Studies On Alternative Methods For Titration Of Virulent FMD Virus Type "O"

Saad M A *, Nermeen Gouda*, Amal I Abdel Hady*, Abeer A Talaat, Bakr A A ** and Elham A El-Ebiary***

*Central lab for evaluation of veterinary biologics, Abbasia, Cairo.

**Vet. Serum and vaccine research institute, Abbasia, Cairo

ABSTRACT

The historical background of FMD vaccine production is briefly described, improvement Achieved through the use of monolayer and suspension cultures outlined elements that are crucial in the production of modern vaccines are discuss such as inactivation of viral antigen, successive concentration and purification of the antigen and the final formulation of the vaccine. To evaluate such vaccine we must be have an identified virulent FMD virus with accurate titer which is the backbone in potency test for evaluation of FMD vaccine these studies shows the comparative between titration of virulent FMDV type O1/3/93 Egypt in cattle and mice. There prepared from the epithelium tongue of infected cattle were titrated in cattle and unweaned baby mice the titers were 6.4, 6.1, 6.3 $\log_{10}$ cattle ID_{50}/ml respectively while in mice the titers in the same batches were 7.3, 7.2, 7.0 $\log_{10}$ mice ID_{50}/ml respectively hence the challenge test was done in cattle. We should depend on the titration in cattle as it is more sensitive, accurate and safe time than in mice.

INTRODUCTION

Foot-and mouth Disease (FMD) is a serious viral disease principally affecting cattle, sheep, goats, pigs, buffalo and deer. The disease exists in 7 serotypes which are clinically indistinguishable but antigenically distinct. FMD has extreme communicability and can spread rapidly through livestock populations and across continents *(1)*. Infection may be through the natural route of infection via the upper respiratory tract or through injection of the virus .Initial virus replication usually occurs in the pharyngeal epithelium resulting in primary vesicles, fever and viraemia can occur with 1-2 days resulting in virus excretion from the respiratory tract, faeces, urine, saliva, milk and semen, virus entering the blood disseminates to various predilection sites such as the mouse, nose, hooves and also sometimes udder and teats any which secondary vesicles occur and from which further virus is released *(2)*.

The disease has very serious consequences including: adverse animal welfare effects due to the formation of acutely painful vesicular lesions of the mouth, feet and udder and fatalities in immature livestock *(3)*. FMD has both direct and indirect economic effects. These include: loss of productivity of meat and milk, mortalities, loss of national trading status and markets for live animals and animal products; interference with agriculture and tourism and the costs of applying control measures. These can encompass movement standstill orders, slaughter and disposal of animals, cleaning and disinfection, compensation and vaccination. To have a potent vaccine we must be apply a restricted control of the product quality whatever the process control results are. As for others inactivated virus vaccine FMD vaccine has the same mean quality criteria which should be considered before release of the vaccine in the field those criteria are o *(4)*:

1- Physical and chemical specifications.
2- Sterility and safety test.
3- Potency test.

In regard to apply the actual potency test we must have an identified field isolated virulent FMD virus with accurate titer to carry out the challenge test from that come the most important demand for design this work. It is desirable, when proposing an assay method to attempt to determine the precision of the method and also to compare the results with those given by established titration procedures this paper represents the results of

a number of comparative titration of some FMDV O1 strain in cattle and mice to establish a sensitive, accurate and rapid method for titration FMD virus.

MATERIAL AND METHODS

1. Virulent FMD virus

Local isolate of foot and mouse disease virus type O1 /3/93 EGYPT has been identified by the animal virus research institute, Pirbright, UK. The virus was inoculated intradermolingually into susceptible calf, and the detached tongue epithelium was collected aseptically in 50 % glycerin–phosphate buffer *(5)*.

2. Calves

Six calves 6-8 months old with 200 – 250 kg body weight were used. This calves were clinically healthy and free from antibodies against foot and mouth disease virus type O1 /93 EGYPT tested by serum neutralization test (SNT).

3. Unweaned baby mice

Unweaned Swiss baby mice, 2-3 days old (Charl's River strain, USA) were used for the titration of virulent FMD virus *(6)*.

4.Maintenance medium

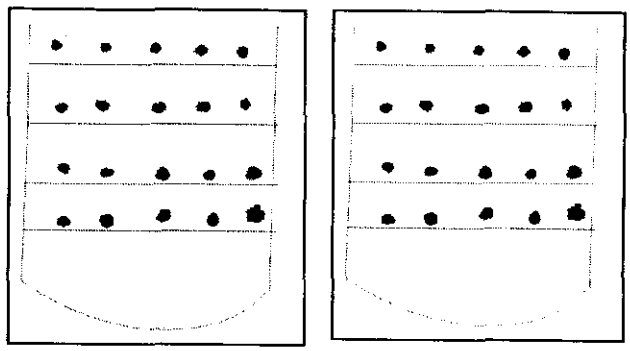
Minimum essential medium (MEM)with Hank's salt ,L-glutamine and without sodium bicarbonate was obtained from GIBCO BRL,UK .It was used as maintenance medium after the addition of 1-2% horse serum and the pH adjusted to 7.2-7.4.

5. Indian ink

Art.no.44357000
Royal talens, Holland used for divide the calf tongue into rows.

Experimental Design

Serial tenfold dilutions in Hank's balanced salt solution were prepared from the virus to be titrated for each dilution a group of 6-8 unweaned baby mice were inoculated I\P with 0.1 ml I\P in each mice *(7)*. Deaths and paralysis in mice were recorded till the 7th day post inoculation. The same dilutions were inoculated in the tongue of calves each dilution in a raw of five sites intradermolingually 0.1 ml for each site the inoculated tongue sites examined and records for 3days post inoculations as vesicles formation reported by *(1, 5, 8)*. The titers were expressed as Log_{10}cattle or mice ID_{50} *(9)*.



The Diagram showed the division of cattle tongue for titration of FMD.V O 1.

RESULTS AND DISCUSSION

FMD virus titers expressed in Log_{10} cattle infective dose 50 (CID_{50}).

Challenge test is the main principle test for potency in concern of FMD vaccine evaluation *(4)*, inoculation of vaccinated and control calves with 10^4 FMD virus particle must have a virulent FMD virus with known accurate titre for apply this test. So proper titration of the virus not only recommended for release of potent vaccine but also for fare judge on the vaccine the present study was intended to compare the obtain titres when use either cattle (CID_{50}) or mice (MLD_{50}). The titer were 6.4 – 6.1- 6.3 log 10 CID_{50} (cattle infective dose 50) for batch 1, 2 and 3 respectively as calculated by **Karber's method** *(9)*, at the third day post cattle tongue inoculation as presented in Table 1. The titer of unweaned baby mice for the same three batches were 7.3, 7.2, 7 log_{10} $MLSD_{50}$ (mice lethal dose 50) respectively as shown in Table 2. The vesicles in the tongue of inoculated cattle were noticed daily carefully for the presence of vesicles to avoid rupture of this vesicles by injection tranquilizer to cattle before tongue examination so the tongue were protruded outside the mouth were the vesicles were counted and recorded in each dilution

rows and this and done for 3 days post inoculation and the titer were calculated as the vesicles were ruptured and fused to form ulcer *(1,5, 10)* 48 – 72 hours post inoculation as seen in photo 1,2 while ruptures of vesicles and ulcer formation at the 4th day post cattle inoculation are seen in photo 3. While photo 4, 5 show the death and paralysis of unweaned baby mice *(8, 11-13)*. In comparison of the three batches titer in the two methods we found that the titration in cattle give more rapid, accurate and fast results than the titration in mice as we seen in Table 3 the titer of the 3 examined batches were differ from cattle and mice from Table 3 it has been shown that cattle gives approximately 1 $\log_{10}$ lower than that in mice which considered high difference that affect the results of the challenge test in vaccine evaluation which is depend on the accurate calculation of the challenge titer of 10^4 $\log_{10}$ *(4)* to be have an exact results for vaccine evaluation and calculation of the protective dose 50 (PD_{50}) or the protection % in a Wright manner. So we can depend on the cattle for titration of virulent FMD virus to carry on the challenge in the potency test of FMD vaccine evaluation and get trusted results *(4)*.

Table 1. Showed titer of 3 batches of the titrated virulent FMD virus type O expressed in $\log_{10}$ cattle ID_{50} (CID_{50}).

hours positive virus inoculation	Batch no.		
	batch 1	batch 2	Batch3
24	5.1	5.4	5.8
48	6.2	6.1	6.3
72	6.4	6.1	6.3
96	Rupture of vesicles and ulcer formation	Rupture of vesicles and ulcer formation	Rupture of vesicles and ulcer formation

FMD virus titre expressed in $\log_{10}$ mice lethal dose 50(MLD_{50})

Table 2. Showed titer of 3 batches for the same titrated virulent FMD virus type O expressed in $\log_{10}$ mice lethal D_{50}

Days positive virus inoculation	Batch no.		
	batch1	batch2	batch3
4	6.3	6.6	6.3
5	6.8	7.0	6.8
6	7.3	7.2	7.0
7	7.3	7.2	7.0

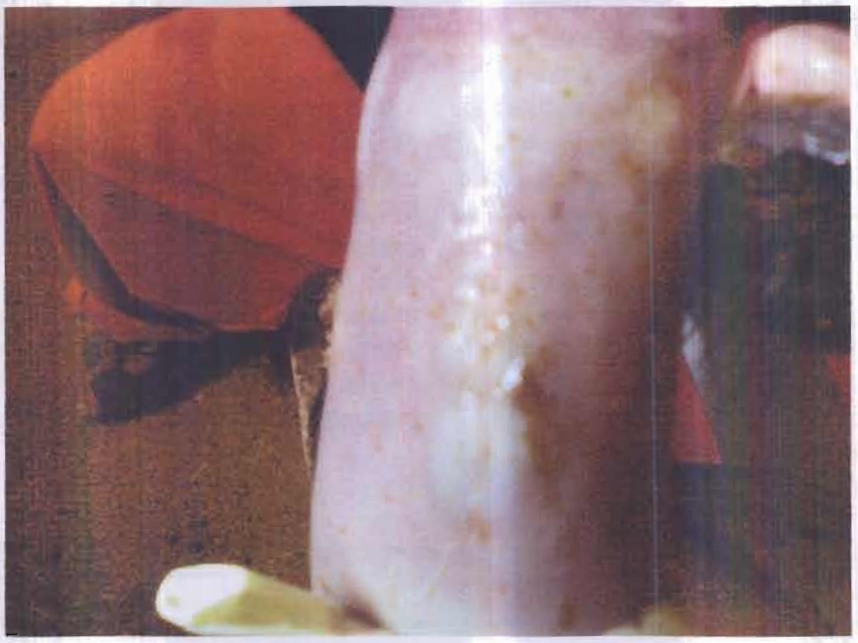


Photo 1. Showed vesicles formed in the tongue of inoculated cattle after 48 hours.

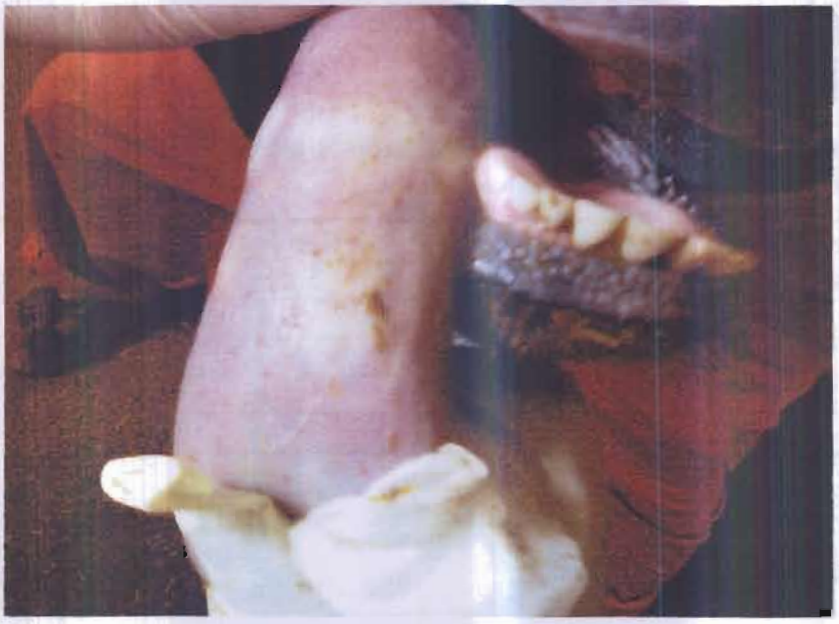


Photo 2. Showed vesicles formed in the tongue of inoculated cattle after 72 hours

Photo 3. Showed rupture of vesicles and ulcer formation in the cattle tongue after 4 days post inoculation

Photo 4, 5. Showed death and paralysis of unweand baby mice inoculated with titrated FMD virus batches.

REFERENCES

1. ***Cox S J , Satya Parida, Voyce C , Reid M S, Hamblin A P , Hutchinas G , Paton D J and Barnett V P (2007):*** Further evaluation of higher potency vaccines for early protection of cattle against FMDV direct contact challenge .Vaccine 25-7687 -7695.

2. ***Barnett P V , Keel P , Reid S , Armstrong R M , Statham R J , Voyce C, Aggarwal N and Cox S J (2004):*** Evidence that high potency of foot and mouth disease vaccine inhibits local virus replication and prevents the carrier state in sheep Vaccine 22: 1221-1232.

3. ***Burrous, R (1996):*** The spictally assay of foot and mouth disease virus in pigs. J. Hyg. Comb., 64: 75.

4. ***OIE (2008):*** Manual of diagnostic tests and vaccine terrestrial animals. 5th Edition 2008 Part 2 Chapter 2 .11.

5. ***Lorenz J and Withmann G (1983):*** The correlation between the potency value obtained by challenge and S.N.T. Zbl. Vet. Med (RCIHEB),19: 45-54.

6. ***Ferreira Maria Etmav (1976):*** Probade Micro-Neutroligocion Boraestudios De Anti Cuerbose Dela Fiebre Aflosa. Bltn. Centrobanamericano Fiebre Aftosa, 21 and 22: 17-24.

7. ***Anderson E C ; Capstick, P B ; Mowztg, N and Lech, F B (1970):*** Suitable Method for Safety Testing of Foot and Mouth Disease. Vaccine, 68, 15-172.

8. ***Skinner H H (1951):*** Propagation of Strain of Foot and Mouth Disease Virus in Cattle and Unweaned white mice. PLOC. CNOY-SOC MED., 44: 1041.

9. ***Karber G (1931):*** Beitrag Zurkillekiven Bhandlung Pharmakologis Reithenver Suche Naungn Schmeidebrgos. Arch-Epx. Path. Pharmak, 162: 280-283.

10. ***Viannafilhoy L , Astudill V , Gomes L , Fermandezg, Eozascee, Ravisauj, A and Alnosoa (1993):*** Potency Control of Foot and Mouth Disease Vaccine in Cattle Comparison of the 50% Protesting Done and the Protesting Against Neutralization. Vaccine, 911 (14): 1424-1428.

11. ***Wagnerg C and Mcvicon J W (1970):*** Foot and mouth disease virus antibodies. Comparison of a tissue culture micro neutralization test with the essay in mice. Liray. Appl. Microliol., 20 (6): 995-997.

12. ***Bernhardt Deruge and Wendrestle H (1977):*** Investigation on the suitability of mice for potency testing of FMD vaccines. Int. Synb on FMD, Dev. Piol. Stan., 35: 285-288.

13. ***Barteling S J (2002):*** Development and performance of fractiterated vaccines against foot and mouth disease. Rev. Sci. Tech. Off. Tnt. Epiz., 21 (3): 577-588.

الملخص العربى

دراسة عن الطرق البديلة لمعايرة الفيروس الضارى لمرض الحمى القلاعية للعترة "O"

*** محمد احمد سعد ، * نرمين جودة شفيق، * أمل اسماعيل عبد الهادى، ** عبير احمد طلعت، ** أحمد عبد الله بكر، * الهام عطا الإبيارى**

* المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية – العباسية - القاهرة

** معهد بحوث الأمصال واللقاحات البيطرية – العباسية - القاهرة

تطوير إنتاج لقاح الحمى القلاعية يتطلب استخدام خلايا زرع نسيجى ومثبطات للأنتيجين على درجة عالية من الكفاءة وللحصول على لقاح ذو كفاءة عالية لابد من تقييم اللقاح طبقاً للبروتوكولات العالمية والتى تشترط إجراء اختبار التحدى لقياس كفاءة اللقاح مما يتطلب توافر الفيروس الضارى للحمى القلاعية بقوة عيارية معلومة ومحسوبة بدقة. لذلك تم إجراء هذه الدراسة لقياس القوة العيارية لفيروس مرض الحمى القلاعية O1/3/93 فى الابقار والفئران عن طريق تحضير ثلاث تحضيرات من الفيروس محضرة من لسان الابقار وعمل تخفيفات لها وحقنها فى الفئران والابقار لتحديد القوة العيارية لها وكانت نتيجتها كالتالى ٦,٣ – ٦,١ – ٦,٤ فى الابقار بينما كانت النتائج فى الفئران كالتالى ٧,٣ – ٧,٢ – ٧,٠ ويتضح من هذه النتائج ان اجراء القوة العيارية للفيروس فى الابقار يعطى نتائج ادق واسرع واكثر حساسية فى الابقار عنه فى الفئران.