# The effect of repeated vaccination in an enzootic foot-and-mouth disease area on the incidence of virus carrier cattle

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### SUMMARY

A comparison was made of the incidence of foot-and-mouth disease virus 'carrier' cattle in an unvaccinated enzootic area and an area where routine 6-monthly vaccination with an inactivated vaccine had been carried out for 3-4 years. The incidence of carriers in the vaccinated area was 0.49% as compared to 3.34% in the non-vaccinated area. The results indicate that, provided the immune status of the vaccinated herd is maintained at a level sufficient to prevent outbreaks of clinical disease and the re-introduction of virus is prevented through livestock movement controls, it should be possible to eradicate the disease from an enzootic area through vaccination.

### INTRODUCTION

Traditional methods used for the control of outbreaks of foot-and-mouth disease in enzootic areas have included either aphthization or vaccination of all in-contact susceptible cattle and vaccination of uninfected farms around the outbreak, together with quarantine procedures and control of livestock movement. These methods are successful in containing the disease but do not necessarily help towards eradicating it. Considering that prophylactic vaccination might give better results, in 1968 Kenya embarked on a 6-monthly vaccination programme of all cattle in a limited part of the country. The area was gradually expanded so that by the end of 1972 most of the large-scale farming areas of the highlands had been included. The object was to attempt to eradicate the two main endemic types of foot-andmouth disease, O and A, by combining routine 6-monthly vaccination, using a bivalent inactivated vaccine<sup>†</sup>, with the normal rigid livestock movement controls. That disease control was being achieved was apparent from the decrease in the number of clinical outbreaks in the area. During the period 1965-7 there was an average of 52 farms affected per year, while in the years 1968-73 the average had fallen to ten farms per year, with the number steadily decreasing each year. The occurrence of clinical outbreaks does not necessarily give a true assessment of the amount of virus in the environment as subclinical or inapparent infection could

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occur, particularly in partially immune cattle. Van Bekkum, Frenkel, Fredericks & Frenkel (1959) demonstrated the prolonged carriage of virus by animals recovered from the disease by collecting oesophageal and pharyngeal fluid and scrapings. The incidence of such virus carrier animals would give a better indication of whether the vaccination programme was eradicating the virus or merely controlling the incidence of clinical disease. It was for this reason that this survey was undertaken and the preliminary results are reported here. The incidence of 'carriers' in the compulsory vaccinated area is compared with that in non-vaccinated areas of the country.

### METHODS

It was shown by Burrows (1966) that the sites in the upper respiratory tract where foot-and-mouth disease virus persists after clinical infection were the pharynx and the dorsal surface of the soft palate. Accordingly, samples were collected from these tissues.

## Collection of oesophageal-pharyngeal scrapings

Pharyngeal scrapings from mature steers from the vaccinated areas were collected immediately after slaughter in the abattoir. The retro-pharyngeal wall and lymphoid tissue were scraped with a curette and the scrapings placed in 5 ml. phosphate buffered saline (PBS), pH 7.4, containing 0.5% (w/v) gelatin, 200 units penicillin, 200 units polymyxin B, 150 g. neomycin sulphate and 50 units mycostatin per ml. The curette was disinfected between samplings by washing in 0.002% (w/v) citric acid and rinsing in tap water. The samples were kept at 4° C. immediately after collection and transferred to a  $-20^{\circ}$  C. refrigerator within 3 hr.

Oesophageal-pharyngeal (O-P) scrapings from cattle in the non-vaccinated areas were collected by means of a probang, as described by Sutmöller & Gaggero (1965). After collection, the sample was placed in 5 ml. 0.08 M phosphate buffer, pH 7.2, containing the same concentrations of antibiotics as the PBS described above. The sample was kept in dry ice until returned to the laboratory, when it was again transferred to a  $-20^{\circ}$  C. refrigerator. The probang was again disinfected after each animal in 0.002 % (w/v) citric acid and then rinsed in tap water.

## Examination of the oesophageal-pharyngeal scrapings for the presence of foot-andmouth disease virus

All the samples were examined for the presence of virus as soon as possible after collection. A 0.2 ml. volume of each sample was inoculated onto each of five monolayers of primary bovine thyroid cells (Snowdon, 1966) in test tubes. These were examined over a period of 72 hr. and the supernatant from any monolayer showing signs of cytopathic effect (CPE) was collected and passaged on to a further five monolayers. If CPE again developed, the presence of foot-and-mouth disease virus in the culture was confirmed by typing in a micro complement-fixation test. The infectivity of a proportion of the positive O-P samples was determined by titration in primary bovine thyroid cells.

## Antibody assay

Sera were collected at the same time as the pharyngeal scrapings and their antibody concentrations to types O and A viruses were assayed in a micro metabolic inhibition test (P. Dawe, personal communication). For this test, modified Eagle's medium containing 4% normal bovine serum, 10% tryptose phosphate broth, 0.3% sodium bicarbonate, 0.2% glucose and 0.004% phenol red was used. This medium was used as the diluent for serum, virus and cells alike. The virus strains used were the same as those used in the vaccine and the cell substrate was baby hamster kidney monolayer cells (BHK 21 cl 13, Macpherson & Stoker, 1962).

A 1/4 dilution of each serum was prepared in the test medium and this was heated at 56° C. for 30 min. A twofold dilution series of this was then prepared in plastic micro-plates, using micro-diluter loops.\* To each well containing the serum dilution was added an equal volume  $(50 \ \mu$ l.) of the virus suspension containing 100 TCID 50 virus. This was then incubated at 37° C. for 30 min. and then  $25 \ \mu$ l. of cell suspension containing  $2 \cdot 85 \times 10^6$  cells per ml. were added. The plates were covered with Sellotape and kept at 37° C. for 48–72 hr., by which time the test cell control wells had turned yellow. At this stage the Sellotape was removed and the medium in the wells allowed to become alkaline. When all the medium had become alkaline, the plates were again covered with Sellotape and replaced in the incubator. This procedure has the effect of removing any partial colour change that develops before any unneutralized virus is able to kill all the cells in the well. The plates were then read 24 hr. later and the titre expressed as the final dilution of serum neutralizing 100 TCID 50 virus.

The between-test standard deviation in this test when the virus input is between 1.5 and 2.5 TCID 50 was 0.35 (log<sub>10</sub> reciprocal serum dilution). The regression coefficient for the correlation between virus input and serum antibody concentration was found to be 0.6.

## RESULTS

The places at which samples were collected or from which slaughter cattle came immediately before sampling at the abattoir are shown in Fig. 1. The details of samples collected from cattle from the vaccinated area are shown in Table 1. Samples were deliberately collected from farms with no recent history of disease, as it was the presence of subclinical infection or symptomless 'carriers' unassociated with clinical disease that this study was concerned with. Even though the number of samples examined formed only a small proportion of the total cattle population, the proportion of virus 'carriers' found was still very low. Only six animals (0.49%) were found to be 'carriers' and of these four came from one farm in the same group of slaughter animals.

Table 2 shows the results of the samples collected in the non-vaccinated areas of the country. Here again, samples were only collected from herds with no recent history of disease. A higher proportion of 'carrier' animals (3.34%) was found. The infectivity titres of the positive samples were all less than  $10^{1.5}$  TCID 50/ml. of sample.

\* Cooke Engineering Company, Virginia, U.S.A.



Fig. 1. Location of sampling points or sources of cattle sampled at the abattoir in carrier survey. The letters O, A and C indicate points at which carrier viruses of these types were isolated.  $\square$ , Vaccinated area;  $\bigcirc$ , sampling points.

The immune status of the animals sampled from the vaccinated areas was also determined and the antibody concentrations to types O and A virus are shown in Table 3. The mean antibody concentrations, expressed as the logarithm of the reciprocal serum end-point dilution, were found to be 1.7 to type O and 1.6 to type A. To assess the significance of these antibody concentrations, sera from

27	1231	46	0.017	1.0	0.49
sampled	samples	per farm	area	cattle	virus carriers
farms	no. of	Average no.	vaccinated	slaughter	found to be
No. of	$\mathbf{Total}$		population in	of mature	tested and
			% of cattle	% of the no.	% of animals
			samples as a	samples as a	
			Total no. of	Total no. of	

 Table 1. Incidence of foot-and-mouth disease virus 'carrier' cattle
 in the vaccinated area

 

 Table 2. Incidence of foot-and-mouth disease virus 'carrier' cattle in the non-vaccinated areas

	No. examined as % of	% of animals tested
No. of cattle	the cattle population	and found to be
examined	in the areas sampled	virus 'carriers'
2219	0.09	3.34

 Table 3. Immune status of the herds sampled in the vaccinated area

		Mean antibody concentrations (log <sub>10</sub> reciprocal titres)		% of cattle with antibody concentrations of 1.35 or greater	
No. of farms	No. of sera tested				
27	1231	19pe 0 1·7	1.6	19pe 0 82	туре н 77

vaccinated cattle that had been challenged with  $10^4$  bovine ID50 of virus were assayed and the serum antibody concentration corresponding to a 50 % protection level was found to be 1.3. This concentration would probably correspond to a 100 % protection level in the field, where the challenge is likely to be much lower. Therefore, if all animals having a titre of 1.35 or greater are considered to be immune, 82% of the sample would be immune to type O infection and 77% to type A.

The duration of the 'carrier' state in the indigenous Boran cattle was also investigated by determining the incidence of 'carrier' animals in a group of 100 Boran steers  $3\frac{1}{2}$ ,  $6\frac{1}{2}$  and 15 months after clinical type C infection. The incidence at these times was, respectively, 8.5%, 6.5% and nil. The number of 'carriers' following type C infection therefore decreased steadily over a period of about a year.

The antigenic relation between two type O 'carrier' viruses, one isolated in the north of the country and the other in the south, and field viruses isolated during clinical outbreaks in these areas was examined by means of a micro cross-complement fixation test (Darbyshire, Hedger & Arrowsmith, 1972). The 'carrier' virus isolated in the north was antigenically dissimilar (R value = 19) to the field virus and could be classed as being a different subtype. This 'carrier' virus had been isolated from an animal that had no history of being associated with a clinical outbreak. The 'carrier' virus from the southern part of the country originated in a herd where there was serological evidence of type O infection within the previous

12 months. This isolate was still antigenically related (R value = 45) to the field outbreak strain from the area.

A type C 'carrier' virus isolated  $6\frac{1}{2}$  months after the clinical outbreak was still antigenically similar to the field virus causing the outbreak (*R* value = 78).

A type A 'carrier' virus isolated in the south of the country two years after the last confirmed clinical outbreak was found to be antigenically related (R value = 53) to one field subtype strain found in that part of the country but dissimilar (R value = 34) to the other subtype strain.

#### DISCUSSION

The incidence of virus 'carrier' animals was found to fall steadily after a clinical outbreak of disease and few, if any, 'carriers' would be found 12 months later. This agrees with previous laboratory observations (van Bekkum *et al.* 1959; Burrows, 1966) and with field studies in Botswana (Hedger, 1970), where the incidence during an outbreak was 68 %. This had decreased to 38 % 6 months later and to 5.4 % 12 months later. The number of 'carriers' found in the enzootic non-vaccinated areas included in this study (3.34 %) was generally lower than that recorded in herds in Botswana (Hedger, 1968), where up to 20 % 'carriers' were found in three herds 7 months after a clinical outbreak and in one herd 12 months after an outbreak of the disease.

It has been demonstrated by Sutmöller, McVicar & Cottral (1968) and Hedger (1970) that vaccinated cattle may become 'carriers' when exposed to virus and that, if the challenge is low, this may occur without overt disease. It could be possible, in an enzootic area where the disease was controlled by vaccination, for subclinical infection to occur in partially immune animals and produce a proportion of 'carrier' animals. The results of this study show that, if total vaccination of the cattle population of the area is carried out and the spread of virus from clinical outbreaks is minimized by livestock movement control and quarantine procedures, then the establishment of the 'carrier' state through subclinical infection does not occur. As the number of clinical outbreaks decreases, so also does the number of virus 'carriers' until a situation is reached where the amount of virus in the environment is greatly reduced or the virus is even eliminated.

The results of the comparative studies on the antigenic relationship between 'carrier' viruses and field viruses indicate that there is an antigenic 'drift' away from the field viruses but these changes occur slowly. As yet there is no evidence that these new 'carrier' subtypes establish themselves in the area to cause clinical disease.

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### REFERENCES

- BURROWS, R. (1966). Studies on the carrier state of cattle exposed to foot-and-mouth disease virus. Journal of Hygiene 64, 81.
- DARBYSHIRE, J. H., HEDGER, R. S. & ARROWSMITH, ANN E. M. (1972). Comparative complement-fixation studies with subtype strains of foot-and-mouth disease virus. *Journal of Hygiene* 70, 171.
- HEDGER, R. S. (1968). The isolation and characterization of foot-and-mouth disease virus from clinically normal herds of cattle in Botswana. *Journal of Hygiene* 66, 27.
- HEDGER, R. S. (1970). Observations on the carrier state and related antibody titres during an outbreak of foot-and-mouth disease. *Journal of Hygiene* 68, 53.
- MACPHERSON, I. A. & STOKER, M. (1962). Polyoma transformation of hamster cell clones an investigation of genetic factors affecting cell competence. Virology 16, 147.
- SNOWDON, W. A. (1966). The growth of foot-and-mouth disease virus in monolayer cultures of calf thyroid cells. *Nature, London* 210, 1079.
- SUTMÖLLER, P. & GAGGERO, A. (1965). Foot-and-mouth disease virus carriers. Veterinary Record 77, 986.
- SUTMÖLLER, P., MCVICAR, J. W. & COTTRAL, G. E. (1968). The epizootiological importance of foot-and-mouth disease carriers. I. Experimentally produced foot-and-mouth disease carriers in susceptible and immune cattle. Archiv für die gesamte Virusforschung 23, 227.
- VAN BEKKUM, J. G., FRENKEL, H. S., FREDERIKS, H. H. J. & FRENKEL, S. (1959). Observations on the carrier state of cattle exposed to foot-and-mouth disease virus. *Tijdschrift* voor Diergeneeskunde 84, 1159.