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Comparison of sensitivity and specificity in three commercial foot-and-mouth disease virus non-structural protein ELISA kits with swine sera in Taiwan

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Abstract

Three commercialized ELISA kits for the detection of antibodies to the non-structural proteins (NSPs) of FMD virus were compared, using sera from uninfected, vaccinated, challenged and naturally infected pigs. The kinetics of the antibody response to NSPs was compared on sequential serum samples in swine from challenge studies and outbreaks. The results showed that ELISA A (UBI) and ELISA B (CEDI) had better sensitivity than that of the 3ABC recombinant protein-based ELISA C (Chekit). The peak for detection of antibodies to NSPs in ELISA C was significantly delayed in sera from natural infection and challenged swine as compared to the ELISA A and B. The sensitivity of the three ELISAs gradually declined during the 6-month post-infection as antibodies to NSP decline. ELISA kits A and B detected NSP antibody in 50% of challenged pigs by the 9–10th-day and 7–8th-day post-challenge, respectively. ELISA B and C had better specificity than ELISA A on sequential serum samples obtained from swine immunized with a type O FMD vaccine commercially available in Taiwan. Antibody to NSPs before vaccination was not detected in swine not exposed to FMD virus, however, antibody to NSPs was found in sera of some pigs after vaccination. All assays had significantly lower specificity when testing sera from repeatedly vaccinated sows and finishers in 1997 that were tested after the 1997 FMD outbreak. However, when testing sera from repeatedly vaccinated sows or finishers in 2003–2004, the specificity for ELISAs A, B and C were significantly better than those in 1997. This effect was less marked for ELISA A. The ELISA B was the best test in terms of the highest sensitivity and specificity and the lowest reactivity with residual NSP in vaccinates.

Keywords: Foot-and-mouth disease; Diagnosis; Non-structural proteins; ELISA (enzyme linked immunosorbent assay)

1. Introduction

Foot-and-mouth disease (FMD) is caused by infection with FMD virus, an *Aphthovirus* genus of

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family *Picornaviridae* and is one of the most contagious infectious diseases of cloven-hoofed animals. The virus strain, O Taiwan 97, however, has been shown to have a species-specific adaptation to pigs (Dunn and Donaldson, 1997) and only caused overt clinical signs in pigs in the March 1997 outbreak of FMD in Taiwan (Shieh, 1997).

FMD causes severe economic losses. The March 1997 FMD outbreak in Taiwan resulted in financial losses in the hundreds of millions of dollars for the Taiwanese pork industry (Chang et al., 1997; Shieh, 1997). The estimated value of the pork export market in Taiwan in 1996 was 1.53 billion US dollars. More than 400 million US dollars was spent to control the disease and compensate farmers for large numbers of pigs killed and incinerated during the outbreak. In addition to this direct cost were the costly control measures and the lost exports for the pork producers in Taiwan during the period needed to regain FMD free status (Yang et al., 1999).

Vaccination has been used successfully in controlling FMD in Taiwan (Yang et al., 1999) and for eradication in Europe (Leforban and Gerbier, 2002). However, differentiation of vaccinated from convalescent animals is still a major problem challenging those countries, which are working toward the eradication of FMD with compulsory vaccination programs. Therefore, there is a great need for reliable approaches to detect infected animals in the vaccinated population.

Antibody to non-structural proteins (NSP) of foot and mouth disease virus, which is produced in infected animals, has been used to differentiate vaccinated from infected cattle in the field (Clavijo et al., 2004; De Diego et al., 1997; Mackay et al., 1998; Moonen et al., 2004; Sorensen et al., 1998). Recently, a number of enzyme-linked immunosorbent assays (ELISA) with high sensitivity and specificity for detection of antibody to NSP in pigs have also been reported as suitable for large scale eradication programs (Chung et al., 2002; Lee et al., 2004; Shen et al., 1999). However, the numbers of validated samples used in the Lee et al. (2004) study for evaluation of the sensitivity of three assays were below the numbers recommended by Office International des Epizooties (O.I.E.) for sensitivity evaluation (O.I.E., 2004a). The study by Chung et al only evaluated an in-house kit based on the 3AB antigen obtained from Dr Sorensen that was not commercially available at that time (Chung et al., 2002). Since the ELISA-based methodology for NSP antibody detection provides many advantages, such as objectivity compared with gel diffusion tests for NSP, high sensitivity and specificity, and the capability for large-scale screening, NSP ELISA has been recommended by the OIE to be used for serologic surveillance in regions or countries that practice FMD vaccination and for monitoring virus circulation in the field (O.I.E., 2004b).

Recently, several NSP-ELISA assays have become commercially available for evaluation. A blocking ELISA has been developed (Sorensen et al., 2005) which has shown high sensitivity and specificity when tested with pig sera collected from naïve, infected, and vaccinated pigs. The test specificity for vaccinated pigs is 99% and can be improved to 99.5% by further treatment by filtration and inactivation at 56 °C for 30 min. For naïve pigs the specificity was 99.8%. Another commercial kit was evaluated by Bruderer et al. who demonstrated the specificity was 99% for 3600 samples tested including bovine, ovine and porcine species. Antibodies specific for 3 ABC could be detected as early as 10 days post-infection (Bruderer et al., 2004). A third kit evaluated uses a 3B synthetic peptide based NSP ELISA which can be used to differentiate convalescent animals from vaccinated animals (Shen et al., 1999; Wang et al., 2001). There were no positive results detected in sera from vaccinated animals. However, preliminary field trials in Taiwan with this kit showed only 98.9% specificity in samples from vaccinated pigs. Comparative evaluations, including the above NSP ELISA kits, have been conducted by a consortium of European and American FMD reference laboratories in 2006 with large panels of sera from cattle that have been vaccinated or vaccinated-and-infected with different serotypes of FMD virus. Some sheep and pig sera were tested in the study but these authors stressed that insufficient numbers of samples from pigs had been tested in order to complete the evaluation of these tests for use with pigs (Brocchi et al., in press).

As these commercialized NSP ELISA kits will be very important for monitoring pigs for active FMD infection in countries using FMD vaccination programs, further evaluation of these tests are needed under different field conditions in order to determine the diagnostic sensitivity and specificity. This study