficaz de controlar y combatir los brotes del virus de la fiebre aftosa, especialmente en áreas endémicas como Egipto. El presente estudio tuvo como objetivo evaluar la eficacia de la vacuna contra la fiebre aftosa que se produce actualmente, frente a la cepa de campo recientemente aislada del virus de la fiebre aftosa SAT2 topotipo VII, linaje Lib-12 (SAT2 Libia), mediante la aplicación de estudios *in vitro* e *in vivo*. Se inocularon en terneros, dos lotes de la vacuna actual contra el virus de la fiebre aftosa. A los 28 días posteriores a la vacunación, se recolectaron muestras de suero y se analizaron contra el virus de la fiebre aftosa SAT2 Libia adaptado a cultivo de tejidos y SAT2/EGY/2/2012 utilizando una prueba de neutralización viral para determinar la relación serológica (valor *r*₁). El ensayo de reto en terneros vacunados se llevó a cabo empleando una cepa virulenta de la fiebre aftosa SAT2 Libia. Se encontró que los títulos de anticuerpos neutralizantes inducidos por los dos lotes de vacuna (1 y 2) y los de animales no vacunados, fueron 0,48, 0,39 y 0,15 log₁₀ DICT₅₀/mL, respectivamente, mientras que la prueba reveló valores de protección de 20%, 0% y 0%, respectivamente. Además, los valores de *r*₁ fueron 0,195 y 0,186 para los lotes de vacuna (1 y 2), respectivamente. Se llegó a la conclusión de que los lotes de vacunas locales comerciales inactivadas disponibles actualmente (SAT2 SAT2/EGY/2/2012) no protegen a los terneros contra el virus circulante de la fiebre aftosa SAT2 topotipo VII, linaje Lib-12 que se aisló recientemente, por lo que es recomendable actualizar las vacunas existentes con la cepa aislada actualmente.

Palabras clave: Fiebre aftosa; potencia de la vacuna; ganado; serogrupo.
