INFECTION OF SOME SPECIES OF AFRICAN WILD LIFE WITH FOOT-AND-MOUTH DISEASE VIRUS

By

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INTRODUCTION

It has been reported that numerous species of African wild animals are either clinically affected with or susceptible to experimental infection with foot-and-mouth disease (FMD) (Macaulay, 1963), while serological surveys (Condy, Herniman and Hedger, 1969) have suggested others are susceptible to the virus. Foot-and-mouth disease virus (FMDV) has been isolated on occasion from lesions from some clinically affected species (Meeser, 1962; Young, Howell and Hedger, unpublished; World Reference Laboratory, Pirbright, unpublished), and also from buffalo in the absence of clinical disease (Hedger, Condy and Falconer, 1969).

Little is known, however, about the course of infection, the significance of antibody titres or the carrier status in wild animals. This paper describes attempts to infect captive groups of different species of wild animals during the course of a natural outbreak of disease in cattle on a ranch in Rhodesia.

MATERIALS AND METHODS

Experimental animals. Groups of buffalo (Syncerus caffer), wildebeeste (Connochaetes taurinus), kudu (tragelaphus strepsiceros), impala (Aepyceros melampus), warthog (Phacochoerus aethiopicus), bush pig (Potamochoerus porcus) and a single elephant (Loxodonta africana) were captured as calves and together with 4 bovine controls were kept in close isolation in a FMD-free area pending an opportunity to carry out the experiment. Pens housing each species were adjoining and of open construction. Animals of the same species were allowed to mix freely and no attempt was made to isolate different species. To avoid the establishment of a fresh focus of infection it was not possible to carry out this experiment except on infected and quarantined premises during a natural outbreak of disease. When such an outbreak occurred all the experimental animals were transported 700 miles by road in cattle trucks to isolation pens in the infected area. These pens, which were of open construction, adjoined one another.

Virus and methods of infection. The virus used for infection was a 1/10 suspension of freshly ground epithelium from tongue lesions of a cow naturally infected during the outbreak. The virus strain was typed as SAT 2 and given the World Reference Laboratory classification Rho. 4/70. Infection was by intradermo-lingual inoculation of 1 ml. of virus suspension at approximately 10 different sites in all species except warthogs and bush pig which were inoculated subdermally in the bulbs of the heels of all four feet.

For infection, and where necessary for sampling and examination, animals were

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anaesthetised or immobilised using sodium thiopentone (Intraval, May and Baker) or M99/etorphine hydrochloride (Reckitt). Following infection all animals were examined clinically twice daily and temperature records were kept. Some animals were left uninoculated to allow contact infection. Oesophageal/pharyngeal (O/P) samples were collected in probang cups similar to that described by Sütmoller and Gaggero (1965). The collection, handling and transport of O/P samples has been described in detail elsewhere (Hedger, 1968). Virus isolation and titration was performed on tissue cultures of primary calf thyroid cells. The specificity of all virus strains isolated was verified by complement fixation (CF) test using the microtitre technique described by Casey (1965).

**Serum neutralisation tests.** Sera were assayed by the cell metabolic or colour test (Martin and Chapman, 1961) using primary monolayers of pig kidney cells and viruses adapted to pig kidney cell cultures by serial passage. The viruses used in test were SAT 1 (strain Rho. 5/66) isolated from an outbreak in Rhodesia in 1966 and SAT 2 (strain SA 3/69) isolated from the most recent SAT 2 outbreak in South Africa in 1969. Neutralisation titres are expressed as the reciprocal of the final dilution of serum present in the serum virus mixture at the 50 per cent. end point estimated according to the method of Kärber (1931).

**RESULTS**

Preinfection determination of the titre of the challenge virus was not possible but, following a 5-day delay in transit to the laboratory, titres of $10^{7}$ calf thyroid tc50 and $10^{8.7}$ mouse id50 per ml. were recorded.

Four additional epithelial samples from cattle in the infected herd were also typed SAT 2 and an assay of 40 sera from convalescent cattle on the ranch revealed the presence of antibodies to SAT 2 only. Unfortunately, 2 of the 6 buffalo in the experiment were known carriers of SAT 1. It was shown later that one other was viraemic with SAT 1 and was probably excreting large quantities of this virus at the time of the experimental infection with SAT 2.

**Cattle**

Two adult cows and 2 eighteen-month-old heifers used as controls showed classical lesions of FMD with generalisation to the feet within 48 hours followed by a normal antibody response to SAT 2 (see Fig. 1). Although no clinical signs of a second virus infection were observed the SAT 2 antibody responses were followed by similar rises in SAT 1 titres and 2 of the animals became long term carriers of the latter virus.

**Buffalo**

The 6 buffalo aged from 1 to 4 years had been screened for FMDV and FMD antibodies on several occasions prior to the experiment. Two had been demonstrated as long term carriers of SAT 1 over a period of 200 days. At the time of arrival on the infected ranch and prior to artificial infection of all groups with SAT 2 one previously uninfected buffalo was shown to be viraemic with SAT 1. Another became viraemic with SAT 1 the following day.

No clinical symptoms were apparent in the buffalo nor were vesicular lesions observed at the sites of inoculation with SAT 2. Detailed examination, however, revealed very small interdigital lesions on one or more feet of 4 of the 6 animals.
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and single very small ruptured lesions, from which SAT 1 was isolated, on the dental pad of 2 of the animals. Antibody responses to both virus types occurred in all animals.

Virus was recovered from O/P samples of the 6 buffalo 46 days after infection. Five were carrying SAT 1 and one SAT 2. A month later one of the SAT 1 carriers was carrying a mixed infection of both virus types. The carrier state persisted in all animals to 206 days after infection and at the conclusion of the experiment, 292 days after infection, 2 animals were still excreting SAT 1. The maximum virus titre recorded was $10^{4.7}$ tcds$_{50}$/ml. of O/P sample (Table 2) and virus titres of up to $10^{3.6}$ tcds$_{50}$/ml. were recorded 7 months after infection.

![Graphs showing antibody responses to FMDV in different species](image)

Fig. 1. Neutralising antibody responses to FMDV in different species

**Kudu**

The ages of the kudu varied from 1 to 3 years. After infection viraemia was demonstrated in 3 of the 4 animals (two SAT 2 and one SAT 1) and clinical infection with vesiculation of the tongue, muzzle and all 4 feet including the accessory digits was observed in all the animals. Lesion material from 1 animal viraemic with SAT 2 was typed as SAT 1 thus demonstrating a mixed infection. Antibody responses to SAT 1, comparable to those detected in cattle and buffalo, occurred in all animals and persisted at a high level throughout the experiment.
SAT 2 responses were of a lower order and duration (see Fig. 1). All the kudu became carriers to SAT 1 and the carrier state persisted from 106 to 140 days.

**Impala**

Type SAT 2 viraemia was demonstrated in 4 of the 5 inoculated impala. Clinical disease with lesions on tongue and 4 feet occurred in all animals including one left uninoculated as a contact control. From this animal, however, which died 10 days after infection, SAT 1 in high titre (10^9.53/g.) was isolated from lingual lesion material. Antibody responses to both virus types were of a somewhat lower order than those in cattle, buffalo and kudu and they did not persist at a significant level beyond 300 days. Carrier virus was not recovered from this species.

**Wildebeeste**

None of the 4 wildebeeste aged from 1 to 4 years used in the experiment succumbed to artificial or natural infection nor were antibody responses recorded. SAT 1 was recovered from O/P samples from the 2 two-year-old animals 45 days after the attempted infection, but not subsequently.

**Warthog**

Artificial infection of all 5 warthog by intradermal inoculation of the bulbs of the heel with SAT 2 was successful. Viraemias (10^3.0 to 10^4.7 tcid₅₀/ml.) were demonstrated and virus of high titre (up to 10^7.0/g.) was isolated from vesicular epithelium from feet lesions. Lesions also occurred on the foreleg kneeling pads and accessory digits. A single lesion on the gum of one animal was seen. Contact infection with SAT 2 took place very rapidly in two uninoculated warthog. Antibody responses to SAT 2 were similar to those of impala, but persisted over a longer period. Warthog did not become virus carriers. Natural cross-infection with SAT 1 did not occur although the warthog were in close contact with infected animals of other species throughout the experiment.

**Bush Pig**

Both bush pigs reacted to SAT 2 inoculation with classical symptoms of FMD. Viraemia was demonstrated in one of the animals and severe vesicular lesions with subsequent thinning were observed on all 4 feet and accessory digits of both animals. Snout lesions were observed in one animal. Virus in high titre was isolated from lesion material. Antibody titres following infection were of a low order and short duration.

**Elephant**

The single elephant, aged 2 years, failed to react either clinically or serologically to artificial or natural infection with either virus. No carrier virus was recovered from subsequent O/P samples.

**Comparative Species Susceptibility**

Severe clinical disease occurred in cattle, kudu, warthog and bush pig. Buffalo and impala were less severely affected although they were shown to be highly
susceptible to the virus. No clinical or serological evidence of infection was observed in either wildebeeste or the elephant. Cattle, kudu, buffalo and impala, but not pigs proved susceptible to natural cross-infection with the SAT 1 strain of buffalo origin. Due to the construction of the pens it is possible that the degree of exposure of the porcines was less than in the other species.

Carrier virus was recovered from kudu and cattle for periods up to 5 months after infection and from buffalo for considerably longer periods. The carrier state was not demonstrated in impala, the pigs or the elephant.

The degree and duration of demonstrable antibody response varied in different species (see Fig. 1 and Table 1). Titres after infection in buffalo, kudu and in impala to SAT 1 were similar to those in cattle. While high titres persisted for only a comparatively short period in impala, their persistence in buffalo and kudu was of longer duration than in cattle. Titres after infection in warthog and bush pig, species which suffered severe clinical infection, were of a lower order and shorter duration.

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<tr>
<td>HIGHEST INDIVIDUAL POST INFECTION RECIPROCAL SERUM TITRES RECORDED</td>
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<td>SAT 1</td>
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<td>Cattle</td>
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<td>Bush pig</td>
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* Group mean of highest individual titres

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<th>TABLE 2</th>
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<tr>
<td>VIRUS TITRES RECORDED IN DIFFERENT SPECIES</td>
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<td>Wildebeeste</td>
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DISCUSSION

While it is unfortunate that superimposed infection with SAT 1 occurred undetected at the beginning of experimental infection with SAT 2, the results emphasise the complexity of the problems associated with areas where FMD may be endemic in animals of varying susceptibility and illustrate the underlying interplay of virus strains which may occur during an outbreak of disease. The difficulties of carrying out this work in primitive conditions in the context of a
field outbreak of FMD were enhanced by distance from the laboratory and the
techniques necessary for handling partially tame and wild animals. Thus, the
titre of the infecting virus suspension was unknown at the start of the experiment
and probably considerably higher than that recorded later. It is also likely that
virus titres of lesion epithelium given for various species are too low. The results are
of value, however, as there is little record of similar work and a similar opportunity
may not readily recur.

Classical FMD occurred in kudu, impala, warthog, bush pig and in the control
cattle, but although very small lesions were observed in some of the buffalo clinical
symptoms were not apparent. Buffalo were highly susceptible to FMDV, how-
ever, and virus of high titre persisted in them for long periods. The lack of
clinical or serological response to both natural and artificial infection by wilde-
beeste supported previous observations (Condy, 1971) that this species may be
highly resistant to infection. This is at variance with reports of many observers
in the field and further quantitative investigations with a wider range of virus
strains is indicated.

The failure to infect artificially the single elephant may have been due to a
number of factors including the strain and titre of the virus used. Its lack of
reaction to contact infection is in accordance with the results of other experiments
of natural transmission in this species (Howell, Young and Hedger, to be published).

The typing of all outbreak virus isolates from cattle as SAT 2, the demon-
stration of only SAT 2 antibodies in convalescent sera from infected ranch cattle,
the pure SAT 2 infection in the pigs, the failure to demonstrate a mixture of
strains in the infecting virus suspension and the absence of FMD from the rest
of the country at this period indicate that the source of the SAT 1 virus could
only have been the long term buffalo virus carriers. While stress imposed by a
700-mile truck journey may have influenced the break-down from carrier state
to subclinical disease, the viraemia with subsequent lesions occurred not in the
carriers, but in previously uninfected and susceptible buffalo in the group. This
is the first record of carrier transmission in buffalo. It is possible that very close
contact during transport may also have been a factor as prior to the experiment
the carrier buffalo had been in close contact in pens with other susceptible
buffalo and susceptible cattle over a considerable period without transmission of
virus. The carrier state was demonstrated in kudu, buffalo and cattle and in these
species antibody titres were highest and of longest duration. This may suggest a
connection between persisting virus and the continuation of high antibody titres
in these species. The persisting and increasing antibody levels illustrate the difficulty
with random sampling of assessing the time of actual infection. In other species,
e.g. impala, warthog and bush pig, which did not carry virus, antibody titres
were of shorter duration and their relationship with time of infection is more
evident.

SUMMARY

The course of infection with foot-and-mouth disease was studied in a number
of species of captive wild animals using a strain of Type SAT 2 virus from a
current outbreak of disease in cattle. During the experiment a superimposed
infection due to Type SAT 1 virus originating from a carrier buffalo was detected.

Clinical foot-and-mouth disease occurred in kudu, impala, warthog and bush pig. Although clinical disease was not apparent in buffalo, very small vesicular lesions were observed in some of the animals. Wildebeeste and elephant did not react to either artificial or natural virus challenge.

The carrier state was demonstrated in buffalo, kudu and cattle, and virus was recovered on one occasion from two of the wildebeeste. Virus persisted longest and at highest titre in the buffalo.

Neutralising antibody titres after infection in kudu and buffaloe were of similar order to those in cattle. Titres in impala were generally lower and did not persist at a high level for so long. Titres in warthog and bush pig were less and of comparatively short duration.

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REFERENCES


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