Isolation and identification of foot-and-mouth disease virus in Cattle and buffaloes in Gharbia governorate

Amal M. A Raof,* Eman, A. Hattb,*Amal S. Gaber *and Abeer, A.H. Boseila**

*Virology Department - Animal Health Research Institute- Dokki**
*National Organization For Drug Control and Research***

Abstract

Foot and Mouth Disease (FMD) is the most important economic threat to the livestock industries. The highly contagious disease affect all cloven hoofed animals and is wide spread through the world. In this study we collected 15 epithelial tissues from 90 infected cattle (60) and buffaloes (30) among total of 150 animals (60% morbidity) from 3 private farms at Gharbia Governorate. Detection of viral antigen and serotyping by indirect sandwich enzyme linked immuno sorbant assay (ELISA). Isolation of positive samples by ELISA on tissue culture BHK-21 for three blind passages, revealed specific cytopathic effect (CPE) for FMDV. Only 8 samples out of 15 (53%) were positive for FMDV antigen detection serotype A and 5 of them were isolated.The differentiation between infection and vaccination is based on the detection of antibody against the non structural proteins (NSPs) of FMDV. The Prio- Check FMD-NS test is validated for the diagnosis of FMDV in cattle, swine, sheep and goats. This test is an ELISA which detects antibodies against the highly conversed non structural (NS) 3ABC protein of the FMDV. The FMDV Prio-Check test was used for detection to NSPs of FMDV in serum of 50 cattle and buffaloes from the same farms. The assay could detect 33 (66%) positive serum. The Prio- Check NS test is simple, rapid, sensitive and specific. Further identification was done for this 50 serum samples which were collected from clinically diseased (35) and apparently health animals (15) for the detection of antibodies against A and O serotypes by using serum neutralization test (SNT), where 30 out of 50 (60%) were positive for serotype A and O.

Introduction

Foot and mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed domesticated and wild animals. Animals infected with FMDV will typically develop a fever followed by formation of vesicles and erosions in the mucosa of the mouth, tongue, lips, soft palate, nostrils gums, dental pads and skin of the inter digital spaces (Radostitis et al., 1994).
FMD is caused by *Picorna* virus of genus *Apthovirus* and divided into seven serotypes (Mehran et al. 2006).

Serotypes O, A, C are widely distributed, where serotypes SAT1, SAT2, SAT3 are normally restricted to Africa where Asia 1 to Asia. The virus is easily spread by several means. Therefore, the disease has serious economic trade impact (Anthony and Werner, 1992). The disease has high morbidity and low mortality (James and Rushton, 2002).In Egypt, FMD was first detected in 1950 when strain SAT2 caused an outbreak, then to 1958 when outbreaks were caused by

strain A. Several foci were detected in years till 1970. No further strains of other than O have been detected since 1961.

In the beginning of year 2006, FMD has taken an enzootic form caused b new exotic strain of FMDV serotype A, where had attached susceptible an showed more sever forms than others caused by dominant serotype O (Ali 2006).

Early detection is essential for effective control of the disease and requ rapid and sensitive method of diagnosis. In addition to the classical techn of virus isolation in tissue culture and antigen detection by enzyme immuno sorbant assay (Kitching, 1992).

The methods for the diagnosis of FMD are theoretically consistent with International des Epizooties (OIE) standards for FMD diagnosis and in antigen-capture ELISA and colloid-gold test strip for viral antigen typing, phase blocking ELISA (LPBE) for detection of antibodies against FMDV, a indirect ELISA for detection of antibodies against the non-structural p (NSP) 3ABC (3ABC-I-ELISA) (Lu et al., 2008).

FMD Virus has 4 structural proteins (SP) (VP1, VP2, VP3 and VP4) formi capsid and when it replicates during infection, results in production of a n of non- structural protein (NSP) of which some are immunogenic (Teser 1989).Differentiation of infection from vaccination is based on antibody to (Rodriguez.et al., 1994). n indirect ELISA was established to specifically i antibodies induced by FMDV infection but not those induced by vaccinatior was more specific to catch antibodies against NSP. The performance assay was validated by commercial FMDV NSP ELISA kits; better to distii between infected and vaccinated cattle (Wang et al., 2010).

Non-structural protein (NSP) 3ABC antibody is considered to be the reliable indicator of present or past infection with FMDV in vaccinated an The method was validated by simultaneous detection of the early an responses to NSP and structural protein (SP) in FMDV Asia 1 in animals.The performance of the method was also validated by detect antibody in reference sera from the FMD World Reference Laboratory (W Pirbright, UK, and comparison with two commercial NSP ELISA kits (Lu 2007).

The present study aimed to determine the causative agent of the field prob Gharbia Governorate where diseased cattle and buffaloes present at 3 farms were suffered from fever inappetance, loss weight, sharp decrease production and ulcers on mucosa of mouth accompanied with sever saliva well as mortalities for young calves. The diagnosis depends on the isola virus and antigen detection by Indirect linked immuno sorbant assay (ELI addition to use of the Prio –Check NS protein of FMD as diagnostic anti against FMDV and to differentiate between infected and vaccinated an The serology confirmation test was applied for further identification by serum neutralization test (SNT) for detection FMDV antibodies in samples.

Materials and Methods

1- Animals:

A total of 150 cattle (100) and buffaloes (50) represented from 3 private farms at Gharbia Governorate were clinically examined.

The examination includes examining temperature, mucosa of mouth and feet for lesions record Table (1).

2- Samples:

a- Epithelial Tissues:

A total of fifteen epithelial tissues were collected from mouth vesicles of fifteen diseased animals used for detection direct viral antigen for determination of main serotypes and for isolation of FMDV.

One gram of epithelial samples per case were preserved in equal volume of glycerol – buffer saline and transported in ice- box at 4°C to virology department, Animal Health Research Institute- Dokki- Giza.

At the laboratory the collect epithelial samples were prepared by grinding and centrifugation to obtain 0.2ml of the inoculums for virus isolation and serotyping determination of antigen according to (Kitching and Doanldson, 1987).

b- Serum Samples:

A total of fifty blood samples were collected from diseased and apparently healthy animals were used for separation of serum that used for serological examination for both Prio- Check ELISA and SNT.

3- Cell Cultures:.

Baby Hamster Kidney Cell (BHK-21) cell line was obtained from National Organization for Drug Control and Research (NODCAR) for virus isolation according to (Clark and Spier, **1980).**

4- Antigen detection and serotyping determination:

All collected epithelial samples were prepared for antigen detection and serotyping determination (serotypes A&O) and tested by indirect sandwich ELISA according the protocol of OIE/FAO WRLab for FMD, Pirbright, UK according to (Hamblin et al., 1986 a, b).

5- Virus isolation:

Virus isolation was successfully done according to (Reid et al., 2002).The positive samples for FMDV antigen detection by ELISA were inoculated on monolayer of BHK-21 Cell line, and observed daily for cytopathic effect (CPE) of FMD for 3 successive passages. The positive samples were kept at -70 °C.

6- Serological examination:

a- Detection of FMD antibodies of infected animals:

The collected 50 serum were tested with FMDV Prio- Check NSP- ELISA (Prionics Lelysted- Netherland) according to (Sorensen et al., 1998).

Test Principle: FMDV- NS is a blocking ELISA.

The wells of the test plate are coated with 3ABC specific monoclonal antibody (mAb), following by incubation with antigen (3ABC Protein). Consequently, test plate of the kit contain FMDV- NS antigen captured by coated mAb. The test is performed by dispensing the test samples to the wells of a plate. After incubation the plate is washed and conjugate is added. FMDV-NS specific antibodies, directed against the non structural protein. After the incubation, the plate is washed and the chromogen substrate is dispensed. After incubation at room temperature the color development is stopped. Color development measured

optically at wave length of 450 nm shows the presence of antibodies direc against FMDV.

b- Serotyping of serum samples:

Serum samples were used for detection of antibodies against FMDV seroty A&O by using Serum neutralization test (SNT) according to (Frey and Li 1971).

Table (1): Number of examined animals and types of samples for diagnosi FMDV :

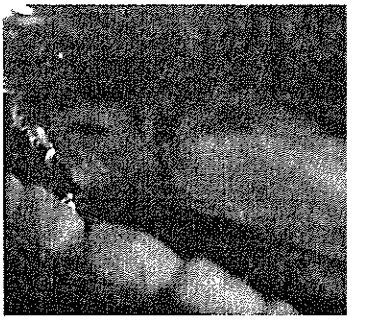
Type of samples	No. of diseased animals	Epithelial samples	Serum Samples
No. of samples	90	15	50

Results

Clinical Findings:

From 150 examined cattle (100) and buffaloes (50), 90 of them demonstra typical clinical symptoms of FMDV. It includes fever salivation, inappeta sever loss of weight, reduction in milk production in dairy animals, ulcers mucosa of mouth at gum and tongue (photo 1), as well as mortality of 3 yo calves.

a)



b)

Photo (1): Showed a) salivation, b) ulcers on mouth cavity of infected buffalc

Virological Findings:

Eight of fifteen prepared tissues were showed positive results for ant detection by indirect sandwich ELISA for serotype A of FMDV (Table 2)

The positive ELISA samples were inoculated on BHK-21 cell line and fiv them showed clear cytopathic effect (CPE) for 3 successive passages.

Serological Findings:

The result of Prio –Check NS Proteins ELISA was 66% positively where 33 of 50 serum samples showed positive for antibodies against FMDV th actually indicated the presence of NS Proteins related to natural infection.

Concerning serotyping of antibodies, the result of SNT was 60% positively where 30 out of 50 serum samples showed antibodies serotypes A, O against FMDV (Table 3).

Table (2): Number of positive epithelial samples for antigen detection and isolation of FMDV:

Type of samples	Epithelial samples	Positive antigen	Positive for isolation
No. of samples	15	8/15 (53%)	5/8

Table (3): Number of positive serum samples for FMDV:

Type of samples	Serum Samples	Positive Prio - Check	Positive for SNT
No. of samples	50	33/50 (66%	30/50 (60%)

Discussion

Foot and mouth disease (FMD) is a notifiable disease in most countries of the world, and any clinical suspicion of disease should be reported to the appropriate authorities. The disease occurs in most of the major livestock producing countries of the world, except North America, Central America Australia, New Zealand, Japan and Ireland (Bhattacharya et al., 2005).
The disease is characterized by the formation of vesicles and erosions in the mucosa of mouth, external nacres in coronary band of claws of the feet; other area including udder and teats. Lameness is seen, reduced lactation, mastitis and abortion are common (Aggarwal et al., 2000).The affected animals suffer from fever associated with depression, anorexia, loss of appetite, lacrimation and excessive salivation, mouth and feet lesions which were attributed to exotic FMDV serotype A, where recognized picture of FMD clinical symptoms was noticed in January 2006. Followed this outbreak, many foci were appeared frequently with long or short intervals (Ali et al., 2006).

This study was planned to through more light on the causative agent of the field problem at Gharbia Governorate where out of 150 cattle and buffaloes suffered from typical symptoms of Foot and mouth disease virus which summarized as fever, loss of appetite, sever salivation, loss of weight, ulcers on mouth cavity drop of milk production as well as mortalities of 3 young calves. In our study we succeeded for isolation the causative agent on tissue culture and for antigen detection and serotyping of FMDV type A by indirect sandwich ELISA. Table 2 showed that positive percentage of antigen detection by ELISA in epithelial tissues was 53% for type A. These findings were in agreement with (Hamblin

al., 1986 a, b). Isolation of FMDV on BHK-21 formed clear CPE after third bl passage. This result was in agreement with (Clark and Spier, 1980).

Antibodies to the structural, capsid proteins of the virus are induced by b vaccination and infection. Therefore, it is possible to differentiate animals t have been infected from those vaccinated based on the detection of antibod to structural protein alone (Mackay et al., 1998).Therefore, serodiagnosis enhanced by the newly developed non structural protein (NSP) assays t enable detection of past or current infection irrespective of vaccination sta (O.I.E., 2004).FMD- Prio Check is primary choice for FMD detection. The te validated for diagnosis of FMDV in cattle, swine, sheep and goats, and can discriminate between infected and vaccinated animals.

The test can be used in mass screening for detection of all FMDV serotypes.T present study is concentrated on detection on natural infection with FMD amc cattle population. The 50 tested serum samples showed 33 (66%) positive natural infection with FMDV. The EU- founded-FMD research group h evaluated the Prio- Check- FMD NS as test with high specificity and sensitiv (Sorensen et al., 1998). This result was in agreement with (Lu et al., 20(where the cedi test FMDV-NS was 98.05% (302/308) and the prevalence 3ABC antibodies reached 71.4% in diseased cattle herd.

Concerning the screening of FMDV serotypes antibodies in cattle sera w successfully done by SNT Table (3), the results revealed 30 out of 50 (60 were positive to serotypes (A and O). These results agreed with (Hamblin et 1986 a, b and O.I.E., 2005).

Our conclusions indicated that FMD could be recognized as one of the ma cattle diseases of economic importance and widely spread among cattle Egypt. More efforts and major measurements must be submitted to prevent a entrance of any exotic strains of disease, in addition cooperation of authorities committee to eradicate the disease.

The diagnosis of FMD is based on recognition of clinical signs, virus isolat and antigen detection by using ELISA. The Prio- Check FMD NS ELISA is addition method can be used to differentiate between natural infection a vaccination. This test is rapid, sensitive and specific for detection of natura infected animals.

References

Aggarwal, N., Z., S. Cox, R. Statham, S. Alzandersen, R.P. Kitching and P.V. Barnett (200(Expermintal studies with foot and mouth disease virus, strain O Vaccines, 20: 250 2515.

Ali, Nawal, M., M. A. Shahein, F. A. Wail and S. A. H. Salem (2006): Natural outbreak of ne exotic serotype A of FMDV in Egypt during 2006. Egypt J. Comp. Path.& Clinic Pat Vol. 19 No. 3, 293-305.

Anthony, E.C. and P.H.E. Werner (1992): Veterinary diagnostic virology. Apractitiners gide.

Bhattacharya, S. R., Banerjee, R., Ghosh, A.P., Chattopadhayay and A. Chatterjee (200 Studies of the outbreaks of foot and mouth disease in West Bengal, India betwee 1985 and 2002. Rev. Sci Tech. Dec., 24 (3): 945-952.

Clark, J. B. and Spier, R. E. (1980): Variation in the susceptibility of BHK-21 populations ar cloned cell lines to three strains of foot and mouth disease virus. Arch. Virol., 63, 1-9.

Frey, H. R. and Liess, B. (1971): Growth curve studies and applicability of high cytopathogenic BVD-MD virus strain for diagnostic purposes using the micro tit method. Zbl. Vet. Med. B 118: 61-72.

Hamblin, C. , Rarnett, I. T. R. and Hedger, S. (1986a): A new enzyme linked immunosorbant assay for detection of antibodies against foot and mouth disease virus. I. Development and method of ELISA. Journal of Immunological Methods 93, 115-121.

Hamblin, C. , Rarnett, I. T. R. and Hedger, S. (1986b): A new enzyme linked immunosorbant assay for detection of antibodies against foot and mouth disease virus. II. Application Journal of immunological methods 93, 123-129.

James, A. D. and J. Rushton (2002): The economic of foot and mouth disease. Res. Sci. Tech. off. Int. Epiz., 21 (3), 637-654.

Kitching, R.P. (1992): Application of biotechnology to the control of foot and mouth disease virus. Br. Vet. J. 148, 375-388.

Kitching, R.P.and A. I. Doanldson (1987): Collection and transportation of specimens for vesicular virus investigation. Res. Sci. Tech. off. Int. Epiz.,6 (1): 263-272.

.(2007): Xie Q, Liu X, Liu Z, Chang H, Ma J, Zhang Q, Li D, Qi S, Guo J, Cao Y, Lu Z Development and validation of a 3ABC indirect ELISA for differentiation of foot-and-mouth Nov 15;125(1-2):157-69. Vet Microbiol.disease virus infected from vaccinated animals.

Xie Q, , Bao H, Qi S, Guo J, Shang Y, Jiang T, Zhang Q, Ma J, Lu ZYin, H. (2008): Techniques developed in China for foot and mouth disease diagnosis. Transbound Emerg. Aug; 55(5-6):196-9.

Mackay, D. K.J., Forssyth, M. A., Davies, P.R., Berlinzarit, A., Belsharn, G.J., Flint, M. and Ryan, M.D. (1998): Differentiating infection from vaccination in foot and mouth disease using a panel of recombinant, non structural proteins in ELISA. Vaccine, 16 (5): 446-459.

Mehran, A., A. Seyed, A. Malahat and S. Reyhaneh (2006): Detection of foot and mouth disease virus and identification of serotypes in East Azerbaijan province of Iran. Veterinarski Archiv, 76 (5), 413-419.

OIE Standard Manual (2004): Manual of Recommended diagnosis techniques and requirements for biological products for lists A and B diseases.

OIE Standard Manual (2005): Manual of Recommended diagnosis techniques and requirements for biological products for lists A and B diseases.

Radostitis, O. M., D. S. Blood and C. C. Gay (1994): Veterinary Medicine, Eighth Edition

Reid, S. M., Ferris, N. P., Hutchings, G. H., Zhang, Z. and S. Alexandersen (2002): Detection of all seven serotypes of foot and mouth disease by Real time, Fluorogenic RT-PCR assay. J. Virol. Methods 10: 67-80.

Rodrignez, A., Dopazo, J., Saiz, J. and Sobrino, F. (1994): Immunogenicity of non structural proteins of foot and mouth disease virus, differences between infected and vaccinated swine. Arch. Virol. 136: 123-131.

Sorensen, K. J., Madsen, K. G., Madsen, E.S., Sal., J.S., Nqundi, J., Machary, D.K.J. (1998): Differentiation of infection from vaccination in foot and mouth disease by the detection of antibodies to the non structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculo virus. Arch. Virol., 43: 1461-1476.

Teser, M., Berger, H. G. and Morquardt, O. (1989): Serological probes of some foot and mouth disease non structural proteins. Virus genes, 3 (1): 29-44.

,(2010): Wei H, Yan Y, Zhao T, Hu X, Luo P, Wang L, Yu Y., Wang H recombinant truncated FMDV 3AB protein used to better distinguish between infected and vaccinated cattle.Vaccine. Mar 1.

ن العربى

صنيف فيروس مرض الحمى القلاعيه فى الابقار والجاموس فى محافظة الغربية

ار عبد الرؤف-*ايمان ابو حطب- *امل سيد جابر-**عبير عبد الحليم بوصيلة
روفوجى- معهد بحوث صحه الحيوان- الدقى- الجيزه
رقابة الدوانية بالعجوزة

الحمى القلاعية من الامراض المهمه والتى تسبب خسائر اقتصاديه. هذا المرض
ا بين الحيوانات ذات الظلف المشقوق ومنتشر خلال دول العالم. فى هذه الدراسة تم
عدد ١٥ نسيج طلائى من ٩٠ من الابقار والجاموس المصابة فى عدد ٣ مزارع بها
يوان بمحافظه الغربيه وذلك للكشف عن انتيجين فيروس مرض الحمى القلاعية وتحديد
المسببه للمرض باستخدام اختبار الساندوتش اليزا الغير مباشر و تم عزل العينات
لوجود انتجين الفيروس على خلايا BHK-21 وذلك لثلاث تمريرات متتاليه والتى
غير خلوى مشابه لمرض الحمى القلاعيه وكانت نسبة الايجابية ٥٣% كما تم اجراء
ات السيرولوجية التاكيديه لعينات السيرم باستخدام اختبار الاليزا اللابنانى وكانت نسبة
٦٦% وكذلك اختبار السيرم المتعادل وكانت نسبة ايجابيته ٦٠%. وقد تم الوصول الى
ر الاليزا المبنى على الكشف عن البروتين الغير تركيبى لفيروس مرض الحمى القلاعيه
ريع – دقيق وبسيط فى ادائه.