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Organic Circulation and persistence of Foot – and – Mouth disease virus type O in guinea pig

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ABSTRACT

Since, the most susceptible laboratory animal to Foot–and–Mouth disease virus (FMDV) is guinea pig, and then one milliliter of FMDV type (O) concentrations of 10^6-$10^{6.5}$ $TCID_{50}$was inoculated intradermally (ID) to 10 plantar surface of guinea pig (right side). Guinea pig adapted virus has been prepared after generalization phase, and then one milliliter of virus was inoculated intradermally to 30 guinea pigs in 5 groups (each consist of six). Samples from different organs including, heart, lung, liver, spleen, pancreas, tongue and plantar epithelium, retropharyngeal and inguinal lymph nodes were collected on days 2, 4, 14, 30, 60 post inoculation (PI) and kept in transport media and sent to FMD department of Ref lab. According to the results of this study, plantar epithelium and tongue as well as lung have been detected to be the original sites for virus survival. In addition, the highest rate of the presence of virus can be seen on 4 days and 14 days PI. By the way there was no sign of presence of the virus in any organs on days 30 and 60 PI.

Keywords: Foot – and – Mouth disease virus, type O, ELISA, PCR, Guinea pig

INTRODUCTION

Foot-and-Mouth disease (FMD) is one of the acute and contagious viral diseases that infect all types of wild and domesticated cloven-hooved ruminants and pigs (livestock). (Thomson 1994). Foot-and-mouth disease is associated with an aphthovirus genus (family Picornaviridae) (Belsham 1993). FMD is transferred very quickly outbreak to healthy animals and can cause a severe economic damage (Alexandersen *et a*l 2002a, Donaldson *et al* 2000, Hughes *et al* 2002, Barnett *et al* 1999, Callens *et al* 1998). Seven types and 85 subtypes of FMDV are well known today for laboratory technician. The main serotypes are: O, A, Asia 1, C, SAT1, SAT2 and SAT3. There are a number of immunological and serological distinct subtypes with different degrees of virulence. As there is no cross-immunity between serotypes, immunity to one type dose not confers protection to others. It is reasonable before outbreak of FMD in local area to take the necessary

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major for vaccination against FMD disease (Kitching *et al* 1998, Kitching *et al* 1989). By the way creating the possibilities of disease in low doses of the virus it would enhances the virus proliferation, virus excretion values, top wind speed and transfer the virus. These causes' problems in terms of control and eradication that is difficult and economically expensive. In fact the farmers are concerned about damage due to the disease. There are considerable points about FMD that after acute phase, sometimes the virus is remained in retropharyngeal lymph node more than 28 days, such animal is so called a carrier. Percentage of carrier animals under experimental conditions has been variable, but the average is about 50 percent. Virus infection titration in samples of esophagus and carriers throat´s liquid is low (by taking probing examination) (10-100 $TCID_{50}$/ ml) (Alexandersen *et al* 2003a). Extensive investigations in a small research laboratory for using in FMD have began in early twentieth century, but in 1920 the researchers inoculated FMDV (ID) to guinea pig´s plantar region then it was known the plantar of this is sensitive (Waldmann & Pape 1920). Since 1949 the research study with more details on the risk factors that influence on quality of FMDV including, vaccination experiments by using these animals had continued. In addition to, many statistically acceptable results could be observed and obtained when guinea pigs are used common for taking the laboratory of FMD vaccine test and hyper immune serum preparation against different type and subtypes of viruses. The purpose of present research was to find out the duration of persistence of FMDV in different organs of guinea pig after ID rout of administration in plantar surface of leg.

MATERIALS AND METHODS

Virus and guinea pig identification. In this study, FMDV type O with 10^6-$10^{6.5}$ $TCID_{50}$/ml was used.

Preparation and adaptation of foot-and-mouth disease virus to guinea pig. Ten guinea pigs were adapted to virus by injection of 1 ml of FMDV type O to right plantar surface (right foot). Several passages of viruses were performed until generalization and adaptation phases to FMDV were appeared. Vesicles in non-injected footpad of guinea pigs, liquid discharge from mouth, nose, eye and tongue lesions, were considered as symptoms of infection, viral generalization and adaptation phases. The epithelium of non-injected footpads were collected and preserved in glycerinated phosphate buffer as a transportable media for the performing of the assay test.

Preparing tissues for injection. The Samples were prepared according to standard methods (Burrows *et al* 1981) and kept in -20 °C until performing the tests.

Guinea pig's injection. Thirty guinea pigs in 5 groups (six for each group) were injected with 10^6-$10^{6.5}$ $TCID_{50/ml}$ of adapted virus to right footpad. In each group only one guinea pig was considered as control case which was injected with normal saline on their right footpads.

Sample Collection methods. Five virus injected guinea pigs and one control animal were sacrificed and samples of different organs such as: planter epithelium, tongue epithelium, heart, lung, liver, spleen, and pancreas and retropharyngeal and inguinal lymph nodes on days 2, 4, 14, 30 and 60 PI were collected. The grinded samples were washed twice by using four micro liters of Minimal Essential Media (MEM) then samples were pulverized in two ml of RNase-free water (Diethylpyrocarbonate Water) and homogenized. One ml was distributed in two micro tubes (1.5 ml) and stored in -20 °c for next stage operation.

ELISA and PCR assays:

ELISA test. Sandwich ELISA is used for virus detection on above mentioned organs according to (Oliver *et al* 1988 and Crowther *et al* 1979). After freezing and thawing in first step, the plates were

coated by rabbit serum against the FMDV type O. After washing 50 micro liter (µl) of prepared samples were added and washed. For the second time 50 micro liters of guinea pigs antibody against the FMDV type O was added and then 50 micro liter conjugated rabbit anti guinea pig antibody was added, and then 50 micro liter substrate and chromogen solution (Orthophenylen diamine) was added. When reaction was stopped the plates were considered and readed at 492 nm wave length by Elisa reader (BDSL Immunoscan MS).

PCR amplification and gel electrophoresis. The Primers which was used in this study are including: NK72 (GAAGGGCCCAGGGTTGGACTC) and C244 GCAGCAAAACACATGTCAAACACCTT) for type O (Reid *et al* 2001, Reid *et a*l 1999, Reid *et al* 1998). cDNA was Prepared from RNA extracted from virus (Viral RNA extraction Kit Roch, Cat NO.185888200). The final volume of RT step performance should be considered only in 40 micro liters for the better performance. All PCR reactions were performed in a total volume of 50 µl. Each mixture contained 5 µl of 10X reaction buffer, 1.5 mM $Mgcl_2$, 5 µl. 4.5 µl of primer, 1.5% gelatin, 1 µl dNTP, 1 µl Taq polymerase, and must be sterile by using of double-distilled water to 32 µl. 5 µl of cDNA, without saying for taken PCR test you should consider the protocol precisely 94 °C for 5 minutes, 30 cycles for 45 seconds at 94 °C, 55°C for 45 seconds, 72 °C for 45 seconds; then 72°C for 10 minutes. Following PCR, 6 µl of amplification products was mixed with 6x loading buffer and subjected to electrophoresis in 1.5% agarose gel in TBE buffer containing ethidium bromide, at 75 v/cm for 1.5 hours. The specific band at 1131 bp location in 1.5% agarose gel was noted (Reid *et al* 1999, Knowles N.J. & Samuel A.R. 1998, Salt 1993).

RESULTS

ELISA assay. Results of ELISA assay (Figure 1) revealed that only there was small amount of virus in the spleen on 2 days PI. However on 4 days PI presence of virus was detected in heart, lung, pancreas, and non-injected plantar epithelial. The amount of virus in non-injected plantar epithelium was more than other organs. Fourteen days PI presences of virus in different organs were detected. The maximum amount of virus was in non inoculated plantar epithelium, lung, liver, inguinal lymph node, tongue epithelium, heart and pancreas respectively. One and two months PI no virus has been found in any organs**.**

PCR assay. Identification of FMDV isolate was confirmed by species-specific PCR. The PCR amplification of isolates produced a single band of 1131 bp with specific FMDV primers (Table1). By the way positive control sample used in this assay was FMDV type O_{manias} (Figure2).

DISCUSSION

As for the laboratory based antigen-ELISA, this test remains very suitable for confirming positive cases (with high specificity). The sensitivity of this assay is about to 100% and the specificity 95% (Hamblin *et al* 1986a, b, 1987). In this study negative typing by ELISA was less than 0.1 OD and positive typing was above 0.1, according to Crowther et al. 1979. The time-course showed that the appearance of vesicular lesions in cattles was coincident with the peak of viraemia and high concentrations of virus in sites where there were clinical lesions (Gailiunas, 1968, Gailiunas & Cottral 1966, 1967). In our study, appearance of vesicle in the guinea pigs was on plantar and tongue epitheliums in peak time of presence of the virus (14 days PI). Interestingly, the contact-infected pigs took 1±2 days longer than those that were inoculated artificially (Oleksiewicz *et al* 2001) to reach peak levels of viraemia and tissue-localized virus and, moreover, the peak levels were approximately 1±2 log units lower, except for epithelial lesions, which had very high and

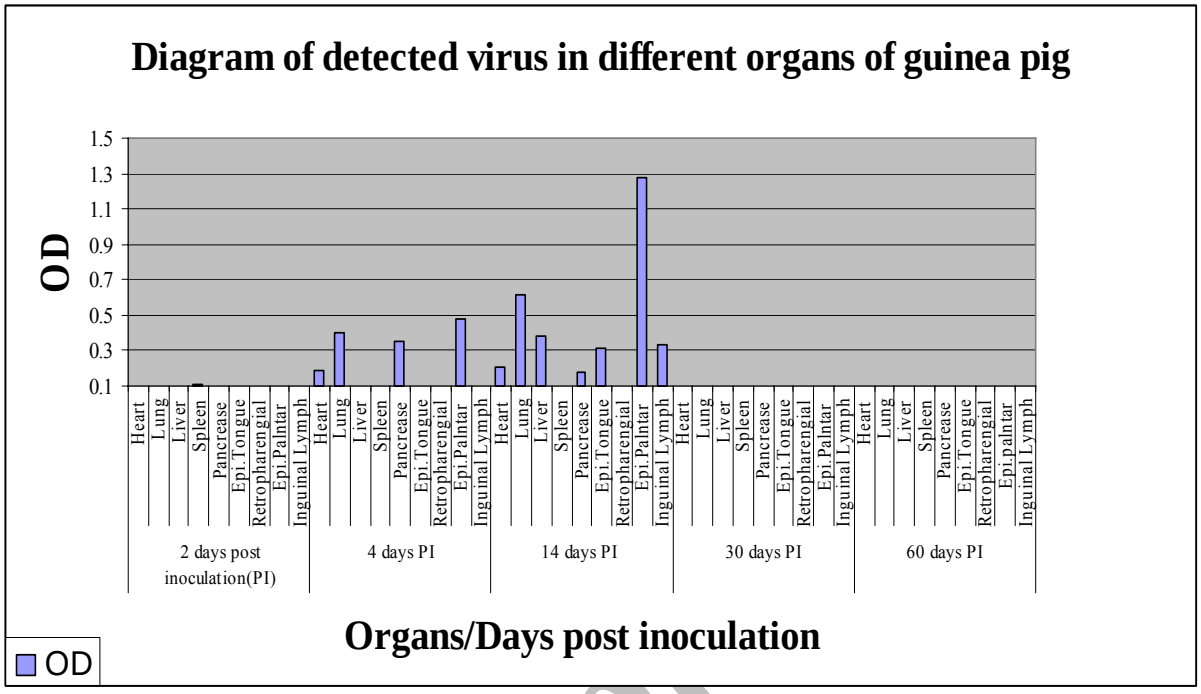


Figure 1. Diagram of virus detection in different organs of guinea pig on different days PI.

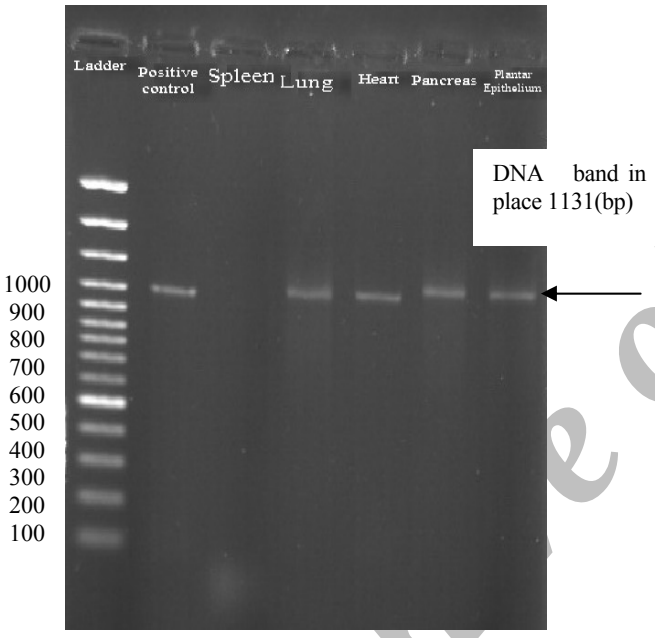
comparable levels in both groups. This may indicate that, when a longer period is required for virus amplification, the host response may reduce virus replication more effectively. As in this study reduction of the presence of virus in 30 and 60 days after inoculation was observed. It is interesting, however, that, when the distribution of virus in pigs infected by different routes, i.e. contact and intradermal (sub dermal heel pad inoculation) (and selected samples from a single pig infected by airborne virus), were compared, similar patterns of tissue distribution were found. However, virus accumulation in the tonsil and perhaps the lung in late infection were unexpectedly high in the pig infected by the aerogenous route. This may suggest that the initial distribution of FMDV in pigs is determined in part by the predilection of virus for certain sites in the pig, as has been established for cattle (Burrows *et al* 1981). By contrast, the virus concentrations in other tissues were low initially and, for certain tissues, increased sharply at days 3 and 4 post exposure. Thus, these data are consistent with tissues of the pharynx being the most likely initial sites of virus replication or deposition, as we seen in plantar and tongue epitheliums in guinea pigs in this study.

Table 1. Results of PCR assay after 14 days post inoculation.

	Organ	Result
14 days post inoculation	Heart	+
	Lung	+
	Liver	+
	Spleen	-
	Pancreas	+
	Tongue Epithelium	-
	Retropharyngeal Lymph Node	-
	Plantar Epithelium	+
	Inguinal Lymph Node	-

However, the differences observed in the kinetics of FMDV replication and accumulation between pigs infected by natural routes and those infected by artificial routes, is that natural FMDV infection in pigs is initiated by the deposition of inhaled virus in the pharynx, in particular the soft palate and the

tonsil. After passage through local lymph nodes, the virus enters the bloodstream at a low, not yet measurable, level of infectivity (Davidson 1997). A cycle of FMDV replication in pigs is of within the 12±24 h duration, so heel pad-inoculated pigs take about 48 h to develop severe disease (and actually around 72 h if given a small dose), while pigs infected by contact require 72±96 h to become severely ill.



In contrast, pigs exposed to natural aerosol (receiving a minimal infectious dose) required about 120 h to develop severe disease. If the infective dose were to be reduced further, we would expect that even more time (more replication cycles) would be required to reach high virus levels and the development of clinical disease (Davidson 1997). In current study in guinea pigs FMDV was detected on 2 days P.I. whereas two weeks P.I was required to reach peak levels of FMDV in organs. In pigs the lungs yielded only small amounts of virus, indicating that they probably play a minor role in FMDV replication. Lymph nodes and the spleen appeared to accumulate virus at days 3 and 4 post exposure (Davidson 1997). This virus was most likely produced elsewhere and altered from the lymph and blood. The liver contained virus at a level that could be explained by the concentration of virus in the bloodstream. In our study, virus was detected 2 days p.i in spleen and 14 days P.I. in inguinal lymph nodes of guinea pigs. In this regard amount of detected virus in guinea pigs' liver can be induced by viremia. According to some studies in cattle that is expressed that pharyngeal area is the usually the primary site of infection except when the virus directly enters into the cornified epithelia or the circulation by damage to the intact integument (Burrows *et al* 1981). Our findings revealed that the primary proliferation location of FMDV is in plantar epithelium. In contact or aerosol- exposed infected cows, virus may be demonstrated in the pharynx for 1 to 3 days before a viremia or clinical disease (Burrows *et al* 1981, Alexandersen *et al* 2002 a & b). Little is known about the relevance of the FMDV receptors to various host range, target cells or persistence. Although it may appear likely that the receptor(s) would be an important determinant of host range, the above mentioned studies have in several instances used human or non-livestock genes apparently allowing efficient entry and replication of FMDV. Consequently, it may be possible that other host factors are important for efficient replication of FMDV in vivo and that the presence of appropriate receptors on a cell is in itself not sufficient to allow replication of FMDV. After initial replication in the pharynx, or in the skin if the virus has entered directly through damaged integument in cattle, virus is spread through regional lymph nodes and into the circulation (Burrows et al 1981, Alexandersen *et al* 2002 a & b). This can be detected as a plasma/serum-associated viremia usually lasting for 4–5 days (Alexandersen *et al* 2002a, Alexandersen *et al* 2003b). In this study after the incubation period in guinea pig, 2 days PI of virus, FMDV survived up to 14 days PI in guinea pig's plantar pad. 14 days PI, virus was visible in inguinal lymph nodes. About of secondary sites,

multiple cycles of viral replication, transmission in particular in the cornified epithelia of skin, tongue and mouth where the main viral amplification occurred in porcine tissues (Oleksiewicz *et a*l 2001, Alexandersen *et al* 2001, Burrows *et al* 1981). Although in pigs vesicular epithelia clearly contain the highest concentration of virus, apparently normal skin, both hairy and hairless, also contains significant amounts of virus (Alexandersen *et al* 2001). Experimental studies in cattle suggest that lymph nodes as well as lymphocytes and macrophages (including alveolar macrophages) play little or no role in FMDV replication and the presence of virus in lymphoid organs, the epithelia of the pharynx, mouth and skin (Burrows *et al* 1981). Virus circulating in guinea pigs in this case is that the virus entered in bloodstream to other organs and then reaches 14 days PI to epithelium of tongue. Virus Entered to other organs such as: lung, pancreas, heart, liver, spleen. In these organs amplified virus could be detected low in very dosage. Totally according to the results of present study it seemed the major sites for virus survival are plantar and tongue epithelium as well as lung that showing the highest doses virus level in four days and 14 days PI. It showed that bound to top match with cattle for FMDV studies.

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