Animal biosecurity in the Mekong: future directions for research and development
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Cover: Children observing a serological survey and learning how the survey can improve their health and the health of the animals in their village (Takeo, Cambodia). (Photo: Domingo Caro III)
Foot-and-mouth disease (FMD) is a severe, highly communicable viral disease of cloven-hoofed animals including cattle, buffaloes, pigs, sheep and goats. Although FMD does not result in high mortality in most outbreaks, the disease is debilitating and leads to significant economic losses from its impact on trade. FMD has a significant impact on livestock systems due to lower production of infected animals resulting from reduced food intake, reduced meat and milk production and draught capacity, and losses from reduced trade and tourism-related activities (Kazimi and Shan 1980; Morris et al. 2002; Perry et al. 2002).

Before this study, there was little epidemiological information on which to formulate logical procedures for control. A further constraint to the development of extension programs for the control of FMD is the lack of information about the disease, including its prevalence and incidence and the direct and indirect losses incurred. The current study was developed to better understand the pattern of FMD and the risk attributed to the spread of the disease in Cambodia. This information will be used to underpin the establishment of a progressive zoning approach to control FMD in the Lower Mekong Basin (LMB).

Materials and methods

Study site T

The study was conducted in eight southern provinces of Cambodia: Kampong Speu, Kampot, Kandal, Koh Kong, Phnom Penh, Prey Veng, Sihanouk Ville and Takeo. These sites were initially identified by the South-East Asia Foot-and-Mouth Disease Campaign (SEAFMD) Program as suitable for developing a zone for the control and eradication of FMD in the LMB.

Sampling design

A two-stage sampling technique was used for selecting the study sites. Survey Toolbox (Cameron 1999), using an expected prevalence of 20%, and a between and within village variance of 0.05 and 0.2, respectively, was used to determine the number of villages and animals to sample. This program determined that 70 villages with 14 animals per village needed to be sampled.

A total of 490 farmers from 70 villages were interviewed. During the blood sampling, seven farmers were randomly selected from each village for questioning.

Questionnaire preparation

The questionnaire covered many aspects of livestock management including species of livestock kept, types of grazing, feeding, watering, possible contact between herds and disease management. After being pre-trialled and revised, the questionnaire was administered.

Laboratory analysis

Two enzyme-linked immunosorbent assays (ELISA)—non-structural protein (NSP) and liquid-phase blocking (LPB)—were used. These tests are serotype-specific and are highly sensitive, and the virus or antigen used in the test is closely matched to the strain circulating in the field (OIE 2008). Both NSP and LPB ELISA kits and techniques were developed by the regional reference laboratory for FMD, Pakchong, Thailand.
Statistical analysis

The seroprevalence was calculated for the NSP ELISA, and the mean antibody titre and type of FMD virus (FMDV) were calculated for the LPB ELISA. A village was considered to be positive if one or more of the sampled animals were seropositive (Bronsvoort et al. 2006). The exact binomial approach was used to calculate the 95% confidence intervals (CI) for the prevalence (Daly 1992).

The relationship between prevalence and age and gender was investigated using linear regression. The association between age and positivity was assessed by using ANOVA and Kruskal-Wallis tests, while a Chi-square test for independence and odd ratios and their 95% confidence intervals were calculated for gender (Armittage and Berry 1987; Martin et al. 1987). The prevalence was also stratified by the age of animals (Bronsvoort et al. 2006).

Putative risk factors for infection and causes of disease spread were analysed using Pearson’s correlation coefficient and logistic regression techniques. Univariate analyses were used to identify any potential risk factors associated with infection by applying a chi-squared test for independence, a Fishers exact test for categorical variables or an ANOVA for continuous variables. Then multivariable analyses were conducted where variables with a P value ≤ 0.25 in the univariate analyses were offered to a logistic regression model using a backward stepwise conditional method (Hosmer and Lemeshow 1989; Frankena and Graat 1997).

Results

Prevalence estimation

The results for the LPB and NSP ELISAs are summarised in Table 1. A total of 277 animals sampled (30%, CI 27.1–33.0%) were seropositive for FMD. The village-level prevalence of FMD in the southern provinces of Cambodia was 87% (CI 79.0–94.9%). There was a significant difference in the distribution of seroprevalence between the different provinces (χ²(1, 7) = 41.027, P = 0.000).

Three FMDV serotypes were present in the southern provinces of Cambodia with a prevalence of 28.5% (CI 25.6–31.5%) for type O, 9.5% (CI 7.7–11.6%) for type A and 9.3% (CI 7.5–11.4%) for type Asia 1. In general, the seropositive animals had low antibody titres to FMDV serotypes A and Asia 1. In contrast, titres to type O were up to 1:5120. Serotype O was present in most villages sampled (84.1%, 58 of 69) followed by type Asia 1 with 59.4% (41 of 69) and type A with 50.7% (35 of 69).

There was no significant difference in the prevalence of FMD in males (20.7%) and females (28.8%) (χ²(1,1) = 2.503, P = 1.114). However, the prevalence between the age groups (χ²(1,10) = 22.544, P = 0.013) was significantly different. The prevalence generally increased with age from 16.7% in the 1-year-old group to 50.0% in the 10-year-old group of animals.

Animals were regrouped into young (up to 2 years old) and adult (more than 2 years old) groups comprised of 209 and 551 individual animals

Table 1. The results of the LPB and NSP ELISA from different Cambodian provinces

<table>
<thead>
<tr>
<th>Province</th>
<th>Samples</th>
<th>LPB ELISA</th>
<th></th>
<th></th>
<th>NSP ELISA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>Prevalence (%)</td>
<td>CI lower (95%)</td>
<td>Positive</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(95%)</td>
<td></td>
<td></td>
<td>(95%)</td>
<td></td>
</tr>
<tr>
<td>Kampong Speu</td>
<td>191</td>
<td>111</td>
<td>58.1</td>
<td>51.1</td>
<td>67</td>
<td>35.1</td>
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<tr>
<td>Kampot</td>
<td>154</td>
<td>81</td>
<td>52.6</td>
<td>44.7</td>
<td>55</td>
<td>35.7</td>
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<tr>
<td>Kandal</td>
<td>135</td>
<td>55</td>
<td>40.7</td>
<td>32.5</td>
<td>28</td>
<td>20.7</td>
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<tr>
<td>Koh Kong</td>
<td>14</td>
<td>6</td>
<td>42.9</td>
<td>16.9</td>
<td>0</td>
<td>0.0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Phnom Penh</td>
<td>14</td>
<td>9</td>
<td>64.3</td>
<td>39.2</td>
<td>7</td>
<td>50.0</td>
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<td></td>
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<tr>
<td>Prey Veng</td>
<td>207</td>
<td>106</td>
<td>51.2</td>
<td>44.4</td>
<td>52</td>
<td>25.1</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sihanouk Ville</td>
<td>28</td>
<td>25</td>
<td>89.3</td>
<td>77.8</td>
<td>19</td>
<td>67.9</td>
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</tr>
<tr>
<td>Takeo</td>
<td>180</td>
<td>109</td>
<td>60.6</td>
<td>53.4</td>
<td>49</td>
<td>27.2</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>923</td>
<td>502</td>
<td>54.4</td>
<td>51.2</td>
<td>277</td>
<td>30.0</td>
</tr>
</tbody>
</table>

CI = confidence interval; ELISA = enzyme-linked immunosorbent assay; LPB = liquid-phase blocking; NSP = non-structural protein
respectively. The seroprevalence in adults was significantly higher (32.3%) than in young animals (21.1%), \( \chi^2(1,1) = 9.278, P = 0.002 \). The antibody level in the adult group was also higher than for the young group. There was a significant difference between seroprevalence in adults and young animals for serotype O \( \chi^2(1,1) = 26.618, P = 0.000 \) but not for serotypes A \( \chi^2(1,1) = 2.440, P = 0.118 \) or Asia 1 \( \chi^2(1,1) = 2.455, P = 0.117 \).

**Determinants of FMD**

The logistic regression model statistics are summarised in Table 2. The results from this analysis revealed that villages where farmers sought assistance from the village animal health worker were 2.8 times more likely to have FMD than in villages where no animal health services were provided. Animals that shared common grazing areas were 2.5 times more likely to contract FMD than those not grazing in common pastures. Similarly, villages where infected cattle were sold were 2.3 times more likely to have FMD than those that didn’t sell infected cattle. Pigs raised under free-range conditions were 1.9 times more likely to result in FMD than those that were penned or tethered. Interestingly, farmers who vaccinated against FMD were 3.9 times more likely to report outbreaks than those who did not vaccinate their animals. Animals that were tethered and fed cut grasses were less likely to get infected with FMD.

**Discussion**

The results from this survey indicated that FMD is endemic in the southern provinces of Cambodia and three FMDV serotypes (type O, A and Asia 1) are responsible for outbreaks in this region. Although these serotypes are widely distributed in the southern provinces of Cambodia, the high seroprevalence of antibodies to type O in animals of all age groups and in most villages indicate that this serotype was responsible for the most recent outbreaks. In contrast, the animal-level and age-stratified seroprevalence for type A and the distribution in the villages sampled suggested that few cattle had been exposed to this serotype. The lower seroprevalence in younger animals may be due to lower opportunities for exposure to FMDV. The presence of this serotype in Cambodia may suggest that FMDV type A is circulating in cattle and bufaloes and may cause outbreaks.

The age-stratified seroprevalence and distribution of type Asia 1 in seropositive villages suggest that there has been less recent exposure to type Asia 1 virus, as none of the sampled animals were seropositive to only serotype Asia 1. If the type Asia 1 virus was not circulating widely—boosting animal and herd immunity—the antibody titres would be expected to be lower in younger animals. The small proportion of samples within villages that were positive and a low age-related seroprevalence can be explained by a combination of waning antibodies, and a decreasing number of older seropositive animals. It is possible that, similar to other countries in the region, serotype Asia 1 may become extinct locally, leaving a highly susceptible population. The low antibody titre detected to FMDV type Asia 1 may reflect the absence of this serotype in recent years, which was last reported in Cambodia in 1997 (Gleeson 2002). Moreover, since the viral properties of FMDV serotype O and Asia 1 have a relatively high homogeneity, the low level of antibody to serotype Asia 1 may have resulted from

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>β</th>
<th>Association with FMD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR</td>
<td>95% CI for OR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.9</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Shared grazing areas</td>
<td>0.9</td>
<td>2.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Intervened by village animal health worker</td>
<td>1.0</td>
<td>2.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Fed cattle cut grasses</td>
<td>-1.9</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Sold infected cattle</td>
<td>0.9</td>
<td>2.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Vaccination against FMD</td>
<td>1.4</td>
<td>3.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Kept free-range pigs</td>
<td>0.7</td>
<td>1.9</td>
<td>1.2</td>
</tr>
</tbody>
</table>

CI = confidence interval; FMD = foot-and-mouth disease; OR = odds ratio

Table 2. Results of a multivariable logistic regression analysis of risk factors for foot-and-mouth disease in southern Cambodia
cross reaction on the ELISA test (Lu et al. 2008). The outbreak of FMD in 2006 in Cambodia may confirm this assumption as only serotypes O (Pan-Asia strain) and A were involved.

The current study also revealed that movement of people and infected animals during the outbreaks, sharing of common grazing land, raising pigs under free-range conditions and vaccination against FMD posed a significant risk to the dissemination of FMDV in the southern provinces of Cambodia. However, movement of animals is probably the most important factor contributing to the spread of FMD (Rweyemamu 1984; Ferris et al. 1992; Perry et al. 2002). Numerous authors have highlighted that the outbreaks of FMD were associated with livestock and livestock products movement (Aidaros 2002; Leforban and Gerbier 2002; More 2002; Bouma et al. 2003; Gloster et al. 2003; Knowles and Samuel 2003). It is possible that lack of biosecurity, poor reinforcement of legislation on movement restrictions in infected areas by the government veterinary authority during outbreaks, and a lack of established on-road checkpoints may contribute to the dissemination of FMDV. Movement of infectious animals that are in a contagious state is known to increase the risk of introducing disease to healthy flocks by both direct or indirect contact arising from mixing (Christley et al. 2005). Since airborne spread has almost no influence on the transmission of FMD in the tropics, distance of possible spread and the size of the epidemic depend very much on direct contact between infected and susceptible animals (Thomson 1995; Sutmoller et al. 2000; Alexandersen and Mowat 2005). Movement of people also revealed a significant level of risk in the spread of disease (Laryea 1975; Rweyemamu 1984).

References


References
disease diagnosis. Transboundary and Emerging Diseases 55, 196–199.


