

Serological comparison of type A foot and mouth disease virus isolates from Thailand

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Summary: Antigenic variation of type A foot and mouth disease (FMD) virus in Thailand was examined using a total of 82 field viruses isolated between 1986 and 1989. A two-dimensional serum microneutralisation test was used to compare these isolates to a reference strain, A_{15} Bangkok 1960 (A BKK/60). Viruses regarded as unrelated to A BKK/60 were compared to another reference strain, A_{22} Nakhon Pathom 1986 (A NPT/86). This approach divided the viruses into two groups. Most of the viruses shared a close antigenic relationship with A BKK/60. Only twelve viruses were regarded as unrelated to A BKK/60, and these were related to A NPT/86. All but one of these twelve isolates were from two provinces in one administrative region of the country. Future type A vaccines in Thailand will need to confer protection against both groups of viruses.

KEYWORDS: Aphthovirus – Foot and mouth disease virus type A – Serological comparison – Thailand.

INTRODUCTION

Foot and mouth disease (FMD) was officially recognised in Thailand in 1953, when an outbreak due to serotype A occurred. This was followed by recognition of type Asia 1 in 1954 and type O in 1957 (1, 2). In 1956, the Royal Thai Government instituted an FMD control programme, which was complemented by appropriate statutes, and by the establishment of diagnostic, research and vaccine production facilities at the Foot and Mouth Disease Centre at Pak Chong, in 1958.

The control programme called for the establishment of a disease-free zone in the two southern regions (regions 8 and 9, comprising 14 provinces) through routine vaccination, movement controls and appropriate animal health measures. A further eight provinces in region 7, immediately north of the disease-free zone, were to be designated a buffer zone. FMD was eradicated from regions 8 and 9 and the disease-free zone declared in 1981. Vaccination in other areas of the country was principally performed in response to local outbreaks.

FMD vaccine production commenced at Pak Chong in 1960. Types A, O and Asia 1 viruses isolated in the Bangkok area were developed as vaccine strains for Frenkel

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vaccine production. The type A isolate was characterised by the World Reference Laboratory (WRL) in Pirbright (United Kingdom), and was assigned the subtype classification A₁₅ Thai/60 (3). This strain was referred to as A₁₅ Bangkok/1960 (A BKK/60) in Thailand.

In 1973, an outbreak of FMD occurred in the southern region of Thailand, subsequently spreading to Malaysia and Singapore. The locally-available A BKK/60 vaccine was unable to control this outbreak in southern Thailand. A virus isolated from this outbreak in Malaysia was classified by the WRL as subtype A₂₂ (5). A contemporary isolate from southern Thailand was shown to be serologically distinct from A BKK/60 and was locally designated A₂₂ Songkla 1973 (A SKL/73) (8). The outbreak was controlled and no subsequent isolations of A₂₂ were made from this region, which was declared an FMD-free zone in 1981.

Viruses isolated from outbreaks of FMD in central Thailand in the late 1970s and early 1980s were found to be more closely related to A SKL/73 than to A BKK/60 (6, 8). This led to the development of vaccines based on locally-isolated strains. The most recent vaccine strain was designated A₂₂ Nakhon Pathom 1986 (A NPT/86). The Department of Livestock Development in Thailand initiated a policy to increase resources and efforts in FMD control in the 1990s. The control strategy includes the development of a new trivalent vaccine based on endemic viruses. A number of recent studies investigating outbreaks of FMD in Thailand have reported that endemic type A virus strains in Thailand are more closely related to the A₂₂ reference vaccine strain than to the A₁₅ vaccine strain (7, 9). This study was undertaken to determine the relationships to the reference A₁₅ virus (A BKK/60) of a wide range of field viruses isolated between 1986 and 1989, and taken from all regions where FMD was considered endemic. It was also hoped that some insight might be gained into the inter-relationships between type A field viruses from Thailand. The comparison was undertaken using the two-dimensional serum microneutralisation test (SNT) (12).

MATERIALS AND METHODS

Viruses

The reference vaccine viruses A BKK/60 (A₁₅), A NPT/86 (A₂₂) and A SKL/73 (A₂₂) were vaccine potency test challenge viruses provided by the FMD Centre in Pak Chong, adapted to growth in BHK 21 cells.

A selection of 82 field virus isolates was made, to cover all seven administrative regions of Thailand where FMD was endemic. Viruses were obtained from the diagnosis section of the FMD Centre in Pak Chong, and from the Northern Veterinary Research and Diagnostic Centre in Hang Chat. Viruses were initially typed by the complement fixation test (6) and/or enzyme-linked immunosorbent assay (15). Primary isolation was made in tissue-culture monolayers of bovine thyroid, bovine kidney, goat kidney or BHK 21 cells. To conduct serum neutralisation tests, all viruses were adapted to growth in BHK 21 cells by four to six serial passages.

Antisera

Bovine antiserum to the A BKK/60 reference virus was provided by the assay section of the FMD Centre in Pak Chong. The serum was obtained after homologous challenge of cattle vaccinated with monovalent A BKK/60 vaccine during vaccine potency trials.

Antisera to the three reference viruses were produced in rabbits using the method of Have *et al.* (4), modified by higher antigen levels and the use of live virus preparations. Briefly, rabbits received 40 µg of 146s antigen in Freund's complete adjuvant and were re-inoculated after 28 days with 20 µg of the same purified virus in either 50% incomplete Freund's adjuvant or phosphate-buffered saline with 0.025 mg/ml of saponin.

Determination of serological relationships

Isolates were assessed for their serological relationship to one or more of the reference viruses by the calculation of *r* values (11), where:

$$r = \frac{\text{titre of reference antiserum against test virus}}{\text{titre of reference antiserum against homologous virus}}$$

The titre of an antiserum against a particular test virus was determined in a two-dimensional SNT as described by Rweyemamu *et al.* (12), with the modifications described below. Virus/serum mixtures were incubated for 1 h at 37°C. BHK 21 cells were then added at a seeding rate of 50,000 cells per microtitre tray well, and the plates were fixed and stained with 0.05% methylene blue in 10% formal saline after incubation for three days at 37°C. Duplicate tests were performed on separate days for all isolates, to determine the mean serum titre to the test virus. If the replicate titres varied by more than 0.25 log₁₀, the test was conducted a third time and the mean value used.

The *r* value was calculated from the titre of the serum at 100 TCID₅₀ (50% tissue culture infective dose) of virus input, as determined from the two-dimensional titration using a linear regression computer program. Criteria for the interpretation of *r* values have been proposed by Samuel *et al.* (14), as follows:

– *r* = 0-0.19: a highly significant serological variation from the reference vaccine strain, best countered by use of a vaccine strain with a closer relationship to the field virus.

– *r* = 0.20-0.39: a significant difference from the reference strain, but protection may be satisfactory if a sufficiently potent vaccine is used.

– *r* = 0.40-1.0: not significantly different from the reference vaccine strain as measured by the test system used.

All reference viruses and field viruses were tested against the reference A BKK/60 bovine antiserum and *r* values were calculated. Viruses deemed to be unrelated to A BKK/60 were tested against one or both of the A₂₂ reference rabbit antisera.

RESULTS AND DISCUSSION

There are 60 provinces in the 7 administrative regions outside the disease-free zone (Fig. 1) and, during the study period, type A virus specimens were submitted to the two source laboratories from 39 provinces. A total of 82 field virus isolates from 32 (82%) of these provinces were included in the study. The isolates were from cattle (52 isolates), buffalo (12), pigs (16) and sheep (1). The species of origin of one isolate was unknown. The sample of virus isolates across the seven regions and over the three-year period was regarded as sufficiently representative to provide an accurate picture of the distribution of the relationships of endemic viruses to the A BKK/60 vaccine strain.

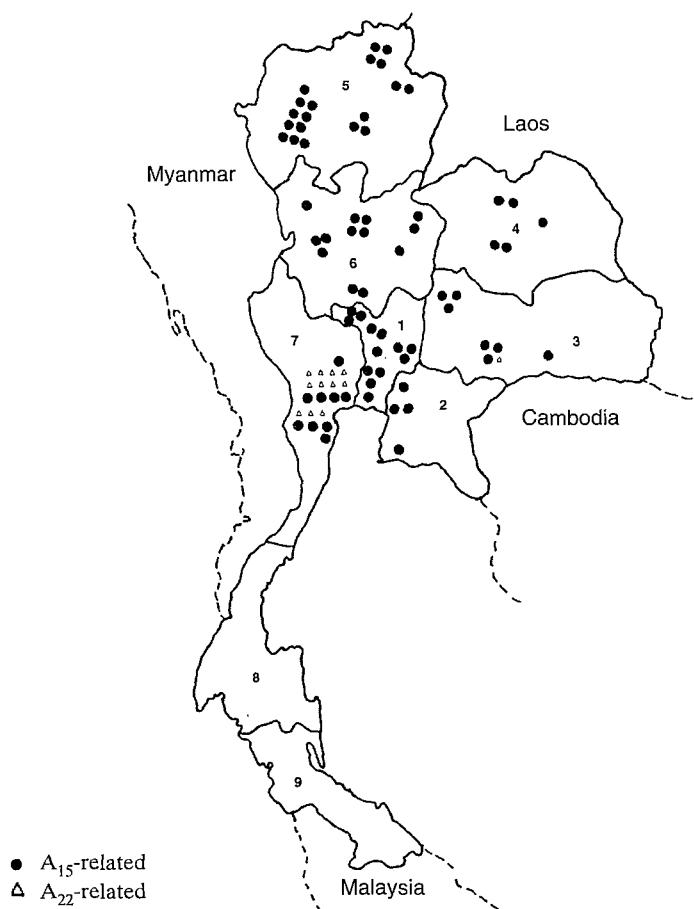


FIG. 1

Geographical distribution of selected foot and mouth disease virus type A isolates in Thailand between May 1986 and January 1989

The origins of the field isolates are shown in Figure 1. The distribution of the r values obtained with the A BKK/60 bovine antiserum is shown in Figure 2.

The r values indicated that 66 (80%) of the type A field viruses were not significantly different from A BKK/60 ($r > 0.39$) and could therefore be regarded as A BKK/60-related. The mean r value of this group of viruses was 0.70, with a standard deviation of 0.22.

Four field viruses (4.8%) had r values to A BKK/60 of 0.2-0.39 with a mean value of 0.32, indicating a significant difference from A BKK/60.

The remaining twelve viruses (15%) had r values to A BKK/60 of 0.113-0.19, indicating a highly significant serological variation from A BKK/60. The mean r value for this group was 0.16, with a standard deviation of 0.03.

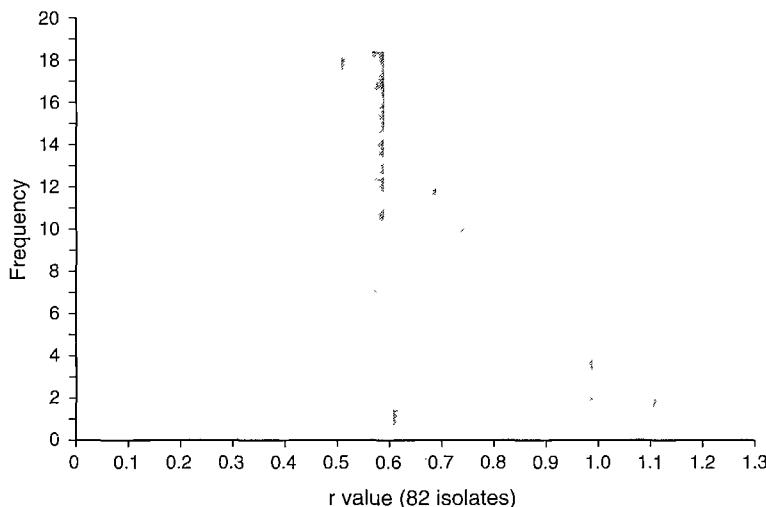


FIG. 2

Distribution of r values to A BKK/60 among foot and mouth disease virus type A field isolates in Thailand between May 1986 and January 1989

The twelve field viruses with r values < 0.2 to A BKK/60 cattle antiserum were tested against rabbit antisera to the A₁₅ and A₂₂ reference viruses. The results are shown in Table I. All of these viruses gave r values ≥ 0.40 to the A₂₂ antisera, and were regarded as indistinguishable from the A₂₂ NPT/86 reference vaccine virus. Eleven of these isolates were collected from cattle (six isolates) and pigs (five) in region 7 between 1986 and 1989, and one was taken from a buffalo in region 3 in 1987. It was noticeable that this group of viruses gave even lower r values (0.01-0.02) with the A BKK/60 rabbit antiserum than with the A BKK/60 bovine antiserum (0.11-0.19). In contrast, 33 isolates with r values > 0.4 and a mean of 0.61 (range 0.41-0.78) to A BKK/60 bovine antiserum gave a mean r value of 0.62 (range 0.29-1.09) when tested against A BKK/60 rabbit antiserum. It seems likely that the rabbit antiserum gave more specific reactions than the convalescent bovine antiserum, and more clearly distinguished those viruses which were significantly different from A₁₅ BKK/60.

Other isolates tested against the reference rabbit antisera showed strong reactions ($r > 0.4$) to both A₁₅ and A₂₂ reference antisera (see Table II). Reactions such as these were discussed by Pereira (10), who proposed designations relating a virus to more than one strain, i.e. A₁₅₋₂₂. Whether these viruses represent a distinct group, an antigenic drift of the established A₁₅ and A₂₂ field viruses, or a mixing of the two groups, will require investigation by other techniques (e.g. nucleotide sequence determination). It was noticeable that three of these isolates were from region 7 (where eleven of the twelve A₂₂ viruses were isolated), two from region 1 and one from region 6: significantly, regions 1 and 6 border on region 7.

The principal purposes of this study were to evaluate the suitability of the existing type A₁₅ vaccine strain (A BKK/60) for continued use in Thailand, and to assess the degree of strain variation among the type A viruses causing outbreaks. The criteria used for this evaluation were already established and accepted (13).

TABLE I

r values of Thai field isolates of type A foot and mouth disease virus significantly different from A₁₅ BKK/60 when compared to various other reference antisera

| Virus | Reference antisera | | | |
|--------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| | A ₁₅ BKK/60 (bovine) | A ₁₅ BKK/60 (rabbit) | A ₂₂ NPT/86 (rabbit) | A ₂₂ SKL/73 (rabbit) |
| 179/86 | 0.16 | 0.02 | 1.10 | 0.93 |
| 9-2/87 | 0.19 | 0.02 | 1.45 | 0.92 |
| 32/87 | 0.18 | 0.02 | 0.64 | 0.66 |
| 44/87 | 0.17 | 0.02 | 0.95 | 0.72 |
| 121/87 | 0.16 | NT | 0.94 | 0.78 |
| S54/88 | 0.13 | 0.02 | 0.63 | NT |
| S55/88 | 0.12 | 0.02 | 0.44 | NT |
| S60/88 | 0.13 | 0.01 | 0.52 | NT |
| S64/88 | 0.19 | 0.03 | 0.90 | NT |
| S65/88 | 0.14 | NT | 0.43 | NT |
| S5/89 | 0.18 | 0.02 | 0.41 | NT |
| S7/89 | 0.11 | 0.02 | 0.61 | NT |

NT: not tested

The frequency histogram (Fig. 2) showed a bimodal distribution, suggesting the presence of two distinct serological groupings of viruses, on the basis of the two-dimensional SNT using bovine antiserum to A BKK/60. Sixty-six (80%) of the viruses examined were not distinguished from the A₁₅ reference virus A BKK/60. Twelve (15%) viruses were significantly different from this A₁₅ reference virus, but were related to the Thai A₂₂ reference virus A NPT/86. Four viruses were different from A₁₅ BKK/60, but not to a highly significant degree.

The data confirmed that the A₁₅ (A BKK/60) and A₂₂ (A NPT/86 and A SKL/73) reference viruses formed two serologically-distinct groups.

There was a distinct geographical localisation of viruses not related to A₁₅ BKK/60 but related to A₂₂ NPT/86, with eleven of these twelve viruses being from outbreaks in region 7. Viruses related to A₂₂ SKL/73 had caused outbreaks in this area of Thailand in 1979, but the continued localisation of this type A strain was unexpected, given the level of livestock movement in Thailand. In contrast, only one of the 62 viruses isolated from regions 1 to 6 was not related to A₁₅ BKK/60. Nine viruses from region 7 were also related to A₁₅ BKK/60.

It can be concluded that the current A₁₅ BKK/60 vaccine strain used in Thailand is capable of providing protection against the type A field strains prevalent in six of the seven regions in which outbreaks occur. In the remaining region this vaccine would be appropriate in less than 50% of outbreaks (9/20), and an A₂₂ vaccine would more readily confer protection against the majority of field viruses. The existing A₂₂ NPT/86 vaccine would be appropriate for this purpose.

The formulation of a vaccine containing both A₁₅ BKK/60 and A₂₂ NPT/86 vaccine strains might facilitate the overall control of FMD outbreaks due to type A virus in Thailand.

TABLE II

r values of selected Thai field isolates of type A foot and mouth disease virus against A₁₅ and A₂₂ reference antisera

| Virus | Reference antisera | | | |
|--------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| | A ₁₅ BKK/60 (bovine) | A ₁₅ BKK/60 (rabbit) | A ₂₂ NPT/86 (rabbit) | A ₂₂ SKL/73 (rabbit) |
| 183/86 | 0.60 | 0.27 | 0.43 | 0.86 |
| 186/86 | 0.55 | 0.32 | 0.57 | NT |
| 187/86 | 0.49 | 0.39 | 0.20 | 0.42 |
| 19/87 | 0.49 | 0.24 | 0.27 | 0.53 |
| 118/87 | 0.69 | 0.79 | 0.19 | 0.57 |
| 32/88 | 0.29 | 0.57 | 0.30 | 0.67 |

NT: not tested

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COMPARAISON SÉROLOGIQUE DE SOUCHES DU VIRUS DE LA FIÈVRE APHTÉUSE (TYPE A) EN THAÏLANDE. - W.J. Doughty, R.A. Lunt, W. Lingchongsubonkoch, L.J. Gleeson et A. Kongthon.

Résumé : Les auteurs étudient la variation antigénique du virus de la fièvre aphtéuse (type A) en Thaïlande à partir de 82 souches sauvages isolées entre 1986 et 1989. Une épreuve de microséroneutralisation à deux dimensions a permis de comparer ces isolats avec une souche de référence, A₁₅ Bangkok 1960 (A BKK/60). Les virus considérés comme non apparentés à A BKK/60 ont été comparés à une autre souche de référence, A₂₂ Nakhon Pathom 1986 (A NPT/86). Les virus ont été divisés en deux groupes. La plupart d'entre eux étaient étroitement apparentés du point de vue antigénique avec A BKK/60. Les autres, au nombre de 12, étaient apparentés à A NPT/86. Onze d'entre eux provenaient de deux provinces situées dans une même région administrative du pays. Les futurs vaccins de type A en Thaïlande devront conférer une protection contre ces deux groupes de virus.

MOTS-CLÉS : Comparaison sérologique – Thaïlande – Virus de la fièvre aphtéuse – Virus de la fièvre aphtéuse de type A.

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COMPARACIÓN SEROLÓGICA DE LOS VIRUS DE LA FIEBRE AFTOSA DE TIPO A AISLADOS EN TAILANDIA. - W.J. Doughty, R.A. Lunt, W. Lingchongsubonkoch, L.J. Gleeson y A. Kongthon.

Resumen: Entre 1986 y 1989 se estudió la variación antigenica que exhibe en Tailandia el tipo A del virus de la fiebre aftosa. Para ello se utilizaron un total de 82 virus salvajes aislados durante dicho período. Una prueba de microneutralización bidimensional en suero fue la técnica empleada para comparar los virus en cuestión con una cepa de referencia, A₁₅ Bangkok 1960 (A BKK/60). Los virus aparentemente no relacionados con A BKK/60 fueron comparados con otra cepa de referencia, A₂₂ Nakhon Pathom 1986 (A NPT/86). Fruto de este enfoque fue su clasificación en dos grupos. La mayor parte de los virus guardaba una estrecha relación antigenica con A BKK/60. Sólo doce de los virus carecían de afinidad con A BKK/60, y estaban emparentados en cambio con A NPT/86. De estas doce cepas, todas salvo una procedían de dos provincias situadas en una sola región administrativa del país. En el futuro será necesario que las vacunas que se apliquen en Tailandia contra la fiebre aftosa de tipo A confieran protección contra ambos grupos de virus.

PALABRAS CLAVE: Aphthovirus – Comparación serológica – Tailandia – Tipo A del virus de la fiebre aftosa.

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