Isolation and Identification of Foot and Mouth Disease Virus from Convalescent Sheep in Jordan

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ABSTRACT

In Jordan, the Foot and Mouth Disease Virus (FMDV) infection among sheep herds resulted in a two intermittent outbreaks at years 2006 to 2008. The present work was undertaken to isolate and serotype the virus from convalescent sheep, which exposed to the natural infection. A total of 113 orp-pharyngeal swabs (OP) were collected from convalescent sheep after the two outbreaks. FMDV was isolated using cell culture of BHK-21 cells that induced clear cytopathogenic changes (CPE) in comparison to the reference control (non-infected cells). Nine out of (113) of OP swabs were showed cellular changes on BHK-21 cells (7.96%). The concentrations of virus isolates that induced CPE which confirmed by the infectivity titration, revealed a significant correlation between the rate and morphological changes of CPE and the virus titers. The cell culture adapted virus with or without CPE formation were serotyped using the capture ELISA. The obtained serotypes were O and A. The O serotype represented the highest percentage of (5.3%), and serotype A represented the least percentage (2.65%).

Keywords: FMD, Jordan, Virus, Serotype.

INTRODUCTION

In the Middle East, FMDV has been a major constraint to the developing dairy industry and sheep flocks. Since 1988, the predominant virus serotype O was intermittently or alternatively accompanied with the previously prevalent types A and Asia-1 (Kitching, 1992 & 1998). Recently, the virus has been eradicated from some European countries, North and Central America, Japan, Australia, South Korea and the British Isles. New introductions caused by type O virus to Italy in 1993, Greece in 1994 & 1996, as well as Bulgaria in 1996. FMDV- type A was introduced to Albany in 1996 and spread to Macedonia. Introductions of the virus types C & SATs to Europe appear unlikely, whereas Asia-1 is as threatening as the types A and O (Marquardt & Hass, 1998 ; Kitching & Hughes, 2002). FMD is an endemic disease in Jordan, the first isolate was grouped to Asia-1 at Pirbright Laboratories, UK (Aidaros, 2002 ; Knowles et al., 2005). Cattle and sheep that have been infected with FMDV may continue to carry the virus in their pharyngeal regions for up to 24 and 8 months respectively (Parida et al., 2005). It may be lifelong in African cape buffaloes as a main reservoir host. Movement of carrier animals plays a major role in the spread of FMD (Reweyemamu, 1984; Sutmoller et al., 2003)
The risk of convalescent animals transmitting disease is probably very low because of the emergency vaccination which prevent or reduce local virus replication and thereby dramatically reduce the amount of virus released into the environment in the all-important early post-exposure period (Cox et al., 2005).

Subclinically infected animals are important in the transmission of FMD. These sheep fail to show clinical signs either because of previous vaccination or very low infecting dose of virus or a natural resistance (Robiolo et al., 2006; Cox et al., 2006). A serotype specific ELISA was developed to detect FMDV and the method is feasible for detecting serotype O FMDV carrier animals (Parida et al., 2005).

This study has been conducted to isolate and serotype the virus from convalescent sheep.

**MATERIALS AND METHODS**

FMDV (Type-O-virus) and BHK-21 cell were obtained from (Abbassia sera and vaccine research institute, Abbassia, Cairo, Egypt). The virus was provided in the form of 2 ml in a sealed vial and of cultured adapted. The cells were cultivated and passaged under the optimum conditions and used for virus isolation. A group of sheep herds of history of previous infection were randomly selected. Sheep herds sampled separately for virus isolation.

ELISA kit for FMD antigen detection, World Reference laboratories for FMDV (WRL/OIE/FAO), Institute for animal health, Pirbright, UK was used. A total of 113 oro-pharyngeal samples fluid (OP) samples were collected during the period from November, 2006 - January, 2009 from 34 sheep herds and different rejoins in Jordan that had clinical signs in the last two outbreaks. Collected samples were transported to the research laboratories at Faculty of Veterinary Medicine at Al baath University as described by Kitching & Donaldson (1987).

The reference serotype O was inoculated into bottom flasks of BHK-21 with maintenance medium for 24 hours, then harvested after complete CPE. The cell suspension centrifuged at 1500 rpm for 15 minutes and stored in –70°C. Virus isolation on BHK-21 Cells, titration and growth kinetic curve were achieved by using the methods of Ferris & dawson (1988); Fulton & confer (1995); Kitching (1987), respectively. The Serotyping of FMDV isolates was done by sandwich ELISA following to the laboratory technical protocol (SOP, WRL, Pirbright, UK) and as described by Voller & Bidwell (1976); Roder & Smith (1987); OIE (2004).

**RESULTS**

Propagation and preparation of FMDV in BHK-21 cells:

Following inoculation of FMDV reference O strain was propagated and adapted to BHK-21 cells. Firstly, mild cytopathic effects (CPE) were developed by the first passage. The virally infected cells developed complete CPE that was characterized by cell rounding and enlarged cell aggregations. Eventually, the virus induced complete CPE With subsequent passages, in a form of aggregation and grape-like clusters with a titer of 1.6x10^6 TCID_{50}/ml. The propagated virus was used as reference in the all diagnostic assays.

Isolation of FMD Virus from OP samples on BHK-21 cells:

Almost the CPE that appeared on BHK-21 cells post-inoculation by OP samples was relatively similar to that observed previously with the inoculated reference virus. The CPE was progressed and developed in the form of granular lytic changes such as cell rounding and aggregation and was less than that seen before. The virus infectivity at the finally adapted passage was 4x10^3 TCID_{50}/ml. A nine OP field samples out of 113 were reproduced and recorded cellular changes with a
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percentage of 7.96%.

Growth kinetic assay and infectivity titration of an FMDV isolates after the fifth a BHK-21 cells respectively:

The growth experiments was performed and titrated on the growth characteristics, and kinetics of FMDV isolates at fifth passage was measured and evaluated on cells at 12 hours intervals. CPE was first visible at 12 hours in BHK-21 cells. The initial titer of the OP isolates on BHK-21 cells was $5 \times 10^2$ TCID$_{50}$/ml and the highest titer was $9 \times 10^4$ TCID$_{50}$/ml.

Serotyping of FMDV isolates from both field tissue and OP samples by Indirect Sandwich ELISA:

The serotyping of FMDV isolates by indirect sandwich ELISA had been carried out on OP samples after propagation and isolation of the virus on BHK-21 cells. The test was able to serotype nine out of 113 field samples (7.96%). The isolates included 6 samples of serotype O (5.3%) and 3 samples of serotype A (2.65%). The comparative percentages of the detected FMDV serotype O and A of positive samples by ELISA post – inoculation on cell culture were 66.65% and 33.35%, respectively.

DISCUSSION

Type O appeared to be the most prevalent according to the Annual Report, MOA (1995-2005). BHK-21 is recommended for FMDV growth, but it is commonly found that cells vary between laboratories and different types of FMDV (OIE, 2004). In this study flat-bottom flasks with LK and BHK-21 cells were used for isolation of FMDV. The virus isolation depended upon the induction and production the CPE. The tested samples induced cellular changes on BHK-21 cells at the fifth passage with a titer of $4 \times 10^3$. This is in agreement with House and House (1989).

The isolation of FMDV on BHK-21 cells has been done successfully as the low amount of virus in OP samples was detected. This is in agreement with Alexandersen et al., (2003) ; Ferris & Dawson (1988) ; Barteling (2004) who recommended that kind of cell culture for isolation of FMDV from field samples and vaccine preparation. The infectivity titration proved to be a reliable and a precise method for the quantitation of the active infectious virus particles in the positive samples by virus isolation on cells. This was compatible with Parida et al., (2005).

The results presented here demonstrated a close similarity between the serotypes, which were responsible for infection in the two outbreaks depending on MOA reports and that isolated from convalescent sheep. This is confirmed by the herd histories which indicated that the sheep had been subjected to infection by field FMDV. This is in agreement with the observations of Moonen et al., (2004). ELISA is a complementary and confirmatory differential technique of FMDV identification and serotyping (Kitching & Hughes, 2002 ; Crowther et al., 1995).

Isolates from infected herds distributed for two outbreaks in Jordan showed to be the carrier status of the virus, this is in agreement with Alexendersen et al., (2002) ; cox et al., (2005); Cristina et al., (2009) who reported that the carrier animals may harbor the virus in cells of upper airway passage for months or even years. The obtained results showed that serotype O was the responsible causative agent of FMD outbreak among herds. This was documented by Pirbright Laboratories (WRL) in the UK and Annual Reports of MOA (1995-2005). The results showed that serotype A is playing a significant role in the two outbreaks in sheep flocks in Jordan. This could be one of the explanation of the occurrence of FMD outbreaks in spite of vaccination of sheep with univalent FMD vaccine contains serotype O ( Kitching, 1992 ; Samuel et al., 1990 ; Kitching et al., 1988). This work has revealed, that the convalescent animals could play an important role in the spread of infection during various epidemics of FMD in Jordan, which has been confirmed by Salt (1993), Barnett et al., (2004).
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الاغنام عند القلاعية الحيم المصابة بفيروس الصرع والإنشقاق الأردني في العلاج بعد الاعيوبة وياسين، ياسين، عمر، وأنور، 1

ملخص الفترة خلال وبين حدوث العيان بعد الأردني في الاغنام عند القلاعية الحيم حالاً ووصف إلى تهدف الدراسة هذه بين الواقعة 2006-2008 حيث الدراسة المنطقية في الاغنام القطع بين متفشية كان المرض.

تجمعت 113 حقلية نحوية على الأعراض عليه التي القطع ضمن الاغنام المعي السائل السائلية المشاكلية وعند الفيتامين 21 BHK 9 ظهر 9 أصل من العيان 113 حقلية بنسبة 7.96% الهمسترن كلياء على وتضح خلوي التغير الأسيميك الأسيميك العيان BHK 21 تأكيد تم حيث ظهر yanı الأحياء اختبار بواستة الفيروس تركيز قياس الخلوي التغيرات معدل بين معنى علاقة هنا لب الفيروس وتركيز.

تم الإنشقاق الاختبار بواستة الحيم عزاولة ليس كور سي إن تشريحة 113 عينة O ونسب 5.3% بينما تشريحة 113 عينة A ونسب 2.65%.

الكلمات الدالة: الفيروس، الاغنام السعزل، القلاعية، الحيم.