The possible role and significance of carrier swamp buffalo in the transmission of Foot and Mouth Disease in South East Asia (SEA)

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This thesis is presented for the degree of Doctor of Philosophy,

Murdoch University, 2011
I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

Blesilda C. Verin
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<td>AlOH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Aluminium hydroxide gel</td>
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<td>ASB</td>
<td>Asian Swamp Buffalo</td>
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<tr>
<td>BTY</td>
<td>Bovine Thyroid</td>
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<td>C-ELISA</td>
<td>Competition Enzyme Linked Immunosorbent Assay</td>
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<td>CPE</td>
<td>Cytopathic Effect</td>
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<td>CT</td>
<td>Threshold cycle</td>
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<td>DOE</td>
<td>Double oil emulsion</td>
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<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<tr>
<td>FMD</td>
<td>Foot and Mouth Disease</td>
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<td>FMDV</td>
<td>Foot and Mouth Disease Virus</td>
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<td>GC</td>
<td>Germinal Centres</td>
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<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Sulphuric acid</td>
</tr>
<tr>
<td>IAH</td>
<td>Institute for Animal Health</td>
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<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
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<tr>
<td>IgG HRPO</td>
<td>Immunoglobulin Horseradish Peroxidase</td>
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<tr>
<td>Lao PDR</td>
<td>Lao People’s Democratic Republic</td>
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<td>LCM</td>
<td>Laser Microdissection</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>LPBE</td>
<td>Liquid Phase Blocking Enzyme Linked Immunosorbent Assay</td>
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<td>Mab</td>
<td>Monoclonal antibody</td>
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<td>MGPs</td>
<td>Magnetic Glass Particles</td>
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<td>NRC</td>
<td>Non-reactive control</td>
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<td>NSP ELISA</td>
<td>Non-Structural Protein Enzyme Linked Immunosorbent Assay</td>
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<tr>
<td>OD</td>
<td>Optical Density</td>
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<td>OIE</td>
<td>Office International Epizooties</td>
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<tr>
<td>OP</td>
<td>Oropharyngeal or Oesophageal</td>
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<td>OPD</td>
<td>o-phenylenediamine dihydrochloride</td>
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<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
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<tr>
<td>PBST</td>
<td>Phosphate Buffer Saline Tween</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PI</td>
<td>Percentage Inhibition</td>
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<td>PI</td>
<td>Post Infection</td>
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<td>RC</td>
<td>Reactive Control</td>
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<td>RNA</td>
<td>Ribonucleic Acid</td>
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<tr>
<td>RRL</td>
<td>Regional Reference Laboratory</td>
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<tr>
<td>rRT-PCR</td>
<td>Real-Time Reverse Transcriptase - Polymerase Chain Reaction</td>
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<td>RT-PCR</td>
<td>Reverse Transcriptase- Polymerase Chain Reaction</td>
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<tr>
<td>SAT</td>
<td>South African Territory</td>
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<td>SEA</td>
<td>South East Asia</td>
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<td>SEACFMD</td>
<td>South East Asia and China Foot and Mouth Disease</td>
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<td>SEAFMD</td>
<td>South East Asia Foot and Mouth Disease</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>Sn</td>
<td>Sensitivity</td>
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<td>SOP</td>
<td>Standard Operating Procedure</td>
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<td>Sp</td>
<td>Specificity</td>
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<td>SPCE</td>
<td>Solid-Phase Competition ELISA</td>
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<td>SVD</td>
<td>Swine Vesicular Disease</td>
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<tr>
<td>TAGS</td>
<td>Test in the Absence of Gold Standard</td>
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<tr>
<td>TSA</td>
<td>Tyramide Signal Amplification</td>
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<tr>
<td>ul</td>
<td>microliter</td>
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<tr>
<td>UN</td>
<td>United Nations</td>
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<tr>
<td>VEV</td>
<td>Vesicular Exanthema Virus</td>
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<tr>
<td>VI</td>
<td>Virus Isolation</td>
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<tr>
<td>VNT</td>
<td>Virus Neutralization Test</td>
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<tr>
<td>VP</td>
<td>Virus Protein</td>
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<tr>
<td>VSV</td>
<td>Vesicular Stomatitis Virus</td>
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<tr>
<td>WRL</td>
<td>World Reference Laboratory</td>
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Abstract

Foot and Mouth Disease (FMD) is a serious trans-boundary livestock disease and is present in many parts of the world. It can result in devastating economic impacts in affected countries or regions. It is endemic in South East Asia (SEA) including Lao PDR and Myanmar. Susceptible animals infected with FMD typically show clinical signs two to four days after exposure to the virus, and in some cases within 24 hours, especially in pigs. However, some animals develop only mild lesions and in others lesions may not be visible. Some animals become persistently infected after recovery. This is the so-called ‘carrier’ state where the virus can still be recovered after 28 days post infection (PI) in the oesophageal-pharyngeal (OP) region.

In SEA, the Asian swamp buffalo (ASB), is important to livestock systems and communities and therefore the presence of FMDV in persistently infected ASB, is potentially of importance in disease control. To investigate the presence and the role of FMD carriers in ASB, cross-sectional and longitudinal studies were conducted in both Lao PDR and Myanmar. The cross-sectional studies were conducted to evaluate the use of tests to detect carriers under field conditions and to determine the percentage of ASB that were seropositive after infection; and the longitudinal studies were conducted to determine the proportion of ASB that remained persistently infected and also measure the duration of persistent infection. The studies were conducted at the sites of FMD outbreaks in 2008. These were due to FMD serotype O as confirmed by FMD laboratory reports from both FMD national laboratories and from the Regional Reference Laboratory (RRL) for FMD in Thailand.

In this study, several tests to detect FMD carrier animals were used and results of the
tests were compared. All laboratory diagnosis of samples collected from both Lao PDR and Myanmar were done at the Institute for Animal Health (IAH), Pirbright Laboratory, United Kingdom. The internationally accepted standard for confirmation of diagnosis of an FMD carrier animal is by recovery of live FMDV from OP fluid collected by probang sampling. This is a highly invasive process and is labour intensive and the recovery of the FMDV in carrier animals is usually intermittent. This makes OP fluid sampling difficult to use as routine diagnosis for carrier identification. An IgA ELISA based test has been developed to detect FMDV-specific immunoglobulin A (IgA) which is present in the serum and also in the saliva of animals after infection with and vaccination by FMDV. It has also been shown that the level of FMD-specific IgA was elevated in carrier animals and this IgA ELISA based test was developed to quantify this elevated level of FMD-specific salivary IgA in persistently infected animals. The non-structural protein (NSP) ELISA which differentiates antibodies due to infection from vaccination and which have been previously used in other species to identify animals that have been infected with FMD and which may still be carrying live virus was validated and used in this study.

This study is the first to validate test performances of the above tests on ASB population previously infected with FMDV. Using the Bayesian statistical analysis, the overall test sensitivity (Sn) of the four NSP ELISAs and the salivary IgA ELISA vary from 60% to 80% only but with high test specificity (Sp), which ranged from 97% to 99%. To maximize detection, a strategy of combining two independent tests, one NSP ELISA (Priocheck) and the IgA ELISA was made. Results of the test Se of the two combined tests showed an increase from 80% to 98% with test Sp of 99%. The performance of the virus isolation (VI) and the real time RT-PCR on ASB
population was validated using 101 OP fluid samples collected eight months PI. Results from VI showed that 14% of the 101 ASB were persistently FMD infected at eight months PI while only 10% of those were persistently FMD infected by real time RT-PCR. Using the strategy of combining two tests and to address specificity issue, only those samples that tested positive to both NSP ELISA and IgA ELISA (Priocheck +ve / IgA +ve) were considered as persistently FMD infected animals. The results showed a higher detection rate (32.7%) compared to VI and real time RT-PCR (10% to 14%).

This study also provided evidence of either silent infection, cross-infection, repeated reinfection, or virus persistence in ASB and that carrier animals may have transmitted the virus to naïve animals in Lao PDR. In this case serotype A was isolated from carrier ASB and their contacts were positive for serotype A in the IgA ELISA test instead of the expected serotype O virus which was the cause of the 2008 outbreak among cattle and ASB in Lao PDR.

The study has also provided evidence for the presence of a carrier state in ASB for at least 20 months PI, which was the end of the study period. Based on these findings a follow up study to investigate further the mechanisms and the epidemiological significance of ASB carriers in the maintenance and transmission of FMD will be necessary to fully understand the epidemiology of FMD in SEA. This will require more controlled laboratory studies using the tools validated in this study to clarify the mechanism for establishment of carriers, the factors influencing transmission and to demonstrate the rates of transmission from carrier ASB. This will be a crucial issue in the control and eventual eradication of the disease in the SEA region.
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