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THE PATHOGENICITY OF BOVINE STRAINS OF FOOT AND MOUTH DISEASE VIRUS FOR IMPALA AND WILDEBEEST

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Abstract: Impala (*Aepyceros melampus*) and wildebeest (*Connochaetes taurinus*) were infected with bovine strains of foot and mouth disease virus by intradermolingual inoculation. No clinical signs developed in the impala but mild atypical lesions developed in the tongues of the wildebeest with generalized spread to one foot in two of the eight animals exposed. All the impala but only some of the wildebeest developed viraemia. No virus could be isolated from any tissues in either species after the 7th day following virus inoculation. Immune response occurred in both species. A field survey revealed few animals of either species with significant antibody titers and no virus 'carriers' were found.

INTRODUCTION

Most of the evidence for the involvement of African wildlife in outbreaks of foot-and-mouth disease is from field observations.[7,8] There are reports of both impala and wildebeest becoming infected and developing lesions. In a serological survey in Rhodesia Condy et al.[4] found 8.6% of the impala and 20% of the wildebeest sampled to have foot-and-mouth disease antibodies. In an attempt to infect impala and wildebeest with a bovine strain of type SAT 2 virus, Hedger et al.[5] found that the impala developed clinical lesions but did not become virus 'carriers'. In impala, there was an immune response with the greatest antibody concentrations occurring about 1 month after infection. The wildebeest did not develop lesions or became virus 'carriers' nor was there any demonstrable immune response. However in another experiment,[4] where live virus of a bovine strain of type SAT 2 virus was inoculated intramuscularly into four wildebeest, there was a significant though small immune response. No lesions developed. The evidence, therefore, suggests that these two species may become involved in foot-and-mouth disease outbreaks but it is not certain whether they can act as reservoirs or transmitters of the disease. As part of a study on the epizootiology of the disease in Kenya, where both these species abound, an attempt was made to confirm and extend the observations made by Hedger et al..[5] and determine whether these species might be involved in the perpetuation and spread of the disease. There have been no confirmed cases of foot-and-mouth disease in either species in Kenya in areas where the majority of field out-

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breaks occur in cattle. Attempts were therefore made to infect impala and wildebeest with bovine strains of virus in the laboratory and the results are reported here. In addition, a field survey was carried out to determine whether virus 'carriers' could be found and to determine the extent to which these and other species are exposed to foot-and-mouth disease virus sufficient to stimulate a detectable immune response.

METHODS

Animals

Adult impala and wildebeest were captured by immobilisation with a mixture of etorphine hydrochloride and acepromazine maleate[3] and xylazine[4] given intramuscularly using Chap-Chur[5] equipment. The dosages used, expressed as mg/kg body weight, were as follows:—

	Impala	Wildebeest
Etorphine hydrochloride	0.02 - 0.025	0.015
Acepromazine maleate	0.08 - 0.1	0.06
Xylazine	0.8 - 1.0	0.23

Following capture, the antagonist diprenorphine was given intravenously at the rate of 3 mg for every 2.5 mg of etorphine hydrochloride used.

For handling and routine sampling, xylazine alone was usually given at the rate of 1.4 mg/kg for the impala and 0.4 mg/kg for the wildebeest.

Following capture in the field oesophageal-pharyngeal (O-P) samples and sera were collected and examined for foot-and-mouth disease virus and antibody, respectively, and if found to be negative the animals were used in subsequent experiments.

Virus Exposure

a) Impala

A type O virus, originally isolated during a field outbreak of the disease in cattle and which had been passaged a further 6 times in cattle in the laboratory, was used. One ml of a suspension containing 10^5 bovine LD_{50}[6] was inoculated intradermolingually into each of six adult impala. Three uninoculated impala were placed in the same loose-box as those inoculated. They were left for 10 days and then moved to a clean loose-box in contact with a susceptible steer.

As no lesions developed in any of these animals, attempts were made to passage the virus in a number of susceptible impala. Virus isolated during the viraemic stage was inoculated intradermolingually into a second impala and the process repeated when it too developed a viraemia. An attempt was similarly made to adapt a type SAT 2 virus.

b) Wildebeest

The same type O virus, dose, and route of exposure as used to infect the impala was used to infect eight adult wildebeest.

Collection of Samples

At intervals after exposure, tissues and body fluids of each animal were examined for the presence of virus. O-P samples were collected by means of a probang as described by Sutmöller and

[3] Immobilon—Reckitt and Colman, Hull, England.

[4] Rompun—Bayer, Leverkusen, Germany.

[5] Palmer Chemical and Equipment Co. Inc., Georgia, U.S.A.

Gaggero[11] for the wildebeest and one similar to that used for sheep for the impala. The O-P samples, nasal swabs and faeces from the impala were collected in 5 ml volumes of phosphate buffered saline (PBS) containing 0.62% w/v gelatin pH7.6, whilst those from the wildebeest were collected in 5 ml 0.04M phosphate buffer pH7.2 because of the greater volume of saliva in these O-P samples. Both solutions contained 200 units/ml penicillin, 200 units/ml polymixin B sulphate, 150 μg/ml neomycin sulphate and 100 units/ml mycostatin. Blood was collected into 0.2%w/v ethylene-diamine-tetra-acetic acid (EDTA) to obtain plasma for the detection of viraemia.

Impala were autopsied at intervals after challenge and tissues collected into the same buffer solution as described above. These tissues were subsequently homogenised and examined for the presence of virus.

In the field survey, when animals were immobilised for the collection of samples, the O-P samples were collected exactly as in the laboratory. When animals were shot, the retro-pharyngeal wall and lymphoid tissue was scraped with a curette and the scrapings placed in 5 ml PBS solution as described above.

Isolation of Virus

Samples were examined for the presence of foot-and-mouth disease virus in primary bovine thyroid monolayers[10] in test-tubes, 0.2 ml aliquots of each sample being inoculated onto each of five monolayers. These were incubated at 37C and examined daily for 3 days. Following the development of cytopathic effect (CPE) the presence of foot-and-mouth disease virus was confirmed by typing in a micro complement-fixation test.

Serum Antibody Assay

Sera collected at intervals throughout the experiments were assayed in a micro metabolic inhibition test.[1]

RESULTS

Laboratory Exposure

a) Impala

No clinical signs developed in any of the impala nor in the susceptible steer introduced with the in-contact control impala on the 10th day after exposure.

The tissues and body fluids from which virus was isolated at varying intervals after exposure are shown in Table 1. A viraemia developed in all the animals inoculated and persisted until the 4th - 5th day. Virus was isolated from the nose and throat only during this period. One of the three uninoculated impala used as in-contact controls developed a viraemia on the 10th day. However, no virus was recovered from the nose or throat of this animal 19 days after the viraemia was detected. At autopsy of the inoculated animals, virus was found in the prescapular, bronchial and mesenteric lymph nodes as well as the lung and spleen 4 days after exposure and in the bronchial lymph node and spleen 7 days after exposure. No virus was detected in any of the tissues collected later than the 7th day after inoculation.

An attempt was made to passage the virus in impala to see if it would become more virulent and produce clinical disease. Type O virus isolated during the viraemic stage from one of the impala used in the above experiments and having a titre of $10^{2.2}ID_{50}$ per ml in primary bovine thyroid cells, was inoculated into the tongue of a second impala. This animal developed a viraemia by the 3rd day which did not persist as long as the 7th day. No virus was isolated from the throat, neither was there any rise in humoral antibody. No lesions developed. The virus isolated during the viraemic stage was then inoculated into the tongue of a third impala. A viraemic again developed but again no virus could be isolated from the throat. No clinical lesions developed but there was an antibody response, the titre 28 days after exposure being $\geq 1/90$.

It was thought that possibly the impala were not susceptible to this type O

TABLE 1. Tissue and body fluids of impala from which virus was isolated at varying intervals after exposure to virus.

Specimen	DAYS AFTER EXPOSURE—NUMBER POSITIVE/TOTAL									
	DAY 2		DAY 4		DAY 7		DAY 10		DAY 29	
	Infected Group	In-contact Controls	Infected Group	In-contact Controls	Infected Group	In-contact Controls	Infected Group	In-contact Controls	Infected Group	In-contact Controls
Plasma	3/6	0/3	4/5	0/3	0/4	0/3	0/3	1/3	0/2	0/3
Nasal Secretions	6/6	0/3	2/5	0/3	0/4	0/3	0/3	0/3	0/2	0/3
Oesophageal Pharyngeal Sample	4/6	0/3	3/5	0/3	0/4	0/3	0/3	0/3	0/1	0/1
Lung	0/1		1/1		0/1		0/1		0/1	0/1
Bronchial Lymph Node	1/1		1/1		1/1		0/1		0/1	0/1
Mesentric Lymph Node	0/1		1/1		0/1		0/1		0/1	0/1
Spleen	1/1		1/1		1/1		0/1		0/1	0/1
Prescapular Lymph Node	0/1		1/1		0/1		0/1		0/2	0/3

virus and so another impala was inoculated with a bovine strain of type SAT 2 virus (titre $10^{6.0}ID_{50}$ per ml in primary bovine kidney cells) by intradermolingual inoculation. A viraemia developed but the virus could not be isolated from the throat nor did any lesions develop. There was a humoral antibody response however, the titre to a heterologous subtype being 1/63 at 11 days and 1/45 at 45 days after exposure. The virus isolated during the viraemic stage from this animal (titre $10^{2.6}ID_{50}$ per ml in primary bovine thyroid cells) was inoculated into the tongue of a second susceptible impala which again failed to develop lesions but did have a viraemia up to the 4th day after exposure. There was a slow antibody response, there being no detectable antibody to a heterologous subtype on the 39th day after infection but a titre of 1/45 on the 67th day. This virus (titre $10^{5.2}ID_{50}$ per ml in bovine thyroid cells) was inoculated intradermolingually into a third impala which not only failed to develop lesions but also did not develop a viraemia nor could virus be isolated from the throat. There was, however, an antibody response, the titre on the 27th day after infection being $\geq$ 1/90.

The humoral antibody response in the first group of six impala following inoculation is shown in Figure 1. As early as the 7th day after exposure there was a significant humoral antibody titre which continued to increase until the 10th day, remaining at this level until the 29th day when the experiment was terminated. Of the three in-contact control animals, only one that developed a viraemia showed any antibody response, although this was small. The susceptible steer that was introduced 10 days after challenge showed no immune response.

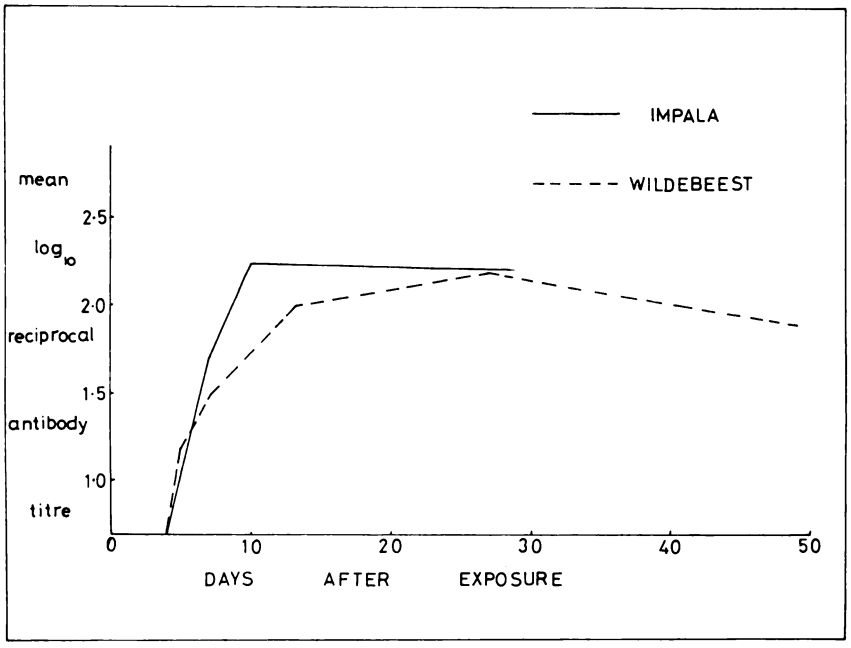

FIGURE 1. The serum antibody response in impala and wildebeest following exposure to type O foot and mouth disease virus.

b) Wildebeest

In all eight of the wildebeest atypical erosions of the tongue epithelium developed around the sites of injection of the virus. No typical vesicles developed. Two animals showed signs of generalised disease in that each developed a vesicle on one foot, which subsequently ulcerated.

The results of tests to determine if virus was multiplying in the body and being excreted are shown in Table 2. Only three of the eight animals developed a viraemia (titres $10^{0.8}$, $10^{1.2}$ and $10^{2.4}$ ID_{50} per ml in bovine thyroid cells) which was present 2 days after infection. Two of these three animals subsequently showed signs of generalised disease. The viraemia did not persist as long as the 5th day after infection. Virus was present in the throat of all eight wildebeest 2 days after infection (mean titre $10^{2.25}$ ID_{50} per ml), in five wildebeest 5 days after infection and in two 7 days after infection. Thereafter, no virus could be detected in the throats of any of the animals. At no time was virus isolated from the nose or faeces.

The humoral antibody response is shown in Figure 1. There was a rapid rise in antibody titre up to the 27th day after infection. It had decreased slightly by the 49th day, when the experiment was terminated.

Field Survey

a) Impala

A total of 262 impala were examined for the presence of foot-and-mouth disease virus in scrapings of the retropharyngeal mucosa and for the presence of humoral antibody. Of these, 238 animals were from an area that had been free from outbreaks of foot-and-mouth disease for at least 5 years. No 'carrier' virus was isolated and no specific antibody was detected.

The remaining 24 animals came from areas where the disease is endemic. Again no 'carrier' virus was isolated and only one animal had a significant antibody titre (1.35 to type A).

b) Wildebeest

A total of 167 wildebeest were examined, all from areas where the disease is endemic. Again, no 'carrier' virus was isolated. Three of the 167 animals (1.8%) showed significant antibody titres. These were, respectively, 1.5 to type A, 1.35 to type SAT 1 and 1.35 to type SAT 2.

DISCUSSION

The results of the laboratory experiments indicate that it is unlikely that either the impala or the wildebeest will develop clinical disease on exposure to bovine strains of foot and mouth disease virus. In these experiments larger doses of virus than would be encountered in the field were given by an optimum route for infection and yet no clinical disease developed.

Virus multiplication occurred in both species and although this stimulated a marked immune response, neither species became a virus 'carrier'. These results are similar to those reported by Hedger et al.,[5] where again no virus 'carriers' were found following exposure of these species to bovine strains of SAT 1 and SAT 2 virus. These authors did, however, describe mild lesions developing in the impala, and the immune response in the wildebeest was not as marked as in these experiments.

In those countries in Africa where most foot-and-mouth disease occurs in cattle and consequently where most of the virus in the environment is adapted to cattle, it seems unlikely that the impala and the wildebeest will readily develop clinical disease. Without clinical disease they will not excrete large amounts of virus and as they do not appear to readily become virus 'carriers' they will not act as reservoirs. There have been cases, however, where the disease has spread well in impala, probably because a mutant arises which readily adapts to multiplication in the species and produces clinical disease. These cases must be ex-

TABLE 2. Specimens from wildebeest from which virus was isolated at varying intervals after exposure.

Animal No.	DAYS AFTER EXPOSURE															
	2				5				7				13			
	Plasma	Throat	Nose	Faeces	Plasma	Throat	Nose	Faeces	Plasma	Throat	Nose	Faeces	Plasma	Throat	Nose	Faeces
33	—	+	—	ND	—	—	—	—	—	—	—	—	—	—	—	—
34*	+	+	—	ND	—	+	—	—	—	—	—	—	—	—	—	—
35	—	+	—	ND	—	—	—	—	—	—	—	—	—	—	—	—
39	—	+	—	ND	—	+	—	—	—	—	—	—	—	—	—	—
40	+	+	—	ND	—	+	—	—	—	+	—	—	—	—	—	—
41	—	+	—	ND	—	+	—	—	—	—	—	—	—	—	—	—
42*	+	+	—	ND	—	—	—	—	—	—	—	—	—	—	—	—
43	—	+	—	ND	—	+	—	—	—	+	—	—	—	—	—	—

* These animals developed lesions on one foot following virus exposure

ceptional and if the disease is controlled in cattle the primary source of virus is removed. The presence of specific antibody is indicative of exposure to foot-and-mouth disease virus but does not necessarily imply that the species concerned is contributing to the spread of the disease.

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