Serological comparison of type Asia 1 foot and mouth disease virus isolates from Thailand

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Summary: Antigenic variation of type Asia 1 foot and mouth disease (FMD) virus in Thailand was examined using a total of 50 field viruses isolated between 1986 and 1992. A two-dimensional serum neutralisation test was used to calculate r values for comparison of these isolates with a reference vaccine strain, Asia 1 Bangkok 1960 (BKK/60). Viruses were also compared to two field isolates, Asia 1 36-2/88 and Asia 1 45/88, and some were compared to another vaccine strain, Asia 1 Nakhon Pathom 1984 (NPT/84). In 80% of cases, field isolates were deemed to be significantly different from the vaccine virus Asia 1 BKK/60, and the distribution of r values indicated that there would be some advantage in selecting a new vaccine strain more closely related to contemporary field strains.

KEYWORDS: Aphthovirus – Foot and mouth disease virus type Asia 1 – Serological comparison – Thailand.

INTRODUCTION

Foot and mouth disease (FMD) is endemic throughout Thailand, with the exception of the southern peninsula (administrative regions 8 and 9), which is a disease control area and experiences only sporadic outbreaks. The endemic serotypes are types O, A and Asia 1. Disease control strategies include animal movement control, animal health measures and vaccination. At the time of this study, three monovalent vaccines formed the basis of the vaccination programme. The Asia 1 vaccine strain was an isolate characterised by the World Reference Laboratory for Foot and Mouth Disease in Pirbright (United Kingdom) in 1960 and locally designated Asia 1 Bangkok/1960 (BKK/60). Outbreaks caused by serotype Asia 1 viruses have generally been less common in Thailand than those caused by the other two serotypes. In 1991, however, Asia 1 was the most prevalent serotype causing outbreaks of FMD in the northern part of the country (unpublished findings).

A study was undertaken to assess the degree of antigenic variation among field strains of FMD virus type Asia 1 which caused outbreaks of FMD in Thailand over a five-year period, and to investigate the suitability of the current vaccine strain for

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continued use. All viruses were compared to the Asia 1 BKK/60 vaccine strain, and most to two recent field isolates, Asia 1 36-2/88 and Asia 1 45/88, using the two-dimensional serum microneutralisation test (SNT). The viruses Asia 1 36-2/88 and Asia 1 45/88 were selected from widely-separated provinces, as examples of the field strains prevalent at the time, for the production of antisera for the FMD-typing enzyme-linked immunosorbent assay (ELISA). Some of the viruses were also compared to another strain, Asia 1 Nakhon Pathom 1984 (NPT/84), which was developed for vaccine production to control outbreaks of FMD in pigs in central Thailand.

MATERIALS AND METHODS

Viruses

The reference vaccine viruses Asia 1 BKK/60 and Asia 1 NPT/84 were vaccine potency test challenge viruses, provided by the Foot and Mouth Disease Centre in Pak Chong (Thailand) and adapted to growth in baby hamster kidney (BHK) cells.

The field strains Asia 1 36-2/88 and Asia 1 45/88 were isolated in BHK 21 cells and passaged a total of six and five times, respectively, prior to use in the two-dimensional SNT.

Field viruses were obtained from the Northern Veterinary Research and Diagnostic Centre in Hang Chat, Lampang, or the FMD Centre in Pak Chong. Samples were regarded as being from distinct outbreaks if they originated from separate provinces or separate districts within a province. Samples were submitted as tongue epithelium and typed using the FMD-typing ELISA (7) or the complement fixation test (4). The 40 virus samples obtained between 1986 and 1988 were isolated in primary bovine thyroid cells (19 samples), secondary bovine kidney (1), goat kidney (2) or BHK cells (18). The majority (35 samples) were passaged a further three to six times in BHK cells prior to use in the SNT. The ten viruses from 1991 were all isolated in Instituto Biológico-rim suino-2 (IB-RS-2) cells and used after two or three passages in these cells.

Antisera

Bovine antiserum to Asia 1 BKK/60 and pig antiserum to Asia 1 NPT/84 were provided by the assay section of the FMD Centre in Pak Chong. The sera were obtained after homologous challenge of animals vaccinated with monovalent Asia 1 vaccines during vaccine potency testing.

Rabbit antisera were produced to viruses Asia 1 36-2/88 and Asia 1 45/88 as described by Have et al. (2), but using higher antigen levels and live virus preparations. Briefly, rabbits received 40 μg of 146s antigen in Freund's complete adjuvant, and were re-inoculated 28 days later with 20 μg of the same purified virus in either 50% incomplete Freund's adjuvant or phosphate-buffered saline with 0.025 mg/ml saponin.

Determination of serological relationships

All isolates were assessed for their serological relationships to the reference viruses, in a two-dimensional SNT (5) with the modifications described below. Virus/serum mixtures were incubated for 1 h at 37°C. BHK 21 or IB-RS-2 cells were added at a seeding rate of 50,000 cells per microtitre 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. The titre of the serum was determined at 100 TCID$_{50}$ (50% tissue culture infective dose) of virus, using a linear regression computer program. Duplicate tests were performed on separate days for all isolates, to determine the mean serum titre to the virus. If the replicate titres varied by more than 0.25 log$_{10}$, the test was conducted a third time and the mean used.

The relationship of any virus to a reference virus (V) was expressed as an r value calculated using the following formula:

$$ r(V) = \frac{\text{titre of reference antiserum against heterologous virus}}{\text{titre of reference antiserum against homologous virus}} $$

Criteria for the interpretation of r values were proposed by Samuel et al. (6), as follows:

- $r = 0-0.19$: a highly significant serological variation indicating a requirement for a vaccine strain with a closer relationship to the field virus.
- $r = 0.2-0.39$: a significant difference from the reference strain, but protection may be satisfactory if a sufficiently potent vaccine is used.
- $r = 0.4-1.0$: not significantly different from the reference strain as measured by the test system used.

**RESULTS AND DISCUSSION**

The study was conducted in two phases. In the initial phase, tests were performed on forty field viruses isolated from separate outbreaks in regions 1, 3, 4, 5, 6 and 7 between June 1986 and November 1988. In the second phase, ten field viruses isolated from outbreaks in regions 5 and 6 between January 1991 and January 1992 were tested.

The origins of the field isolates are shown in Figure 1. No isolates were available from region 2. Forty-four of the isolates were from cattle, three from buffalo and two from pigs. The species of origin of one isolate was not known.

The fifty viruses were tested for their serological relationship to the vaccine virus Asia 1 BKK/60. Many isolates gave low r values to Asia 1 BKK/60, and these were then tested against antisera to two recent field isolates, Asia 1 36-2/88 and Asia 1 45/88.

The frequency distribution of r values to Asia 1 BKK/60 antiserum is shown in Figure 2, and to Asia 1 36-2/88 and Asia 1 45/88 antisera in Figure 3. One virus isolated from cattle in region 7 in 1986 (Asia 1 163/86) was clearly distinguishable from all three reference viruses (all r values < 0.06). This virus had an r value to the Asia 1 NPT/84 pig vaccine virus of 0.22. The virus had been re-isolated from original bovine tongue epithelium, purified by two limiting dilution passages and typed as Asia 1 by ELISA, after initial testing had produced very low r values. Figures 2 and 3 show the majority of r values at the lower end of the range to Asia 1 BKK/60, at a slightly higher range to Asia 1 45/88 and distinctly higher for Asia 1 36-2/88. The mean r value to Asia 1 BKK/60 was 0.29, with 32% of values $< 0.2$ and 80% $< 0.4$. The mean r value to Asia 1 45/88 was 0.33, with 14% $< 0.2$ and 65% $< 0.4$. The mean r value to Asia 1 36-2/88 was 0.83, with only one value $< 0.2$ (2.3%) and none between 0.2 and 0.4.
The r value distribution showed a significant antigenic drift away from the vaccine strain Asia 1 BKK/60, which was originally isolated in 1960. But the study also demonstrated that, while 80% of field isolates examined were significantly different from Asia 1 BKK/60, the pattern of r values to the three reference viruses showed little antigenic variation across the Asia 1 field viruses causing FMD outbreaks. This pattern was essentially similar for the viruses isolated between 1986 and 1988 and those isolated in 1991, suggesting that no new strains had caused outbreaks in that time. No subsequent isolates were detected with r value patterns similar to the very low values observed for the 1986 isolate Asia 1 163/86. A similar observation was made with Asia 1 isolates from India, where antigenic drift from the historical vaccine strain India 8/79 was demonstrated by two-dimensional SNT, but a close relationship was found between field strains and one contemporary vaccine candidate strain (1).

The bovine antiserum to Asia 1 BKK/60 was the most appropriate reagent for assessing the ability of the vaccine strain to control disease outbreaks (3). The rabbit antisera to the two field isolates were used to study further the serological relationships of the outbreak viruses. Although it might have been preferable also to use bovine antiserum in these neutralisation tests, the rabbit antisera were useful for showing relationships between the field isolates. A small study using ten of the Asia 1 viruses,
Frequency distribution of r values to the Asia 1 vaccine strain BKK/60 of fifty Asia 1 viruses isolated from foot and mouth disease virus outbreaks in six regions of Thailand (1, 3, 4, 5, 6 and 7) between January 1986 and January 1992

and comparing r values obtained with bovine and rabbit antiserum to Asia 1 BKK/60, gave mean r values of 0.3 (range 0.113-0.99) and 0.36 (range 0.06-0.776), respectively, indicating similar results in both test systems.

This study demonstrated that, while there was little antigenic variation across the population of Asia 1 field viruses causing FMD outbreaks in Thailand, as assessed by serum neutralisation, the r values to the vaccine strain Asia 1 BKK/60 were generally low, with 80% of the viruses regarded as significantly different from Asia 1 BKK/60 (6). This vaccine strain could be expected, in an emergency, to give protection against the field strains if a vaccine of good potency was administered more than once. But it appears that the efficacy of any Asia 1 vaccine used in Thailand would be enhanced by incorporating a vaccine strain more closely related to the majority of current field strains. While it must be acknowledged that no conclusions should be drawn with regard to the likelihood of vaccine efficacy on the basis of data derived using a rabbit antiserum, the r values suggested that a strain such as Asia 1 36-2/88 could confer greater protection against the majority of field strains.

Vaccination programmes are a key element in FMD control strategies, and laboratory investigations of relationships between field viruses and vaccine strains make a critical contribution to the success of these programmes. This study demonstrates the need for continual monitoring of these relationships.
Frequency distribution of r values to two Asia 1 field viruses isolated in 1988, of Asia 1 viruses isolated from foot and mouth disease virus outbreaks in six regions of Thailand (1, 3, 4, 5, 6 and 7) between January 1986 and January 1992

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Résumé : Les auteurs étudient la variation antigénique du virus de la fièvre aphteuse (type Asia 1) en Thaïlande à partir de 50 souches sauvages isolées entre 1986 et 1992. Une épreuve de séroneutralisation à deux dimensions a permis de calculer les valeurs r et de comparer ces isolats avec une souche vaccinale de référence, Asia 1 Bangkok 1960 (BKK/60). On a également comparé les virus aux isolats de terrain, Asia 1 36-2/88 et Asia 1 45/88, et certains d’entre eux à une autre souche vaccinale, Asia 1 Nakhon Pathom 1984 (NPT/84). Dans 80 % des cas, les isolats de terrain se sont révélés radicalement différents de la souche vaccinale Asia 1 BKK/60. Quant à la distribution des valeurs r, elle indiquait qu’il y aurait intérêt à sélectionner une nouvelle souche vaccinale plus étroitement apparentée aux souches de terrain actuelles.

MOTS-CLÉS : Comparaison sérologique – Thaïlande – Virus de la fièvre aphteuse – Virus de la fièvre aphteuse de type Asia 1.

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Resumen: Entre 1986 y 1992 se investigó la variación antigénica que exhibe en Tailandia el tipo Asia 1 del virus de la fiebre aftosa. Para la realización de dicho estudio se utilizó un total de 50 virus salvajes aislados durante aquel periodo. La aplicación de una prueba de neutralización bidimensional en suero permitió calcular los valores r de comparación entre los aislados y una cepa de referencia para vacunas conocida como Asia 1 Bangkok 1960 (BKK/60). Los virus fueron comparados además con otras dos cepas salvajes, Asia 1 36-2/88 y Asia 1 45/88, y algunos de ellos con otra cepa utilizada para la producción de vacunas, Asia 1 Nakhon Pathom 1984 (NPT/84). Los resultados indicaron que los aislados eran, en el 80% de los casos, significativamente distintos de la cepa de vacuna Asia 1 BKK/60. A tenor de la distribución de los valores de r, puede afirmarse que sería ventajoso seleccionar una nueva cepa para la producción de vacunas, más afin a las cepas salvajes contemporáneas.

REFERENCES


