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Haemagglutination activity of chick embryo chorio-allantoic membrane experimentally inoculated with foot and mouth disease aphthous virus

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Abstract
This study was designed to investigate the clinical and gross pathological features of the porcinophilic strain of Foot-and-Mouth Disease virus (FMDV) (O/Iraqi/014) in experimentally infected chicken embryo. Fifty-five specific pathogen free (SPF) chick embryo of 10-11 days age, were used in this study. All chick embryos were inoculated in the chorio -allantoic membrane with 0.1 ml of the porcinophillic strain (O/Iraqi/014/FMDV), the viral suspension containing $10^{4.7}$/ ml Egg Infectious Dose fifty (EID50). This virus strain was isolated from the epizootic of FMD in Iraqi sheep in 2014. Five chick embryos were inoculated with 0.1 ml of phosphate buffer saline only and acted as the control. The EID50 was calculated according to Reed and Munch method. The results approved the gross pathological changes in the chorio-allantoic membrane. In addition, haemagglutination test was approved the virus activity in the chick embryos harvest.

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Introduction

Foot-and-mouth disease (FMD) (FMDV; family Picornaviridae; genus Aphthovirus) is believed to be the most significant constraint on international trade of cloven-hoofed (two-toed) animals (e.g,. cattle, pigs, sheep) livestock and wildlife and their derived products (Sumption *et al*., 2008). The pathogenesis of FMDV has been studied previously (Yilma, 1980; Burrows *et al*., 1981; Alexandersen *et al*., 2003a). Examination of lesions in cattle by an immunofluorescence technique revealed that the process of vesicle formation was somewhat analogous to that of plaque development in tissue culture (Yilma, 1980). The in situ hybridization studies were demonstrated that the early FMDV lesions localize in multicellular segments and laminae of the epidermis in experimentally infected guinea pig and cattle (Brown

et al., 1995). The infection studies in swine revealed that FMDV was widely disseminated in all epidermal tissues regardless of histological apparent cellular disruption (Brown *et al*., 1995). The histology and the distribution of virus were comparable with those reported in cattle (Gailiunas, 1968). Experimentally infected pigs were approved to excrete airborne FMDV, as well as the virus in nasal fluid and saliva, at maximal levels during the early phases of clinical disease (Alexandersen *et al*., 2003b). Monaghan *et al*., (2005) demonstrated replication and the mechanism of FMDV lesion in the epithelium of pigs. The virus concentration in a material is often expressed as ID 50 (50% infective dose) per a certain weight or volume of the material, e.g. per gram or per ml. By definition, the ID50 is the dilution of material which would infect 50% of the individuals (Sutmoller and Vose, 2001).

Human's susceptibility to the FMD virus was debated for many years. The virus has been isolated and typed (type O, followed by type C and rarely A) in more than 40 human cases in Valentini in Germany that keep FMD as a zoonosis disease (Bauer, 1997). FMD is considered as global disease (Kesy, 2002). The symptoms of FMD in humans are included: malaise, fever, vomiting, red ulcerative lesions (surface-eroding damaged spots) of the oral tissues, and sometimes vesicular lesions (small blisters) of the skin (Canadian Food Inspection Agency, 2013). There are scarce studies regarding the experimental infection of FMD virus in Iraq. So this study was designed to investigate the clinical and histopathological features of the porcinophilic strain of Foot-and-Mouth Disease virus (FMDV) (O/Iaqi/014) which were isolated from FMD outbreak in sheep, in experimentally infected chicken embryo.

Materials and methods

Samples collection

A total of 100 vesicular samples were collected from vesicles blister of the feet and mouth of sheep suffering from clinical signs of FMD, in addition the infected sheep revealed profuse salivation and severe lameness. The detection of foot and mouth disease virus were performed by rapid test (Immuno-chromatography) supplied from Biochek company –USA. The positive samples for aphthous by the rapid test were diluted with phosphate buffer saline or normal saline and used subsequently for virus isolation and experimental infection.

Virus isolation

The supernatant fluids of the vesicular suspensions were obtained by centrifugation at 1000 g/minute for 10 minutes at a temperature not exceeding 25°C and inoculated into the chorio-allantoic membrane (0.1 ml/ for each) of at least five embryonated SPF fowl eggs of 10–11 days age. These were incubated at 35–37°C for 2–4 days, after inoculation (Alexander and Senne, 2008). To avoid the contamination, all samples were treated by incubation with increased antibiotic concentrations for 2–4 hours (Streptomycin 500mg/ml, Penicillin 500 I.U/ml, Mycostatin 500mg/ml) for every 9ml suspension. One positive sample of the aphthous virus was diluted in a

ten-fold serial dilutions according to the method described previously by (Mackay *et al*., 1998). An adaptation of the mathematical technique devised by Reed and Muench (Reed and Muench, 1938) was used to calculate the dilution of the virus suspension, which being tested to produce the end point of the results of the Haemagglutination (HA) test. The end point contains one unit of infectivity of 50 percent Embryo Infectious Dose (1 EID 50). This dilution is then used to calculate the Infectivity Titer (Thayer and Beard, 2008), on each of the inoculated eggs. Five embryonated eggs are inoculated with each dilution. After incubation for four days, the HA test is used to determine the presence of the virus. This data is used to calculate the Infectivity Titer.

Experimental study

A total of 50 fertilized eggs (10-11days old) were divided into ten groups, each group with five embryonated eggs. The first group of the chick embryo were inoculated directly on the chorio-allantois with 0.1ml of aphthous virus suspension (this suspension was collected from one positive FMD sample). The second group was inoculated with 0.1 ml of sterile phosphate buffer saline (PBS) and acted as control group. The other group were acted as control group and were injected with $0.1X10^{4.7}$ EID_{50} ml. All inoculated eggs were incubated at 36.5 ^{0}C. All inoculation procedure were done according to the method described previously by (OIE, 2011). The gross pathological changes and lesions were reported after 4 days and the chorio-allantois membrane harvested. All other procedures of haemagglutination test were done according to (Briody and Stannard, 1951).

Results

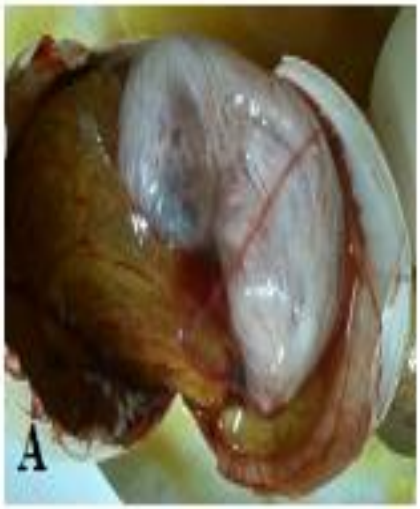
The gross pathological changes were appeared on the chorio-allantois membrane of the chick embryo inoculated with aphthous virus suspension, in addition, dead chick embryo were also seen within inoculated groups (Figure. 1). The dead embryo were appeared as small gray-black color accompanied with changes in the chorio-allantois membrane (Figure 2) in compare to normal embryo in control group (Figure. 3 A&B). The result of the EID 50 for each dilution were recorded as the number of eggs infected (HA +ve) and the number of eggs not infected (HA -ve) by the inoculum (Table.1).

Discussion

World Organization for Animal Health (OIE) are released different methods that use to identifying the FMD status in different countries. In Indonesia, a formal survey of eight international FMD experts have been reported different opinions from each individual experts to investigate the finding of FMD. In addition, the experts' responses was relatively accurate (91% sensitivity and 85% specificity) at identifying the FMD status of Indonesia, (Garabed *et al*., (2009) in compare to methods that employed by OIE. The virus infectious dose of chick embryos are reported previously by scientists (Briody and Stannard 1951; Maitland and Tobin,

1956). Maitland and Tobin, (1956) found that the virus increased in the allantoic fluid and play important role in the results of the haemagglutination test. (Other researcher discussed also the ability of chick embryos inoculation method to study the hydropericardium syndrome virus (Mansoor *et al*., 2009).

Figure 1. Shows the gross pathological changes appeared on the chorio-allantoic membrane in the chick embryo inoculated with aphthous suspension.
Figure 2. Shows the dead chick embryo inoculated with aphthous suspension.



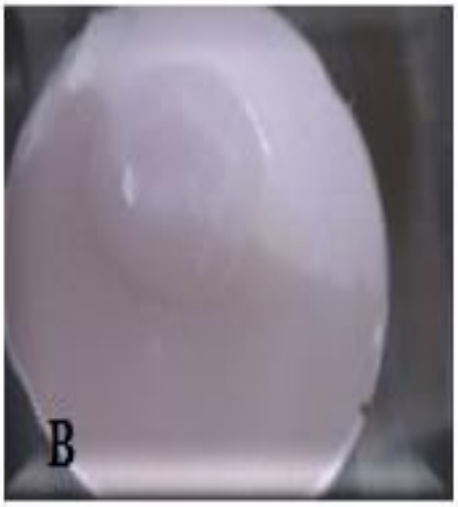


Figure 3. Shows normal: A) embryo and B) chorio-allantoic membrane of control eggs treated with phosphate buffer saline.
Table 1: Shows the results of the HA positive and negative infected embryo (the equation is extracted from completed Reed Muench working sheet).

Dilutions	Number of eggs infected (HA +ve)	Number of eggs not infected (HA -ve)	Accumulated numbers			Percentage infected A/(A+B) × 100
			Infected (A)	Not infected (B)	Total number tested (A+B)	
10^{-3}	5	0	11	0	11	11/11 = 100%
10^{-4}	4	1	6	1	7	6/7 = 86%
10^{-5}	2	3	2	4	6	2/6 = 33%
10^{-6}	0	5	0	9	9	0/9 = 0%

From this data, the percentage cases entered in the Reed and Muench formula are as follows:
Dilution of 10^{-3} infected at dilution immediately above 50% = 86 percent
Dilution of 10^{-4} infected at dilution immediately above 50% = 33 percent
The index was calculated as follow:
Index = (86% - 50%) ÷ (86% - 33%)
Index = 36 ÷ 53 = 0.7
The index of 0.7 is applied to this dilution that provided the 50 % infection of eggs or 1 EID_{50} is $10^{-3.7}$.
The reciprocal of this dilution is the amount of virus contained in the 0.1 mL of the original suspension
= $10^{3.7}$ EID_{50}/0.1 ml
= $10^{4.7}$$EID_{50}$/ml

The results of the present study approved the effects of aphthous virus on chick embryo that was inoculated in the chorio-allantoic membrane. The aphthous virus revealed the characteristic gross pathological changes in the chorio- allantoic membrane of the infected embryos. In addition, to the haemagglutination activity that appeared in this membrane. This results is compatible to previously reported studies by Alexandersen, *et al*., (1997), when matured Minimal aerosol infectious dose were prepared from the O1 Lausanne strain of foot-and-mouth disease virus for pigs. In conclusion, this study approved the ability of the porcinophilic strain of Foot-and-Mouth Disease virus (FMDV) (O/Iraqi/014) to induce the gross pathological changes in the experimentally infected chick embryos. Moreover, haemagglutination activities were also seen in the infected chick embryos.

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