A comparison of type O foot and mouth disease virus field isolates from northern Thailand

S.D. BLACKSELL *, L.J. GLEESON *, CHANPEN CHAMNANPOOD **, NARONGCHAI NAKARUNGKUL ** and CHAOWANA MEGKAMOL **

Summary: A survey of type O foot and mouth disease (FMD) virus isolates from northern Thailand was undertaken to determine the relationship between field viruses and the vaccine in use, and to gauge the range of antigenic variation among field viruses. Isolates were collected from the two most recent epizootics, 1986-1987 and 1989-1990, and assessed using a two-dimensional neutralisation test to determine their relationship to FMD type O1 Bangkok 1960 (O BKK/60) reference (vaccine challenge) virus. The critical r value for the survey was 0.259 and all isolates tested were found to have an r value considerably greater than this (range 0.66 to 0.80). The results showed close antigenic relationships between the isolates and the reference virus, and indicated a relatively small range of antigenic variation between the isolates.

KEYWORDS: Antigenic variation  Aphthovirus  Foot and mouth disease Thailand - Two-dimensional microneutralisation test - Type O.

INTRODUCTION

Foot and mouth disease (FMD) virus is an important disease of livestock in Thailand. Though usually mild in nature, the disease is economically significant due to the inability of infected animals to work, the reduction of milk yields and restrictions on international trade (6, 15). The first confirmed diagnosis of FMD in Thailand was of type A in 1953 (5) and currently three of the seven serotypes of FMD virus (type O, type A and type Asia 1) are endemic in Thailand.

The control of animal movement and mass vaccination have assisted in eradication of the disease in southern Thailand, with no reported cases of FMD south of latitude 12° since August 1981 (1). At present, the disease is restricted to north, north-east and central Thailand. The most prevalent of the FMD viruses in South-East Asia is reported to be type O1 (2), and outbreaks of FMD caused by type O are periodically reported in northern Thailand. A recent report (10) suggested that type O field isolates were beginning to display a degree of antigenic variation from the vaccine reference strain, sufficient to cause concern about the efficacy of the vaccine in use. Samples from the outbreaks occurring in 1986-1987 and 1989-1990 were compared to the type
O Bangkok 1960 (O BKK/60) vaccine challenge strain by the microneutralisation test. Information obtained would lead to some appreciation of the likely efficacy of the current type O vaccine in controlling FMD in northern Thailand, and indicate whether incursions of new field viruses had occurred in the region.

MATERIALS AND METHODS

Viruses

Field virus

All samples used in this study were submitted to the Northern Veterinary Research and Diagnostic Centre in Hang Chat, Lampang, as tongue epithelium samples. Routine samples were tested in an indirect sandwich enzyme-linked immunosorbent assay (ELISA) (11, 16), and aliquots of processed samples were stored at −80°C in the period prior to tissue culture propagation. Samples containing type O virus were selected from five provinces from each of the two regions served by the Northern Veterinary Research and Diagnostic Centre. Where possible, a sample from both the 1986-1987 and 1989-1990 epizootics for each province was tested. One pig, three buffalo and sixteen cattle isolates were selected from specimens submitted between October 1986 and October 1989. The isolates chosen for evaluation are detailed in Tables I and II. Isolates were removed from 80°C storage and passaged no more than seven times in primary bovine thyroid (BTY) cells, second passage bovine kidney (BK) cells or baby hamster kidney (BHK) cells until they reached a satisfactory infectivity titre.

TABLE I

<table>
<thead>
<tr>
<th>Field virus</th>
<th>Passage</th>
<th>Province</th>
<th>r value</th>
<th>r value range</th>
</tr>
</thead>
<tbody>
<tr>
<td>471/87</td>
<td>BTY1/BK1/BHK2</td>
<td>Phayao</td>
<td>0.75</td>
<td>0.74 0.76</td>
</tr>
<tr>
<td>796/89</td>
<td>BTY1/BK3</td>
<td>Phayao</td>
<td>0.72</td>
<td>0.70 0.74</td>
</tr>
<tr>
<td>478/87</td>
<td>BK2/BHK2</td>
<td>Phrae</td>
<td>0.73</td>
<td>0.71 0.75</td>
</tr>
<tr>
<td>789/89</td>
<td>BK6</td>
<td>Phrae</td>
<td>0.73</td>
<td>0.71 0.75</td>
</tr>
<tr>
<td>475/87</td>
<td>BK1/BHK2</td>
<td>Chiang Mai</td>
<td>0.74 0.75</td>
<td></td>
</tr>
<tr>
<td>770/89</td>
<td>BK5</td>
<td>Chiang Mai</td>
<td>0.80 0.81</td>
<td></td>
</tr>
<tr>
<td>335/86</td>
<td>BTY1/BK6</td>
<td>Lampang</td>
<td>0.67</td>
<td>0.66 0.68</td>
</tr>
<tr>
<td>803/89</td>
<td>BTY2/BHK2</td>
<td>Lampang</td>
<td>0.73</td>
<td>0.71 0.74</td>
</tr>
<tr>
<td>390/87</td>
<td>BTY1/BHK4</td>
<td>Chiang Rai</td>
<td>0.67 0.69</td>
<td></td>
</tr>
<tr>
<td>857/89</td>
<td>BTY1/BHK3</td>
<td>Chiang Rai</td>
<td>0.69</td>
<td>0.67 0.70</td>
</tr>
</tbody>
</table>

Note: Chiang Mai, Chiang Rai, Lampang, Lamphun, Mae Haeng, Nan, Phayao and Phrae province suffix denotes year of sample collection. Critical t value: 0.259.
TABLE II  
_Evaluation of virus isolation samples_ 
Region 6*

<table>
<thead>
<tr>
<th>Field virus **</th>
<th>Passage</th>
<th>Province</th>
<th>r value ***</th>
<th>r value range</th>
</tr>
</thead>
<tbody>
<tr>
<td>480/87</td>
<td>BTY1/BHK2</td>
<td>Tak</td>
<td>0.69</td>
<td>0.69 - 0.69</td>
</tr>
<tr>
<td>919/89</td>
<td>BK5</td>
<td>Tak</td>
<td>0.75</td>
<td>0.73 - 0.77</td>
</tr>
<tr>
<td>392/87</td>
<td>BK2/BHK2</td>
<td>Kamphaengphet</td>
<td>0.71</td>
<td>0.68 - 0.74</td>
</tr>
<tr>
<td>930/89</td>
<td>BTY1/BK2</td>
<td>Kamphaengphet</td>
<td>0.67</td>
<td>0.64 - 0.69</td>
</tr>
<tr>
<td>516/87</td>
<td>BK2/BTY1/BHK2</td>
<td>Phetchabun</td>
<td>0.74</td>
<td>0.72 - 0.76</td>
</tr>
<tr>
<td>913/89</td>
<td>BK6</td>
<td>Phetchabun</td>
<td>0.73</td>
<td>0.71 - 0.75</td>
</tr>
<tr>
<td>437/87</td>
<td>BTY1/BK1/BHK2</td>
<td>Uttaradit</td>
<td>0.71</td>
<td>0.70 - 0.71</td>
</tr>
<tr>
<td>872/89</td>
<td>BTY1/BK1/BHK2</td>
<td>Uttaradit</td>
<td>0.66</td>
<td>0.64 - 0.67</td>
</tr>
<tr>
<td>849/89</td>
<td>BK5</td>
<td>Uthai Thani</td>
<td>0.67</td>
<td>0.66 - 0.69</td>
</tr>
<tr>
<td>875/89</td>
<td>BK5</td>
<td>Nakhorn Sawan</td>
<td>0.71</td>
<td>0.68 - 0.73</td>
</tr>
</tbody>
</table>

Reference virus

O NPT/64 P13/BHK9  0.82  ND

* comprises Kamphaengphet, Nakhorn Sawan, Phetchabun, Phuchit, Phutsaluk, Sukotai, Tak, Uthai Thani and Uttaradit provinces
** suffix denotes year of sample collection
*** critical r value 0.25
ND no data

Reference viruses

The reference viruses used in this study were obtained from the Department of Livestock Development, Foot and Mouth Disease Centre in Pak Chong, Nakhon Ratchasima. Type O BKK/60 vaccine challenge virus had thirty four cattle intradermolingual passages followed by six BHK cell line (C34/BHK6) passages. Type O Nakhon Pathom 1964 (O NPT/64) had thirteen porcine intradermolingual passages followed by nine BHK cell line (P13/BHK9) passages.

Antiserum

Convalescent antiserum to type O BKK/60 collected at 21 days post infection was also obtained from the Foot and Mouth Disease Centre in Pak Chong.

Microneutralisation test

All isolates were assessed for their serological relationship to the reference virus (type O BKK/60) in a two-dimensional neutralisation test (13) using antisera raised against the reference virus. Duplicate tests were carried out on all isolates except reference virus O NPT/64. The O BKK/60 reference virus was tested on eighteen occasions, usually concurrent with field isolate evaluations.

Calculation of the r value

The relationships of the field virus isolates to O BKK/60 are expressed as an r value. The r value was calculated by employing the following formula:

$$ r = \frac{\text{titre of O BKK/60 antiserum against field (heterologous) virus}}{\text{titre of O BKK/60 antiserum against O BKK/60 reference (homologous) virus}} $$
Significance of the r value

An r value close to 1.0 indicates that the field virus isolate has a close antigenic relationship with the reference virus.

As the r value decreases, this indicates a corresponding decrease in the homology of the field virus isolate with the reference virus. The critical r value is the highest value of r which is distinguishable from 1.0 at a set probability for a given number of test replicates (14). Using the values tabulated by Rweyemamu and Hingley (14), for the parameters of this study (i.e. eighteen test replicates of homologous reference virus and duplicate testing of heterologous field viruses) at \( P = 0.01 \), the critical r value is 0.259.

RESULTS

For the 20 field isolates studied, the range of mean r values obtained was 0.66 to 0.80. The mean value for the 1986-1987 viruses was 0.71 (±0.03) and for the 1989-1990 viruses 0.70 (±0.03). In addition, the r value for the pig type O challenge strain (O NPT/64) was determined. The individual values are shown in Tables I and II.

Employing the critical r value of 0.259 as the lower limit of statistically significant relatedness, none of the virus isolates tested varied significantly from the reference virus. The mean titre of the reference serum against O BKK/60 over the eighteen test replicates was \( 10^{3.51} \) at virus input of 100 TCID\(_{50}\).

DISCUSSION

Antigenic variation of FMD virus has been widely investigated (3, 4, 12). It occurs both in the field and on laboratory passage of the virus, and is of great importance to vaccine efficacy. A number of studies have been performed to determine the relationships of FMD type O virus isolates to reference viruses in the South East Asia region (2, 7, 8, 9, 10). Some significant antigenic differences relative to reference viruses have been reported (8), while other investigations have reported antigenic changes of lesser significance (7, 9, 10). A survey of ten FMD type O isolates collected during 1980 and 1981 from a wide geographical area of Thailand indicated that all isolates except one were comparatively closely related to at least one of three type O vaccine strains (7). However, on examining the relationship between South-East Asia regional isolates and O\(_1\) BFS 1860, Ouldridge and colleagues (10) suggested that a major change had occurred in the type O\(_1\) virus in Thailand, and that this had contributed to an extensive outbreak of FMD in central Thailand between 1979 and 1981.

In the course of virus propagation in this study, the passage of virus isolates in the laboratory was minimised to restrict the possibility of antigenic change which could influence r values (4). Furthermore, isolates were selected from a number of geographically distinct outbreaks in each epizootic to achieve a broad sample of field viruses from the northern region.

The practical significance of the r value is to indicate the likelihood that \textit{in vitro} protection could be obtained when using a particular vaccine against a given field virus. The critical r value is a well defined value, but not directly related to \textit{in vivo}
protection. The formulation of the critical \( r \) value is based on the assumption that quantitative differences in antigenic composition of the compared virus strains gives rise to measurable serological differences. These measurable differences, and the potential to detect them, are dependent on the inherent errors of the serological tests employed. These errors, in turn, are influenced by the number of replicate tests performed for each of the homologous and heterologous viruses assessed, and by inter-laboratory variability (12). The results presented show that there is a close antigenic relationship between all the field viruses and the reference virus, because for all viruses tested the \( r \) value was considerably greater than the critical \( r \) value. This data also indicated that no major change in the antigenic relationship had occurred in the interval between epizootics.

The variance of \( \log_{10} (\text{titre}) \) for the eighteen estimates of the reference bovine serum was 0.04. This accords with the criteria for use of the table of critical \( r \) values for varying numbers of replicates used (14).

It was of particular interest that type O field viruses circulating in the late 1980s bore a close and uniform antigenic relationship to O BKK/60, a virus isolated almost 30 years previously and used in vaccine production. While no direct comparison was made, the results also pointed to a relative antigenic homogeneity amongst field viruses, both within and between epizootics, and suggested that such antigenically stable field viruses were probably from a common enzootic source. These observations also suggested that the antigenic relationship between field viruses and vaccine virus was not an important epidemiological variable contributing to type O FMD outbreaks in northern Thailand in recent years. The limited extent of variation between \( r \) values indicated that a single vaccine strain can be used to control FMD in northern Thailand.

**CONCLUSION**

Based on the \( r \) values determined, the field virus isolates were not significantly different from the O BKK/60 reference virus. The small amount of variation in \( r \) values between epizootics suggested that little antigenic variation occurred in type O virus and, therefore, that field strains from both epizootics and the vaccine challenge strain arose originally from a common source.

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Résumé : Les auteurs ont étudié des souches du virus aphteux de type O isolées dans le nord de la Thaïlande pour les comparer au vaccin actuellement utilisé et pour estimer l’importance de la variation antigénique parmi les souches trouvées sur le terrain. Les virus provenaient des deux épizooties les plus récentes (1986 1987 et 1989-1990). Un test de neutralisation bidimensionnel a été utilisé pour les comparer au type O1 Bangkok 1960 (O BKK/60) qui sert de référence (essais d’efficacité des vaccins). La valeur critique de r déterminée au cours de cette étude était de 0,259. Toutes les souches examinées ont présenté une valeur de r très supérieure (de 0,66 à 0,80). Les résultats ont révélé des relations antigéniques étroites entre les souches trouvées et le virus de référence et une variation antigénique relativement limitée entre les souches.

MOTS CLÉS : Aphthovirus - Fièvre aphteuse - Test de microneutralisation bidimensionnel Thaïlande - Type O Variation antigénique.

COMPARACIÓN DE VIRUS AFTOSOS DEL TIPO O AISLADOS EN EL CAMPO EN TAILANDIA DEL NORTE. S.D. Blacksell, L.J. Gleeson, Chanpen Chamnanpood, Narongchai Nakarungkul y Chaowana Megkamol.

Resumen: Se llevó a cabo un estudio de virus aftosos del tipo O aislados en el campo en Tailandia del norte para determinar la relación entre esos virus y la vacuna utilizada y para evaluar la amplitud de variación antigénica entre los virus de campo. Los virus aislados procedían de las dos epizootias más recientes (1986 1987 y 1989 1990) y se evaluaron utilizando una prueba de neutralización bidimensional para determinar su relación con los virus aftosos del tipo O1 Bangkok 1960 (O BKK/60) de referencia (prueba de la vacuna). El valor r crítico utilizado para el estudio fue de 0,259 y la prueba demostró que todos los virus aislados presentaban un valor r mucho más elevado (entre 0,66 v 0,80). Los resultados indicaron una estrecha relación antigénea entre los virus aislados y los de referencia, así como una amplitud de variación antigénica relativamente pequeña entre los aislados.

PALABRAS CLAVE: Aftovirus - Fiebre aftosa - Prueba de microneutralización bidimensional - Tailandia - Tipo O Variación antigénica.

REFERENCES


