The Role Of FMDV Carrier Buffalos In Transmission of The Disease To In-Housed Animals

Ismail M.[1], Salama A.[1], Daoud A.[2] and Abu-Elnaga H.[2]

[1] Department of Virology, faculty of Veterinary Medicine, Zagazig University

[2] Department of Foot and Mouth disease, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo

ABSTRACT

Foot-and-Mouth Disease virus (FMDV) is a long known virus in Egypt, but the crucial role of the virus carrier state in the disease spreading is still elusive, especially in buffalos. FMD virus carrier water buffalos experimentally infected with the virus strain O/EGY/93 were detected by virus isolation in tissue cultures, inoculation of Swiss baby mice and RT-PCR. They were penned with susceptible FMDV livestock for about three months. However, clinical findings and sero-conversion of the in-housed animals did not reveal any aspects of FMDV transmission to them.

INTRODUCTION

Foot-and-mouth disease (FMD) is the most economically significant animal viral disease worldwide *(1)*. FMD virus has a wide host range, an ability to infect in small doses, a rapid rate of replication, a high level of viral excretion and multiple modes of transmission, including spread by wind *(2)*.

Ruminant animals that have recovered from infection with FMDV and vaccinated ruminants that have had contact with live virus may retain infection in the pharyngeal region for a variable period of time. The carrier is defined as an animal from which live-virus can be recovered after 28 days following infection *(3)*.

There is field evidence to indicate that carrier African buffalo can precipitate new outbreaks of disease, and more anecdotal evidence also implicates carrier cattle and sheep in disease recrudescence or in starting new outbreaks *(2,4)*.

Establishment of persistent infection in unvaccinated as well as vaccinated animals, particularly buffalo and cattle *(5)*, are well documented; and these carriers can shed virus intermittently leading to FMD outbreaks *(6)*. Identification of such carriers and animals harboring sub-clinical infection has never been easy with conventional assays *(7)*.

Therefore, it was imperative to detect carriers and investigate the possibility of transmitting of FMDV from carriers buffalo to in-housed livestock.

MATERIALS AND METHODS

1- Experimental infection

Three water buffalos (*Bubalus bubalis*) were inoculated subdermolingual with tongue epithelial tissue (T.E.) suspension of FMD strain (O/EGY/93).

2-Exposure of some cloven-hoofed farm animals to carrier buffalos

Fifty-seven days after the experimental infection of the buffalos, one male water buffalo, one female cattle, two male sheep and two female goats were in-housed with the three experimentally infected buffalos for about 3 months (Fig. 1). The three buffalos became FMDV carriers after 28 days following disease infection as previously defined *(8)* and as proved by isolation of FMDV on tissue cultures, via inoculation of unweaned Swiss baby mice and detection of the virus by RT-PCR.

3- Clinical examination and collecting of samples

Clinical examination and body temperature of the experimentally infected

buffalos were recorded daily during the first two weeks (incubation period of FMDV) from their infection. Also, the same was carried out on the in-housed farm animals during the time of the experiment. Serum samples were collected from all animals in the study during two weeks interval time to demonstrate immune response to FMDV using virus neutralization (VN) test. In addition, oesophageal-pharyngeal fluids (OP) samples were collected using probang cup to detect carrier buffalos *(9)*.

4- Isolation and detection of FMDV in carrier buffalos:

a) Virus isolation in cell line

Pig kidney cell (IB-RS-2) was used *(10)*.

b) Detection of the virus in lab experimental animal

Unweaned Swiss baby mice were inoculated with OP fluids intraperitoneal as stated by *(11)*. Paralysis and death of the inoculated suckling mice indicate positive virus detection.

c) Detection of FMDV by RT-PCR

Extracted RNA from OP samples of infected buffalos was checked by one-step RT-PCR using serotype O specific primers derived from 1D/2B gene of 402 bp expected fragment size *(12)*.

5-Demonstration of seroconversion by virus neutralization (VN) test

The test was performed as prescribed for international trade *(11)*. The quantitative VN microtest for FMD antibody is performed with BHK-21 or IB-RS-2 in flat-bottomed tissue-culture grade microtitre plates.

RESULT AND DISCUSSION

The experimentally infected buffalos showed FMD typical clinical signs. The carrier buffalo (28 days after acute phase of FMD), which was proved by tissue cultures virus isolation (Fig. 2), baby mice virus detection (Fig. 3) and RT-PCR virus identification (Fig. 4), were penned with FMDV susceptible buffalo, cattle, sheep and goat for three months

(Fig. 1). During three months, clinical observation of the penned animals with the carrier buffalos did not reveal any symptoms of FMD infection. In order to confirm the clinical examination, sero-conversion monitoring of FMDV using VN test was carried out to determine whether the infection had occurred or not. VN test declared zero level of antibody to FMDV in the in-housed animals. Where there is no history of vaccination, seroconversion can serve as indicator of infection *(11)*. The absence of clinical findings and sero-conversion in the in-housed livestock proved no FMD transmission from the carrier buffalos.

FMDV RNA is localized within the epithelial cells of the soft palate and pharynx during persistent infection without causing lysis. It was suggested that the lack of epithelial cell lysis despite of the virus persistence in the cells seems more likely to be due to a change in the virus than to viral persistence within specific cells that are insusceptible to the lytic action of FMDV *(13)*.

FMDV in the pharyngeal area tissues could be a source for the virus present in oesophageal-pharyngeal fluids, OP fluids *(14)*. The titer of virus in the OP fluids of carrier animals is low, and virus is not consistently recovered from individual animals *(15, 16)*. Currently, virus isolation from OP fluids is the most sensitive method to detect carrier animals, but reverse transcription- polymerase chain reaction (RT-PCR) assays are being developed to attempt to increase sensitivity *(17)*.

Circumstantial evidence dictates that buffalo are the usual source of FMDVs that result in outbreaks of the disease in cattle in Southern Africa *(18)*. A natural transmission of FMDV type SAT1 to cattle in a wildlife area of Zimbabwe where a close relationship between three viruses, one virus isolated from cattle and two viruses isolated from buffalo, was demonstrated by partial nucleotide sequencing of the gene coding for the capsid protein 1D *(19)*. In addition, experimental transmission of FMD virus (SAT2) occurred from three male African buffalo (Syncerus caffer) to four

female cattle after five months of holding together on island of Zimbabwe *(20)*.

Also, the transmission of FMD serotype SAT2 from two inoculated buffalos to two susceptible buffalos and two susceptible cows in the same pen within 7-10 months was recorded. It was postulated that African buffalo were not only the usual source of SAT type infections for domestic livestock in southern Africa but also continuously generate antigenic variants, which complicates control of the disease through immunization *(21)*.

Other wildlife species can transmit FMD in southern Africa, the most important species seem to be impala *(Aepyceros melampus)*. It was shown most likely that antelope (impala or kudu), infected through contact with the buffalo herd within the conservancy, had jumped over the fence and transmitted the virus to the cattle *(22)*.

In earlier work, it was observed the transmission of type SAT3 virus to cattle after the carrier buffalo had been removed, transported by road and returned into contact with the cattle *(23)*. On the other hand, carrier buffalo and cattle have sometimes been in direct or close contact for long periods of time without transmission occurring *(24-26)*.

SAT-1 viruses have been recovered from persistently infected buffalo at a higher rate *(27)*. Most FMD outbreaks in the southern Africa region are caused by SAT2 followed by SAT1 and lastly SAT3 which had led to speculation that SAT2 is better adapted for spread to other species such as cattle and impala *(28)*, and SAT1 may be better adapted for spread among buffalo *(29)*.

The discrepancy of the study result with what previously reported in the sub-Saharan of Africa might be attributed to the differences in the FMD serotypes (SAT types) and strains, the buffalo breed *(Syncerus caffer)* and the relatively longer period experiment (five-ten months) . Also, each carrier buffalo in this study was secured in its ring and was not allowed to move freely inside the pen whereas the possibility of sexual transmission of FMD from carrier African buffalo to cattle was previously assumed *(30)*. In this experiment, buffalos varied between 6 months and 1 years of age. It was known that buffalo heifers reach sexual maturity between 2 and 3 years of age, whereas spermatogenesis commences in buffalo bulls at 2.5 years of age *(29)*.

Egypt has an ambitious policy to control FMD via vaccination against FMD. In the mid of 2006, the previously used monovalent vaccine against FMD was substituted by the bivalent vaccine against FMDV serotype 'O' and 'A'. To help attain the goal of our country control and eradication of FMD there is a need to establish what can be done to prevent the development of the carrier animal, to improve the identification of carriers, to limit the duration of the carrier period in individual animals and to reduce risk of transmission of FMDV to susceptible stock.

Finally, it is recommended to further study FMDV carrier among non-secured sexually matured cloven-hoofed animals.

ACKNOWLEDGMENTS

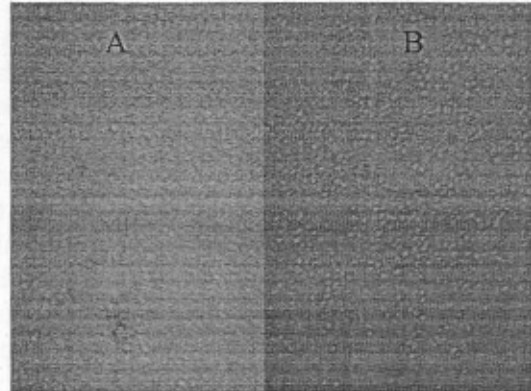**Fig 1.** Set up of the in-housed livestock with carrier buffalos.

Fig. 2. Established cell line (normal and infected cells) used in FMD virus isolation. A) Normal IB-RS-2 cells; B) infected IB-RS-2 cells. The photographs were acquired through 10 objective lens for the cells.

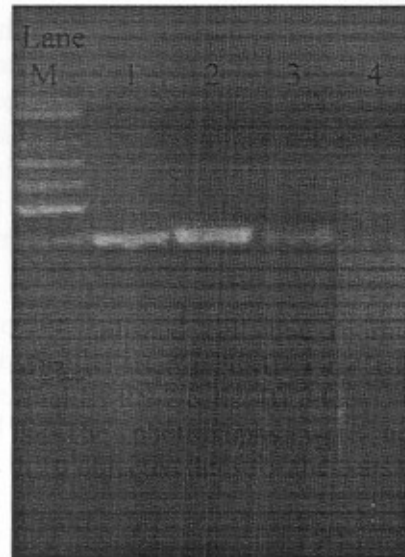**Fig. 3.** The unweaned baby mouse shows paralysis in hind limb

Fig. 4. Detection of FMDV in OP by RT-PCR. M: 100 bp DNA marker (100, 200, 300, 400, 2*500, 600,......1000 bp). Lane 1, 2 & 3: positive FMDV RNA in Buffalos OP with 402 bp amplicon for FMDV serotype O 1D gene; Lane 4: negative control tissue sample.

REFERENCES

1-Chénard G., Miedema K., Moonen P., Schrijver R.S. and Aldo Dekker (2003): A solid phase blocking ELISA for detection of type O foot and mouth disease virus antibodies suitable for mass serology. J. of virological Methods, 107 (1): 89-98.

2-Alexandersen S., Zhang Z., Donaldson A.I. and Garland A.J.M., (2003): The pathogenesis and diagnosis of foot-and-mouth disease. J. Comp. Pathol. 129: 1–36.

3-Kitching R.P. (2002): Identification of foot and mouth disease virus carrier and subclinically infected animals and differentiation from vaccinated animals. Rev. sci. tech. Off. int. Epiz., 21(3): 531-538.

4-Alexandersen, S., Zhang, Z. and Donaldson, A. I. (2002): Aspects of the persistence of foot-and-mouth disease virus in animals – the carrier problem. Microbes Infect 4, 1099–1110.

5-Salt J.S., Samuel A.R. and Kitching R.P. (1996): Antigenic analysis of type O foot-and-mouth disease virus in the persistently infected bovine. Arch. Virol. 141, 1407–1421.

6-Lubroth J. and Brown F. (1995): Identification of native foot-and-mouth disease virus nonstructural protein 2C as a serological indicator to differentiate infected from vaccinated livestock. Res. Vet. Sci. 59: 70–78.

7-Mohapatra J.K., Sanyal A., Hemadri D., Tosh C., Palani G., Rasool T.J. and Bandyopadhyay S.K (2006): Development and comparison of genome detection assays for the diagnosis of foot-and-mouth disease suspected clinical samples. Journal of Virological Methods 137: 14–20.

8-Salt J.S. (1993): The carrier state in foot and mouth disease virus. An immunological review. Br.Vet. J. 149: 207-223.

9-Kitching R.P. and Donaldson A.I. (1987): Collection and transportation of specimens for vesicular virus investigation. Rev. sci. tech. Off. int. Epiz., 6: 251-261.

10-Ferris N.P., King D.P., Reid S.M., Hutchings G.H., Shawa A.E., Paton D.J., Goris N., Haas B., Hoffmann B., Brocchi E., Bugnetti M., Dekker A. and De Clercq K. (2006): Foot-and-mouth disease virus: A first inter-laboratory comparison trial to evaluate virus isolation and RT-PCR detection methods. Veterinary Microbiology 117: 130–140.

11-OIE (2004): Foot and mouth disease. In: OIE Standards Commission (5th Eds.), Manual of Standards for Diagnostic Tests and Vaccines. Office International des Epizooties, Paris, France (Chap. 2.1.1).

12-Reid S.M., Ferris N.P., Hutchings G.H., De Clercq K., Newman B.J., Knowles N.J. and Samuel A.R. (2001): Diagnosis of foot and mouth disease by RT-PCR: use of phylogenetic data to evaluate primers for the typing of viral RNA in clinical samples. Arch Virol 146: 2421-2434.

13-Zhang Z.D. and Kitching R.P. (2001): The localization of persistent foot and mouth disease virus in the epithelial cells of the soft palate and pharynx. J. Comp. Pathol. 124: 89–94.

14-Zhang Z., Murphy C., Quan M., Knight J. and Alexandersen S. (2004): Extent of reduction of foot-and-mouth disease virus RNA load in oesophageal–pharyngeal fluid after peak levels may be a critical determinant of virus persistence in infected cattle. Journal of General Virology 85: 415–42.

15-Kitching R.P. (2002): In foot and mouth disease, control strategies, symposium proceedings, 2-5 June 2002, Lyons, France: 353-359. Problems of diagnosis of foot and mouth disease in domestic animals.

16-Abu-Elnaga H.I. (2004): Studies on experimental infection of goats with foot and mouth disease virus. M.V.Sc. thesis, Zagazig university, Egypt.

17-*Grubman M.J. and Baxt B. (2004):* Foot-and-Mouth Disease. Clinical Microbiology Reviews 17 (2): 465–493.

18-*Thomson G.R. (1995):* Overview of foot and mouth disease in Southern Africa. Rev Sci Tech. 14(3):503-20. Review.

19-*Dawe P.S., Flanagan F.O., Madekurozwa R.L., Sorenson K.J., Anderson, E.C. Foggin C.M., Ferris N.P. and Knowles N.J. (1994):* Natural transmission of foot-and-mouth disease from African buffalo (Syncerus caffer) to cattle in a wildlife area of Zimbabwe. Vet. Rec. 134: 230–232.

20-*Dawe P.S., Sorensen K., Ferris N.P., Barnett I.T.R., Armstrong R.M. and Knowles N.J. (1994):* Experimental transmission of foot and mouth disease virus from carrier African buffalo (Syncerus caffer) to cattle in Zimbabwe. Vet. Rec. 139: 211–215.

21-*Vosloo W., Bastos A.D., Kirkbride E., Esterhuysen J.J., van Rensburg D.J., Bengis R.G., Keet D.F. and Thomson G.R. (1996):* Persistent infection of African buffalo (Syncerus caffer) with SAT-type foot-and-mouth disease viruses: rate of fixation of mutations, antigenic change and interspecies transmission. J. gen. Virol., 77: 1457-1467.

22-*Hargreaves S.K., Foggin C.M., Anderson E.C., Bastos A.D.S., Thomson G.R., Ferris N.P. and Knowles N.J (2004):* An investigation into the source and spread of foot and mouth disease virus from a wildlife conservancy in Zimbabwe. Rev. sci. tech. Off. int. Epiz., 23 (3), 783-790.

23-*Hedger R.S. and Condy J.B (1985):* Transmission of foot and mouth disease from African buffalo virus carriers to bovine. Vet. Rec.17: 205.

24-*Condy J.B. and Hedger R.S. (1974):* The survival of foot and mouth disease virus in African buffalo with non-transference of infection to domestic cattle. Research in Veterinary Science 16, 182-185.

25-*Bengis R.G., Thomson G.R., Hedger R.S., De Vos V. and Pini A. (1986):* Foot-and-mouth disease and the African buffalo (Syncerus caffer). I. Carriers as a source of infection for cattle. Onderstepoort J. Vet. Res. 53 (2): 69–73.

26-*Gainaru M.D., Thomson G.R., Bengis R.G., Esterhuysen J.J., Bruce W. and Pini A. (1986):* Foot and mouth disease and the African buffalo (Syncerus caffer). II. Virus excretion and transmission during acute infection. Onderstepoort. J. vet. Res., 53 (2): 75-85.

27-*Thomson G.R., Vosloo W. and Bastos A.D. (2003):* Foot-and-mouth disease in wildlife. Virus Res. 91, 145–161.

28-*Thomson G.R. and Bastos A.D.S. (2004):* In: Coetzer, J.A.W., Tustin, R.C. (Eds.), Foot and-Mouth Disease. Infectious Diseases of Livestock, Second ed. Oxford University Press, South Africa, pp. 1324–1365.

29-*Vosloo W., de Klerk L.-M., Boshoff C.I, Botha B., Dwarka R.M., Keet D. and Haydon D.T. (2007):* Characterization of a SAT-1 outbreak of foot-and-mouth disease in captive African buffalo (Syncerus caffer): Clinical symptoms, genetic characterization and phylogenetic comparison of outbreak isolates. Veterinary Microbiology 120: 226–240.

30-*Bastos A. D., Bertschinger H.J., Cordel C., van Vuuren C.D., Keet D., Bengis R.G., Grobler D.G. and Thomson G. R. (1999):* Possibility of sexual transmission of foot-and-mouth disease from African buffalo to cattle. Vet. Rec. 145:77–79.

الملخص العربى
دور الجاموس الحامل لفيروس مرض الحمى القلاعية في نقل المرض إلى
الحيوانات المقيمة في نفس العنبر

محمد البكري عبد الرحيم إسماعيل¹، على عبد الرشيد على سلامة¹،
أحمد محمود داود²، هاني إبراهيم جبر أبوالنجا²
¹قسم الفيرولوجى كلية الطب البيطري-جامعة الزقازيق
²قسم الحمى القلاعية معهد بحوث الأمصال و اللقاحات البيطرية بالعباسية- القاهرة

الملخص العربي

بالرغم من أن فيروس مرض الحمى القلاعية معروف في مصر منذ زمن بعيد، ما زال الدور القاطع للحالة الحاملة للفيروس في نشر المرض محيرا و خاصة في الجاموس. لذلك تم العدوى التجريبية للجاموس بفيروس المرض عترة O/EGY/93 وتم الكشف عن الحالة الحاملة للفيروس بتجارب عزل الفيروس على المزارع النسيجية، و على الفئران السويسرية الرضيعة و الكشف عنه بتجربة النسخ العكسي و تفاعل البلمرة المتسلسل. وضع الجاموس الحامل للفيروس بعد ذلك مع حيوانات المزرعة ذو الحافرين (جاموس – بقر – أغنام – ماعز) الخالية من المرض أو أجسام مناعية له في نفس العنبر لمدة حوالي ثلاث شهور، و مع ذلك لم يوضح الكشف الطبي على هذه الحيوانات أو الرد الفعل المناعي أي مظاهر انتقال مرض الحمى القلاعية لهم.