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Observations on the carrier state and related antibody titres during an outbreak of foot-and-mouth disease

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SUMMARY

An outbreak of foot-and-mouth disease in a partially immune population of cattle in Botswana is described. The results show that when cattle immunized by vaccination were presented with natural field challenge of FMD, many animals with immunity sufficient to protect them against clinical disease were, however, susceptible to pharyngeal infection and subsequently became virus carriers. The proportion of animals becoming carriers appeared to vary with the degree of severity of the challenge.

Vaccination before exposure to virus appeared to have little effect on the duration of the carrier state. No evidence was obtained of the spread of carrier virus to immune herds following the outbreak.

Antibody titres during the outbreak were higher in the clinically infected animals than in the carrier animals and the uninfected animals. Evidence suggested that natural challenge boosted the titres of immune animals. After the outbreak, however, it was not possible to distinguish by their antibody titres between the carrier animal and the virus-negative animal.

Antigenic studies on the strains of virus isolated are described.

INTRODUCTION

The carrier state in foot-and-mouth disease (FMD) is now well established as a sequel to infection (van Bekkum *et al.* 1959; Sütmoller & Gaggero, 1965; Burrows, 1966; Hedger, 1968), but the infection may not necessarily involve the appearance of lesions at the predilection sites. Thus Sütmoller, McVicar & Cottral (1968) found that the exposure of susceptible cattle to minimal amounts of virus could establish the carrier state with only a subclinical infection with fever and viraemia. They had also shown that immune cattle exposed to contact infection became carriers without developing lesions or viraemia. Hedger (1968) suggested on the basis of his results in Botswana that transmission of virus from carriers in a herd may occur without the appearance of overt disease.

The observations presented here were made during a natural outbreak of FMD caused by a type SAT 1 virus. They show that when animals in a herd partially immune following vaccination are challenged by natural infection in the field, many with immunity sufficient to protect them from clinical disease do nevertheless become carriers of virus. The carrier state was also demonstrated in vaccinated

herds which, although contiguous to naturally infected herds, did not show any clinical evidence of FMD either during or after the outbreak. No evidence was obtained of the spread of carrier virus to immune herds after the outbreak.

Antigenic studies were made on the virus strains isolated.

Sera were assayed for FMD antibodies and the levels demonstrated are correlated with the state of the immunity of the various groups.

Previous history

The outbreak of FMD was confirmed on 1 January 1968 in cattle in the Satau area of the Chobe River district of Botswana close to its northern border with the Caprivi Strip. The virus was typed SAT 1. In this remote area, isolated from the remainder of Botswana by a game reserve to the east and south and by tsetse fly country to the west, FMD has occurred infrequently. The last recorded outbreak was in 1950 and the virus type was SAT 1. In recent years some movement of cattle into the area has taken place, but at the time of the outbreak there were no reports of FMD occurring or having recently occurred in other parts of Botswana or in neighbouring territories.

In September 1967, the Chobe River area was included in the annual prophylactic FMD vaccination campaign which covers the whole of northern and western Botswana. The herds in the area had received their first vaccination with a bivalent types SAT 1 and SAT 3 inactivated vaccine.

Contact of domestic stock with wild life, generally indirect, does occur in the area and there was a reported contact of some of the infected herds with buffalo in November 1967. A type SAT 1 virus strain was recovered from a clinically normal buffalo some months after the outbreak (Hedger, Condy & Falconer, 1969). Although this strain of virus was shown to be antigenically similar to the outbreak strain, it is not possible to state whether the infection passed from the cattle to the buffalo or vice versa.

MATERIALS AND METHODS

Virus

Samples of epithelium from animals with lesions were collected in 50 % glycerol phosphate buffer.

Oesophageal/pharyngeal (O/P) samples for the isolation of carrier virus were taken and transported on dry ice to the laboratory. The collection, transport and the isolation of virus from O/P samples on bovine thyroid cell tissue culture monolayers has already been described in detail (Hedger, 1968).

Whole blood with an anticoagulant (EDTA) was taken from the clinically normal animals to check for the presence of viraemia. These samples were also transported on dry ice and virus isolation attempted on bovine thyroid monolayers.

Virus specificity

The specificity of all the virus strains isolated was checked by complement-fixation (CF) tests using the microtitre technique described by Casey (1965). Subtype variations were checked by screening in CF test all virus strains isolated against a range of subtype-specific antisera.

A virus strain isolated from one of the epithelial samples was adapted to guinea-pigs for the production of a specific antiserum and, using cross-complement-fixation tests (Davie, 1964), antigenic differences between this strain, the vaccine strain, carrier virus strains and previously known subtype strains were studied.

Serum neutralization tests

Sera were assayed by the cell metabolic inhibition test or colour test (Martin & Chapman, 1961), using primary monolayers of pig kidney cells and a type SAT 1 virus strain (Rho 5/66) isolated from an outbreak of FMD in Rhodesia in 1966. Before its use in the test the virus was adapted to grow in pig kidney cell cultures by serial passage.

Neutralization titres are expressed as the reciprocal of the final dilution of serum present in the serum-virus mixture at the 50 % end point estimated according to the method of Kärber (1931).

Vaccine

The vaccine used during the prophylactic vaccination campaign and for control during the outbreak was a bivalent A.E.I. inactivated B.H.K. suspension culture vaccine incorporating a saponin adjuvant. The virus strains used were type SAT 1 (Rho 5/66) and type SAT 3 (Bec. 1/65).

Cattle and area

The cattle (*Bos indicus*) were native-owned indigenous Tswana stock ranched with a low standard of husbandry. Grazing is communal and herds mix freely. Herds were examined in three areas: (1) the infected area (Satau), (2) the contiguous area (Katchekau), (3) the clean area (Kazangula).

The village of Satau is about 16 miles from the village of Katchekau, but the grazing areas of the two villages overlap and some movement of stock takes place between the two areas. Kazangula, although ecologically similar and administratively part of the Chobe District, is approximately 70 miles from Satau and there was little likelihood of recent intermingling of stock between the two areas. There were no sheep and only a few goats in the areas selected for study.

The stocking rate in the district was low and in recent years had been reduced to well below the carrying capacity of the land, probably owing to the combined effects of streptothricosis, tick-borne disease and the tsetse fly.

The examination and sampling of herds in these three areas made it possible to study any evidence of previous undetected infection in the district as a whole and also to study the possible subsequent transmission of carrier virus into cattle in the contiguous area following the withdrawal of the quarantine restrictions after the outbreak.

RESULTS

General survey

Clinical examination of several hundred head of cattle in the clean and contiguous areas failed to show evidence of current or recent disease. O/P samples from 60 head of cattle at Kazangula (clean area) and 30 head at Katchekau (contiguous area) were taken at random from different herds. No foot-and-mouth disease virus (FMDV) was recovered from any of these animals. There was thus no evidence of previous undetected infection in the district and it may be assumed that antibody levels in the cattle in these two areas were due to vaccination and not infection.

The immune and partially immune herds in the infected area (Satau) comprised about 1200 head and fell roughly into three groups: (1) herds with an approximate 70 % morbidity, (2) herds with an approximate 25 % morbidity, (3) herds in contact with diseased herds but in which no clinical disease was recorded.

Carrier virus was recovered from O/P samples from clinically normal animals in all three groups. Table 1 shows the incidence of carrier virus recovery in each of the groups.

Table 1. *The recovery of carrier virus from clinically normal cattle which resisted natural challenge with foot-and-mouth disease virus*

Morbidity in groups	No. of animals sampled	No. virus-positive	% carriers in clinically normal animals
Group 1, 70 %	27	23	85
Group 2, 25 %	18	6	33
Group 3, 0 %	20	6	30

As it was possible that, in the presence of active infection, some of these apparently immune animals were in the prodromal phase of the disease (Burrows, 1968) or were undergoing subclinical infection, whole blood was examined in parallel with the O/P samples for the presence of viraemia. No viraemia was detected.

Available animals in the infected area and the contiguous area were resampled at periods of 6 and 12 months after the outbreak. Table 2 summarizes the results of the follow-up samplings.

All the virus strains isolated were typed SAT 1. Of the 13 virus-positive animals

Table 2. *Percentage of virus-positive animals during and after the outbreak*

	During outbreak			6 months after outbreak			12 months after outbreak		
	No. examined	Virus-positive	%	No. examined	Virus-positive	%	No. examined	Virus-positive	%
Infected area (Satau)	93*	63	68	34*	13	38	37*	2	5·4
Contiguous area (Katchekau)	30	0	0	30	0	0	30	0	0

* Includes both clinically normal and clinically infected animals at time of outbreak.

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at 6 months after the outbreak, five had not been clinically infected. One of the two positive animals at 12 months had not been clinically infected.

Serology

Serum was collected from all animals sampled for carrier virus. In addition, in the infected area numbers of animals actually undergoing infection or having recently undergone infection were also bled.

Table 3 presents a summary of the antibody titres to type SAT 1 virus (strain Rho 5/66) at the termination of the outbreak. The results are recorded as the geometric means of the reciprocal antibody titres of each of the groups. The numbers of the animals sampled and tested in each group are given in parentheses.

Table 3. *The geometric mean reciprocal antibody titres of groups of cattle in infected and non-infected herds*

	Clinically infected animals	Carriers (no overt disease)	Virus-negative animals
Infected area (Satau)			
Group I. 70 % morbidity	631 (24)*	247 (23)	1318 (4)
Group II. 25 % morbidity	3090 (4)	513 (6)	513 (12)
Group III. 0 % morbidity	—	794 (6)	166 (13)
Contiguous area (Katchekau)			
Group IV	—	—	112 (29)
Clean area (Kazangula)			
Group V	—	—	112 (22)

* The numbers in parentheses are the numbers of cattle in each group.

Serum samples were taken again at 6 months and 12 months after the outbreak. As expected, antibody titres had dropped considerably. There was no significant difference between the antibody titres in the carrier animals and the virus-negative animals, nor was it possible by their antibody titres to differentiate between those animals which had undergone clinical disease and those which had resisted infection.

Antigenic studies

In an earlier survey of FMDV carrier animals previously infected with a type SAT 3 virus (Hedger, 1968), antigenic variation between different strains of carrier virus from animals in the same herds was observed. It was not possible to state whether there had been a variation in the virus at the time of the outbreak or whether such variation had occurred while the virus was in the carrier state after clinical disease.

Therefore, in this survey, in addition to the various carrier strains of virus isolated during the outbreak, lingual epithelium was collected from a number of affected animals and virus isolations were made. All the virus strains isolated were typed SAT 1.

Virus from one of the epithelial samples was adapted to grow in guinea-pigs for the production of a specific antiserum. Using a range of type SAT 1 subtype-specific antisera and including this homologous antiserum, all the strains of virus

isolated during the outbreak were tested in complement-fixation tests for possible antigenic variation. With each virus strain the serum titres were plotted as histograms and compared with each other and with histograms from other type SAT 1 subtype strains. No antigenic variation was seen among the 40 strains isolated during the outbreak. A number of the carrier virus strains recovered at periods after the outbreak have also been tested. Antigenic variation from the original outbreak strain has not been observed in any of these strains so far examined.

In cross-complement-fixation tests the outbreak strain was shown to be antigenically similar to strain Rho 5/66 with a complement-fixation product of 0·83 (Davie, 1964) equivalent to an R value of 91 % (Ubertini *et al.* 1964). Rho 5/66 is a standard World Reference Laboratory subtype strain originating from an outbreak in Rhodesia in 1966 and used since as a vaccine strain.

DISCUSSION

The results show that when cattle immunized by vaccination were presented with a severe natural field challenge with FMDV (Group 1), nearly all those animals (85 %) with sufficient immunity to protect them against clinical disease were, however, susceptible to local pharyngeal infection and became virus carriers.

In Group II immunity was higher and a smaller proportion of animals became carriers.

Most significant was the finding of carrier animals in vaccinated herds which had been in close contact with field virus but in which no clinical disease was observed.

The percentages of demonstrable persisting carrier animals at 6 and 12 months after the outbreak (Table 2) are of a similar order to those found in a previous survey involving a type SAT 3 virus (Hedger, 1968). The results suggest that vaccination before exposure to virus has little effect on the subsequent duration of the carrier state.

Repeated sampling in the vaccinated contiguous herds failed to reveal carrier animals. This indicates that after an outbreak there may be little likelihood of carrier virus spreading in a vaccinated population under natural conditions.

While it is not possible to differentiate by the antibody level of an individual animal between infection and resistance to infection with or without an accompanying carrier status, differences in the mean antibody level of the groups in the areas are worthy of comment.

Experience has shown that, following infection, where high antibody levels are found in a sample of a population, carrier virus will also be recoverable from that population. Conversely, if significant antibody levels cannot be demonstrated in some animals in a population it is highly unlikely that carrier virus will be recovered. Thus the antibody levels in the contiguous area (Katchekau) and the disease-free area (Kazangula) where no carrier virus was isolated may be accepted as due to vaccination.

The antibody levels of Groups I and II in the infected area where animals have been either infected or in direct contact with natural infection are considerably higher. In Group III, however, where there was no apparent clinical disease, but

where there had been sufficient contact with infection for some of the animals to become virus carriers, the mean antibody titre of the negative animals is of the same order as the control groups (IV and V) and is markedly lower than the other virus-negative animals in the herds where frank clinical disease occurred. This supports the observation that clinical disease had not in fact occurred in these herds, although some of the animals had assumed the carrier status.

In Groups I and II where overt disease had occurred, the mean antibody titres in the clinically affected animals were, as expected, higher than in those which had resisted challenge. The mean titres of the negative animals in these groups were, however, also considerably higher than in the control groups in the non-infected areas. This suggests that the presence of virulent infection in an immune or semi-immune population actively boosts the immunity of animals which resist challenge.

The very high antibody titres in the virus-negative animals in Group I are not fully understood, but they may suggest that if animals possess a strong enough induced immunity, they may not only resist clinical disease but also carrier infection.

The close antigenic similarity of all the strains of virus isolated at the time of the outbreak was expected, and is similar to the findings when multiple strains have been examined during other outbreaks. For example, no significant antigenic differences were recorded in the many strains of virus isolated during the British type O_1 epizootic of 1967/68 (J. Davie, personal communication). However, the close similarity of all the carrier virus strains isolated after the type SAT 1 outbreak is at variance with the findings of an earlier survey involving a type SAT 3 virus, when antigenic differences were observed not only between some of the carrier strains and the outbreak virus strain but also between carrier strains isolated from different animals in the same herd (Hedger, 1968). The evidence suggests that the SAT 1 virus strain involved in this outbreak and in the previous outbreak in Rhodesia is more stable than the SAT 3 strain mentioned, in which antigenic variations were recorded in the carrier phase. The possibility of antigenic change occurring in the SAT 1 carrier virus at a later date, however, is not precluded.

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