Onderstepoort J. vet. Res. 40 (2), 41–52 (1973)

FOOT-AND-MOUTH DISEASE IN THE AFRICAN ELEPHANT (*LOXODONTA AFRICANA*)

P. G. HOWELL[1], E. YOUNG[2] and R. S. HEDGER[3]

ABSTRACT

HOWELL, P. G., YOUNG, E. & HEDGER, R. S. Foot-and-Mouth disease in the African elephant (*Loxodonta africana*). *Onderstepoort J. vet. Res.* 40(2), 41–52 (1973).

A strain of SAT 2 foot-and-mouth disease virus which was experimentally inoculated into the epidermis of the tongues of captive African elephants produced vesicular lesions at the site of inoculation.

After a short period of viraemia, secondary lesions developed in the mouth and on the feet giving rise to extensive tissue damage and the separation of the soles. In spite of close contact there was no spread of the disease to other elephants and by conventional sampling techniques no carrier virus could be demonstrated. The neutralizing antibody response was of a low order and this finding together with the observations made during the course of the experimental disease are discussed in relation to the possible role of the elephant in the epizootiology of foot-and-mouth disease in Africa.

INTRODUCTION

The epizootiology of foot-and-mouth disease (FMD) on the continent of Africa is probably more complicated than in any other part of the world. Over large areas, the susceptible domesticated stock share the pastures with the indigenous fauna, many of which are believed to play an important role as a source of infection and as a mechanism for further dissemination of the disease. Amongst the domestic animals, the relative susceptibility of individual species has been established and the role they play in an epizootic in any particular region of the world can be anticipated. There is still insufficient information, however, on the susceptibility of the various wild fauna of Africa and the role which they play, in the evolution of an epizootic.

Attempts have been made to assess the situation by surveys of virus neutralizing antibody in game (Brooksby, 1968; Condy, Herniman & Hedger, 1969) as well as by the recovery of carrier virus, for example from the buffalo (Hedger, Condy & Falconer, 1969; Hedger, 1972) but the information provided by these investigations is incomplete and has only served to identify the more important hosts.

In order to implicate a particular species in the epizootiology of the disease it is necessary to determine:

(a) Whether the animal can be readily infected or not;
(b) The nature and severity of the clinical reaction which follows;
(c) The success with which the animal can pass on the infection to its own species or other species in contact with it and whether the virus can persist in the animal and create a reservoir for possible future dissemination;
(d) The nature of the immune response, in order that this information can be used to interpret field surveys.

In areas where game conservation is practised the population density of certain species has increased tremendously and falling within this category is the African elephant (*Loxodonta africana*). This report contains the results of an experiment which was conducted with a view to establishing the possible role of this animal in the epizootiology of the disease in Africa.

Previously Ramiah (1935) described a case of FMD with very severe symptoms in an Indian elephant cow, which it was stated had been in close association with active infection amongst cattle. No virological data were presented and the report remains to be corroborated by further work. More recently Piragino (1970) reported an outbreak of FMD amongst a group of 15 African elephants in a circus in Italy. The disease was confirmed by the recovery and identification of a type A_7 strain of FMD virus and again it was suspected that the source of virus was infected cattle in the vicinity.

In so far as field evidence is concerned, 353 African elephant sera collected at random from different countries in Africa and tested against the appropriate viruses have yielded negative results, notwithstanding the fact that these sera were frequently taken during outbreaks of FMD in domestic stock and in other game, in which neutralizing antibodies were demonstrated. These results are further substantiated by the fact that no confirmed clinical observations of the disease in elephants have been reported from this continent.

MATERIALS AND METHODS

Virus

The virus used in this experiment was identified as a type SAT 2 strain and was collected from a vesicle on the tongue of a buffalo showing clinical evidence of FMD. This animal was one of a number of buffalo and impala involved in an epizootic of the disease in the northern regions of the Kruger National Park during the autumn of 1970.

A portion of the sample was inoculated intradermally into the tongue of a bovine heifer. The epithelium from the vesicle which developed 18 hours later was finely ground and an approximate 1:15 m/v suspension was prepared in chilled M/25 phosphate buffer pH 7,6. The supernatant virus suspension obtained after centrifugation at 2 000 rpm for 10 minutes was dispensed into bottles which were then sealed and stored on dry ice for use in various phases of the experiment.

Sampling procedures

Inspection and the collection of samples necessitated the tranquilization and casting of each individual elephant. This was accomplished by the administration of etorphine hydrochloride (M99, Reckitts) in combination with acetyl promazine maleate (Boots) in projectile syringes, fired into the gluteal region. The dosage rates were based on an estimate of mass as suggested by Pienaar, Van Niekerk, Young, Van Wyk & Fairall (1966). In order to obtain rapid recovery, narcosis was

[1]Veterinary Research Institute, Onderstepoort
[2]Veterinary Investigation Centre, Skukuza, Kruger National Park
[3]The Animal Virus Research Institute, Pirbright, England

Received 17 February 1973 - Editor

terminated by the intravenous injection of cyprenorphine (M285, Reckitts).

Where specific lesions were observed, suitable portions of tissue were either scraped or dissected away and placed in a solution of 50% glycerol in phosphate buffer. Oesophageal/pharyngeal samples for the isolation of carrier virus were collected by probang according to the technique described by Sütmoller & Gaggero (1965). The collection, handling and transport of these samples have been described in detail elsewhere (Hedger, 1968). Whole blood was collected from an ear vein into bottles containing diamino-ethane-tetra-acetic acid (EDTA) and immediately after collection all samples of tissue and blood intended for the assay of infective virus were placed on dry ice.

Assay of virus in samples

The isolation and titration of infective virus were performed by standard techniques using a logarithmic series of dilutions. At Pirbright monolayer cultures of calf thyroid cells (Snowden, 1966) were used as the primary assay system. For the purpose of comparison duplicate titrations were also performed in 5 to 6 day-old suckling mice or by intradermo-lingual inoculation of the tongues of cattle accommodated at Skukuza. The specificity of the virus recovered from the samples was checked by complement fixation tests, conducted according to the microtitre technique described by Casey (1965).

Serum-virus neutralization tests

Serum samples were assayed at Pirbright for neutralizing antibodies by the cell metabolic inhibition test (Martin & Chapman, 1961) modified by the use of BHK 21 cells in microtitre plates.

The strains of type SAT 1, SAT 2 and SAT 3 virus used in the neutralization tests were isolated from the most recent outbreaks of the disease amongst cattle in the southern regions of Africa.

The SAT 2 reference strain of virus (SA 3/69) considered to be homologous with the virus used in the experiment, had been isolated 6 months previously from an outbreak of disease close to the border of the Kruger National Park. Before inclusion in the test these viruses were adapted to grow in BHK 21 cells by serial passage.

Experimental animals

During the course of culling operations in various regions of the Kruger National Park, young weaned orphaned elephants were captured and transported to an isolation camp, where they were held for a period of 9 to 12 months before the commencement of the experiment. Their ages were estimated to vary from $1\frac{1}{2}$ to 3 years.

For the purpose of the experiment the elephants were divided into four groups of three. Each group was accommodated in a separate pen.

Experimental procedure

On the basis of a preliminary titration of the infectivity of the stock virus suspension in suckling mice three dilutions were prepared. A volume of 0,5 ml of the virus suspension selected was equally distributed through six needle tracks over two sites, three on the anterior and the other three on the posterior aspect of the dorsum of the tongue. In each group, one elephant remained uninoculated while the remaining two received one of the three dilutions of virus. When retitrated in cattle tongues the stock virus gave an infectivity titre of $10^{8,5}$ cattle ID_{50}/ml ($10^{9,37}$ TC ID_{50}/ml in calf thyroid cells). In the volume of 0,5 ml inoculated into the tongues of the elephants, the amount of virus administered therefore covered the range of 2×10^6, 2×10^4 and 2×10^2 cattle ID_{50} respectively.

Results

A summary of the clinical observations considered to be specific to the experimentally produced disease is given in Appendix Table 1. In this table the pulse and respiratory rates are given as well as the rectal temperatures of the elephants, taken immediately after all three animals in the pen had fallen under the effect of the tranquilizing drugs. Illustrations of the predominant features of the lesions described in particular animals are given in Fig. 1-12.

Examination of the tongues at the time of injection revealed a high incidence of traumatic damage to the epithelium. The position and appearance of these lesions were noted in order to distinguish them from those produced by the virus.

After experimental infection the incubation period was short. In the elephants which received the highest concentration of virus, vesicular lesions or signs of tissue damage were present at the site of inoculation when they were first inspected 24 hours after infection. On the next day all the elephants were examined and with the exception of two, all those that had been inoculated, exhibited lesions of variable size at the site of infection. There was a distinct relationship between the rate of progress in the development of the lesion and the dose of virus administered. The epidermis of the elephant tongue is very thin and delicate, but the vesicles which developed were distended and very firm. When punctured the contents did not readily flow out and it appeared as if they contained numerous fine septa between which the fluid was trapped.

Of the two elephants which failed to react, one (Elephant 1) had received only 2×10^2 ID_{50} of virus while the second (Elephant 8) although it had received 2×10^6 ID_{50} only developed a localised and superficial area of necrosis, which extended for 1 to 2 mm on either side of the needle tracks, as well as one small indistinct vesicle approximately 1 cm in diameter.

At the 72nd hour the primary vesicles had ruptured and a diphtheritic membrane covered the sites of inoculation. The process of vesiculation, however, appeared to have continued in a centrifugal fashion and the original sites became surrounded by separate smaller, additional vesicles, which gave the periphery of the lesion an undulating appearance. The extension to one such lesion eventually covered an area 15 cm in length. It appeared as if the lesions on the posterior aspect of the tongue were more extensive and severe. During the acute stage of development of the vesicles the behaviour of the affected animals indicated that the mouth was sensitive and painful and the intake of food was temporarily reduced. Secondary vesicles observed after 6 to 7 days in some of the animals were smaller but otherwise similar in appearance to those associated with the original inoculation sites.

Continued examination of the elephants between the 6th and 13th day revealed areas of necrosis a few cm in diameter, involving the mucous membrane of the palate, cheeks, sides of the tongue and particularly the commissures of the lips. The affected areas were a pale dirty white in contrast to the pale pink of the unaffected tissue. No virus was recovered from samples taken from these areas, and their association with the disease process remains obscure.

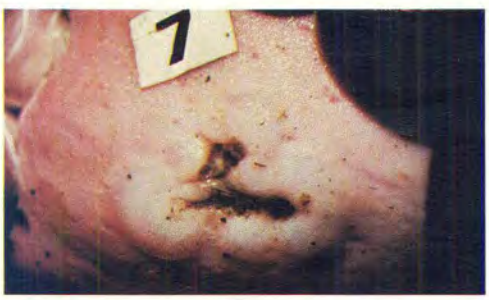

FIG. 1 Multilocular vesicle 48 hours after infection. Central portion discoloured through removal of sample of epithelium 24 hours earlier

FIG. 2 Progressive development and extent of primary lesion 96 hours after infection

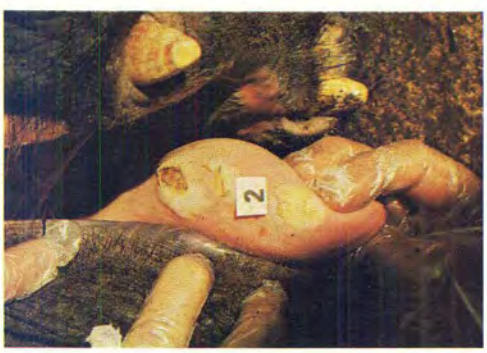

FIG. 3 Vesicles on the dorsum of the tongue in various stages of development 96 hours after infection

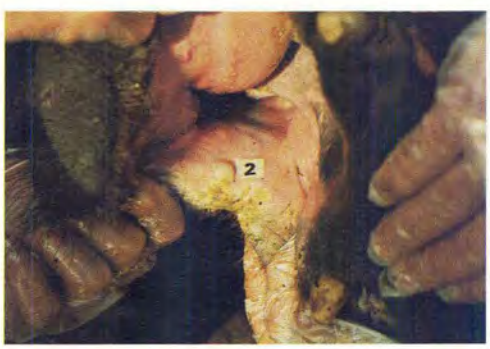

FIG. 4 Smaller unruptured secondary vesicle on left commissure of mouth 7 days after infection

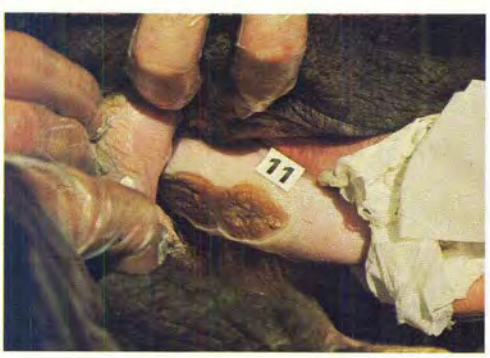

FIG. 5 Early stages in the healing process of two coalescent vesicles, 6 days after infection

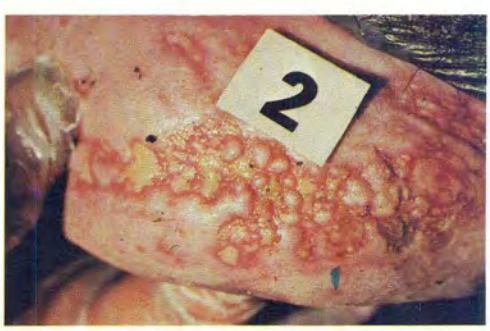

FIG. 6 Islands of regenerating epithelium as seen 9 days after infection

p. 44 is a blank page

FIG. 7 First appearance of secondary vesicle on the foot. A soft swelling as seen on the 6th day of the disease

FIG. 8 Enlargement of interdigital vesicle on point of rupture, 9 days after infection

FIG. 9 Generalized swelling and enlargement of foot with aspiration of vesicular fluid from the coalesced vesicles on the foot

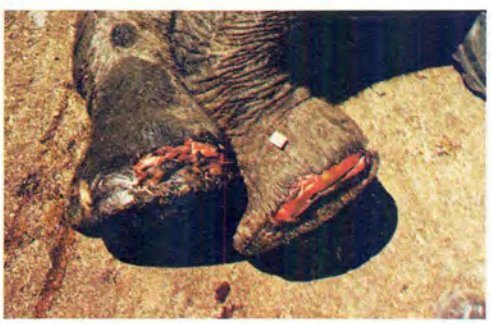

FIG. 10 Early rupture of skin of foot and separation of the sole 9 days after infection

FIG. 11 Exposure of subcutis to show extent to which vesicles have coalesced and separated the sole from the foot

FIG. 12 Extensive detachment of the sole of an affected foot

p 46 is a blank page

TABLE 1 Assay of infectivity of tissue and blood samples taken from elephants experimentally infected with foot-and-mouth disease virus

Dose of virus (Cattle ID_{50}/ml)	Elephant No.	Sample	Days after infection									
			1	2	3	4	6	7	9	10	13	17
2×10^2	1	Blood	NVR	NVR	—	NVR	—	NVR	NVR	NVR	—	—
2×10^2	4	Tongue Epith.	—	—	—	—	6,0(m)	—	—	—	—	—
		Pedal Epith.	—	—	—	—	—	—	—	—	—	1,83
		Blood	—	NVR	4,03	—	5,02	—	NVR	—	—	—
Control	3	Blood	NVR	NVR	—	NVR	—	NVR	NVR	NVR	—	—
2×10^4	2	Tongue Epith.	—	—	—	—	—	4,72(m)	—	—		
		Pedal Epith.	—	—	—	—	—	—	7,0(m)	—	Euthanasia.	
		Blood	NVR	NVR	—	NVR	—	≥1,0(m)	NVR	NVR		
2×10^4	5	Tongue Epith.	—	8,2	—	—	—	—	—	—	—	
		Pedal Epith.	—	—	—	—	—	—	—	—	3,37	
		Blood	—	TR	≥1,0(m)	—	1,0(m)	—	NVR	—	—	Died
Control	6	Blood	—	NVR	NVR	—	NVR	—	NVR	—	—	—
2×10^6	7	Tongue Epith.	7,2	8,4	—	—	—	—	—	—	—	—
		Pedal Epith.	—	—	—	—	—	—	—	—	—	4,03
		Vesicular fluid	—	8,2	—	—	—	—	—	—	—	—
		Blood	NVR	TR	—	NVR	—	NVR	—	NVR	—	—
2×10^6	8	Blood	NVR	NVR	—	NVR	—	NVR	—	NVR	—	—
Control	9	Blood	NVR	NVR	—	NVR	—	NVR	—	NVR	—	—
2×10^6	10	Tongue Epith.	6,37	—	—	—	—	—	—	—	—	—
		Blood	NVR	—	3,93	—	NVR	—	NVR	—	—	—
2×10^2	11	Tongue Epith.	9,03	—	—	—	—	—	—	—	—	—
		Pedal Epith.	—	—	—	—	—	—	—	—	3,37	—
		Pedal Ves. Fld.	—	—	—	—	—	—	1,0	—	—	—
		Blood	1,2	—	3,0	—	2,2	—	NVR	—	—	—
Control	12	Blood	NVR	—	NVR	—	NVR	—	NVR	—	—	—

NVR No virus recovered
(m) Titration undertaken in 5-day old unweaned mice
TR Trace amounts
All titration results expressed as the logarithmic index (log 10) of the 50% end point dilution/ml sample.
End points calculated according to the method of Kärber (1931)

Lameness was first observed between the 5th and 8th days after infection, when the feet felt warm and the animals showed evidence of pain. At this stage a generalized swelling of the feet could be seen from a distance and closer examination revealed raised blanched areas around the toe nails. When pressure was applied they felt turgid and fluid could be detected within. Over a further period of 2 to 5 days the blisters increased in size and the feet became more swollen. At the junction of the skin and sole the developing vesicles were not so obvious, but at this point the skin ruptured and a considerable amount of serous fluid exuded. The break in the continuity of the skin progressed around the periphery of the feet and the cornified epithelium of the sole became completely separated. The necrosis was extensive and from one third to complete sloughing of the sole took place. In some of the elephants the separation of the soles appeared to have taken place before the skin was broken and in their efforts to alleviate the pain, by reducing the mass on their legs, the extremity of the limb moved independently within what appeared to be a loose fitting bag of fluid.

A loss of mass was more noticeable in some of the elephants than in others and no doubt the animals would have been more severely affected under natural conditions. The most serious complication was cardiac involvement since it is believed that a myocarditis precipitated the death of Elephant 5 and also accounted for the irregularities detected in the pulse and heart rates.

Regeneration of the epithelium in the mouth was slow and small disseminated islands of new epithelium first appeared 1 to 2 weeks later. Scars from some of the mouth lesions were, however, still distinctly visible after 3 to 4 weeks. After supportive treatment the soles of the feet regenerated and acquired a normal thickness within a few weeks, but they remained badly deformed and the earlier signs of infection could still be clearly identified, when the animals were destroyed 10 months later.

The samples taken from the affected elephants during the course of the disease yielded relatively high concentrations of virus (Table 1). Tongue epithelium gave infectivity titres of between $10^{6,3}$ to $10^{8,4}$ TC ID_{50}/g while vesicular fluid from one of the elephants gave a titre of $10^{8,2}$ TC ID_{50}/ml. Epithelial samples from the vesicles on the feet taken at an early stage of development were less infective and titres of $10^{1,8}$ to $10^{3,4}$ TC ID_{50}/g were obtained. On occasions volumes of up to 50 ml of fluid were withdrawn from the vesicles on the feet and when this fluid was titrated, the virus concentration varied from $10^{1,0}$ to $10^{4,0}$ TC ID_{50}/ml.

The detection of virus in the blood samples, taken at intervals during the course of the disease, showed that viraemia was present in Elephant 11, 24 hours after infection, while in the others virus was recovered 24 to 48 hours later. Viraemia persisted for up to 6 days in elephants which had received both high and low concentrations of virus. The titre of virus in the blood reached peaks of $10^{4,0}$ to $10^{5,2}$ TC ID_{50}/ml and appeared to be independent of the original concentration of virus inoculated. On no occasion during the 21 day period after infection, in which sampling was undertaken, was virus detected in the blood of the uninoculated controls or the two elephants which failed to respond clinically to infection.

Probang samples of oesophageal and pharyngeal material taken from the elephants at intervals of 38, 62 and 91 days after infection were all negative for carrier virus.

The immune response of the infected elephants is given in Table 2. Examination of the pre-infection serum samples revealed no trace of antibodies to either of the three selected SAT type antigens. With the exception of Elephants 1 and 8 which remained serologically negative, maximum antibody titres were detected on the 21st day after infection. The absence of any immune response in the control animals confirmed the observation that these animals had failed to become infected through contact. When compared with other species, the immune response appears to be relatively poor and the decline in the antibody concentration was rapid.

Discussion

Previous evidence has created the impression that clinical FMD does not occur naturally amongst ele-

Table 2 Detection of virus neutralizing antibodies in the sera of elephants after experimental infection with foot-and-mouth disease virus

Dose of virus (Cattle ID_{50}/ml)	Elephant No.	Neutralizing antibody titre to antigen SA3/69 (SAT 2) Days after infection							
		0	7	8	18	21	38	63	91
2×10^2	1	≤6	—	≤6	—	≤6	≤6	≤6	≤6
2×10^2	4	≤6	≤6	—	—	45	22	11	16
Control	3	≤6	—	≤6	—	≤6	≤6	≤6	≤6
2×10^4	2	≤6	—	≤6	Euthanasia				
2×10^4	5	≤6	≤6	—	22	Died			
Control	6	≤6	≤6	—	—	≤6	≤6	≤6	6
2×10^6	7	≤6	—	11	—	45	32	16	16
2×10^6	8	≤6	—	6	—	≤6	8	≤6	≤6
Control	9	≤6	—	6	—	≤6	≤6	≤6	≤6
2×10^6	10	≤6	≤6	—	—	45	32	22	16
2×10^6	11	≤6	≤6	—	—	64	32	22	22
Control	12	≤6	≤6	—	—	≤6	≤6	≤6	≤6

phants on this continent. This work, however, has confirmed the susceptibility of this species to experimental infection and has shown that the pathogenesis of the disease follows a similar pattern to that which has been determined in other susceptible animals after inoculation. This typical sequence of events was illustrated by the development of vesicular lesions at the site of inoculation, the viraemia which followed and which persisted for up to 6 days, and the eventual appearance of vesicular lesions on the feet.

The clinical picture and especially the severe involvement of the feet, which may be related to the structure of the foot and the considerable mass which it bears, suggests that if the disease had occurred frequently amongst elephants in the wild, it would have been recognised. The severe nature of the lesions and the inability to walk on account of the pain would have rendered an animal immobile under natural conditions.

Quantitative assay showed that the virus had multiplied in the susceptible cells to produce high concentrations of infective virus in both the vesicular fluid and the blood and large quantities of virus must have been shed by individuals. It was therefore surprising that transmission did not take place to any of the four susceptible elephants, kept in close confinement with the infected animals. The close contact of these experimental animals compared with the chance of contact within a free-living herd suggests that spread of this strain of virus would not have taken place, assuming that infection was in fact possible by the natural route.

On the 21st day all the animals which were infected showed a type specific neutralizing antibody response of a low order, which appeared to decrease rapidly. It was unfortunately not possible to determine how long these low antibody levels persisted, but the results suggest that caution is necessary in the interpretation of similar low titres or possibly negative results which might be obtained from the examination of random serum samples collected during surveys. This decline in antibody titres also suggests that the animals do not become carriers. Attempts to demonstrate the carrier state, using the probang technique, did not entirely rule out the possibility that the elephant may harbour virus at a site other than that identified in the majority of species.

Notwithstanding the fact that scar tissue from the experimentally produced disease was found to persist for more than 10 months, no similar lesions of this nature have been observed in some 3 535 elephants examined during capturing and culling operations conducted in the Kruger National Park during the past 4 years. Taking into consideration the fact that three extensive outbreaks of FMD have occurred in the Kruger National Park during this period, it is therefore not unreasonable to anticipate that at least some evidence of infection would have been found in the elephant population estimated to number 8 800, if they were in fact susceptible to natural infection. This experiment and the failure to detect any evidence of the disease during the course of routine examination of captured or dead elephants, suggest that the elephant does not play an important role as a natural host for the spread of FMD in the enzootic regions of Africa.

Acknowledgements

We wish to express our appreciation to the National Parks Board of Trustees for making the experimental animals and the numerous facilities available for this project. In addition we are indebted to Miss S. M. Golding of the Animal Virus Research Institute, Pirbright and Messrs P. J. L. Bronkhorst and C. J. Lombard, Skukuza, for their technical assistance.

References

Brooksby, J. B., 1968. Wild animals and the epizootiology of foot-and-mouth disease. *Symp. zool. Soc. Lond.*, 24, 1-11.

Casey, Helen, L., 1965. Standardised diagnostic complement fixation method and adaptation to micro test. *Publ. Hlth Monogr.* 74, 1-34.

Condy, J. B., Herniman, K. A. J. & Hedger, R. S., 1969. Foot-and-mouth disease in wild life in Rhodesia and other African territories, *J. comp. Path. Therap.*, 79, 27-31.

Hedger, R. S., 1968. The isolation and characterisation of foot-and-mouth disease virus from clinically normal herds of cattle in Botswana. *J. Hyg. Camb.*, 66, 27-36.

Hedger, R. S., 1972. Foot-and-mouth disease and the African buffalo (*Syncerus caffer*). *J. comp. Path. Therap.*, 82, 19-28.

Hedger, R. S., Condy, J. B. & Falconer, J., 1969. The isolation of foot-and-mouth disease virus from African buffalo (*Syncerus caffer*). *Vet. Rec.*, 84, 516-517.

Karber, G., 1931. Beitrag zur kollectiven Behandlung pharmakologisher Reihenversuche. *Arch. exp. Path. Pharmak.*, 162, 480-483.

Martin, W. B. & Chapman, W. G., 1961. The tissue culture colour test for assaying the virus and neutralising antibody of FMD and its application to the measurement of immunity in cattle. *Res. vet. Sci.*, 2, 53-61.

Pienaar, U. de V., Van Niekerk, J. W., Young, E., Van Wyk, P. & Fairall, N., 1966. The use of oripavine hydrochloride (M-99) in the drug immobilization and marking of wild African elephant (*Loxodonta africana* Blumenbach) in the Kruger National Park. *Koedoe*, 9, 108-124.

Piragino, S., 1970. Un focolaia di afta epizootica in elefanti da circo. *Zooprofilassi*, 25, 17-22.

Ramiah, B., 1935. An outbreak of foot-and-mouth disease in elephants. *Indian vet. J.*, 12, 28-29.

Snowden, W. A., 1966. The growth of FMDV in monolayer cultures of calf thyroid cells. *Nature, Lond.*, 210, 1079-1080.

Sütmoller, P. & Gaggero, A., 1965. Foot-and-mouth disease carriers. *Vet. Rec.*, 77, 968-969.

APPENDIX TABLE 1 Summary of observations recorded during examination of experimentally inoculated elephants during the acute stages of the disease

Dose of virus (Cattle ID 50/ml)	Elephant No.	Period after infection	Clinical observations	Pulse/min. Resp./min. Temp. °C
2×10^2	1 Age 1 to 2 years Sex female Condition good	0	Diphtheritic ulcer 2 × 1,5 cm on dorsum of tongue 23 cm from tip	64/12/36,5
		25 hours	1 to 2 mm blanched foci at points of inoculation	64/12/35,5
		49 hours	Blanched needle tracks 2 cm × 1 to 2 mm without vesiculation or inflammatory changes	60/8/35,5
		4 days	No lesions	72/10/35,5
		7 days	No lesions	64/12/36,5
	4 Age 2 years Sex Female Condition good	0	Superficial excoriation right antero-dorsal surface of tongue	64/16/36,5
		47 hours	Two linear scars 2 cm × 1 mm at posterior inoculation site without blanching	68/8/35
		71 hours	No development of lesions visible at anterior inoculation site. Lobulated vesicle formation on periphery of posterior needle tracks surrounded by blanched epithelium (2,5 × 1 cm)	84/16/37
		6 days	Extensive posterior lesion with necrotic central area surrounded by turgid multilocular vesicles. Secondary vesicles developing on right cheek and extreme posterior aspect of tongue	64/24/36,5
		9 days	Lesions healing with pale pink islands of regenerating epithelium. No apparent involvement of the feet	52/8/35
		12 days	Noticeable loss of mass. Uneasiness but feet appear normal.	
		13 days	Mild diarrhoea. Feet appear normal with no signs of pain.	
		15 days	Ruptured blanched vesicle posterior to 3rd toe of left hind foot.	
		17 days	Ruptured vesicle behind 4th toe of right front foot. Distinct scar formation with healing buccal lesions	60/12/36,5
		22 days	Further vesicles between toes with portions of necrotic skin around old lesions sequestrating. Separation of sole from subcutis	68/24/36,5
		28 days	Buccal lesions still visible as deep scars. Separation of soles continuing with process complete in right fore foot.	
		31 days	Soles regenerating after surgical removal	54/12/36,5
		33 days	Further portions of soles of affected feet removed	57/20/35,5
		62 days	New soles completely regenerated.	
2×10^4	2 Age 1–2 years Sex female Condition fair	24 hours	No lesions with site of inoculation barely visible.	
		48 hours	Two distinct pale brown needle tracks at anterior site surrounded by clearly demarcated blanched area 1 cm in diameter. One pale needle track at posterior site with no signs of vesicle formation.	
		4 days	Anterior site - one confluent multilocular vesicle with pseudomembranous centre measuring 3 cm. Posterior site - Two discrete vesicular lesions, one ruptured with free epithelium 2 cm in diameter, the other turgid, measuring 5 × 2 cm with pseudomembranous centre and raised periphery 3 mm above the unaffected epithelium. Raised areas pale white in colour (Fig. 3)	48/12/36
		7 days	One coalesced lesion 10 × 5 cm with dirty yellow necrotic surface and vesicular extensions on posterior extremity. Various smaller independent vesicles on the dorsum of the tongue, hard palate and left commisure of mouth (Fig. 4)	56/11/36
		8 days	Oedematous swelling of all four feet, trembling of limbs, continuous shifting of mass, difficult movement and unwilling to walk. Ears hanging forward, trunk immobile and white frothy saliva in corners of mouth and from trunk. Dejected, irritable and in acute pain.	
		9 days	Buccal lesions healing with islands of regenerating epithelium (Fig. 6). Swelling of interdigital vesicles increasing (Fig. 8). Extensive rupturing of skin above toe nails on all four feet (Fig. 10, 11). Up to 50% of the posterior perimeter of the right fore foot involved. Clear yellowish fluid exuding from ruptured visicles with exposed red, congested subcutis	80/12/37
		10 days	Extensive detachment of soles from subcutis (Fig. 12) Animal in extreme discomfort. Euthanasia.	76/12/37,5
	5 Age 2 years Sex female Condition fair	0	Old reversed S-shaped scar, extreme posterior aspect of dorsum of tongue. Small depressed erosion with well defined edges on sole of left fore foot	80/12/36,5
		24 hours	Three distinct linear needle tracks at anterior site 1 mm × 1 cm. Necrotic area adjoining vesicular lesion of 2 × 1 cm with red, raw area remaining with haemorrhage after removal of epithelium from posterior lesion. Entire site of inoculation surrounded by white blanched epithelium	68/8/35
		72 hours	Anterior lesions healing with very small areas of necrosis over barely visible needle tracks. Posterior lesion increasing in size with lobulated periphery of clear vesicles. Subcutis from sampled area covered by pale yellow brown diphtheritic membrane	84/13/36,5
		6 days	Lesions coalesced. Secondary vesicles in advanced state of development on dorsum of tongue	76/8/37
		8 days	Lameness in right hind limb.	
		9 days	Buccal lesions healing with no further vesicles visible. Swelling of 15 × 5 cm extending from behind the first toe to between second and third toe nail of left fore foot. Smaller swellings of 3 × 2 cm between toe nails of right fore and hind feet	76/9/35
		12 days	Vesicles on various feet ruptured.	
		13 days	Further vesicles appearing between toes. Entire sole of left and right fore feet detached from subcutis. First observation of new secondary vesicle of 2,5 × 2 cm on posterior aspect of tongue	80/12/36,5
		15 days	All buccal lesions now in process of healing. Majority of vesicles on feet ruptured with necrotic rims, although generally appear to be healing	60/12/36,5

APPENDIX TABLE 1 (continued) Summary of observations recorded during examination of experimentally inoculated elephants during the acute stages of the disease

Dose of virus (Cattle ID 50/ml)	Elephant No.	Period after infection	Clinical observations	Pulse/min. Resp./min. Temp. °C
		17 days	Recumbent with respiration barely perceptible, pulse weak and irregular, collapsed after tranquillisation. Post mortem examination shows buccal lesions have almost healed. Extensive tissue damage and separation of the soles of all except the left hind foot. Internal organs including lungs, liver, kidneys, adrenals and alimentary tract show no apparent macroscopic changes. On section myocard shows alternating yellow and dark red striations . .	32/0/35,5
2×10^6	7 Age 3 years Sex female Condition fair	24 hours	Anterior site - Dull yellow area of necrosis 0,5 cm which after curretting leaves haemorrhagic surface. Posterior site - Two small vesicles of 1 cm × 1 mm one of which had ruptured and is surrounded by white zone. Epithelium readily removed leaving red haemorrhagic surface	56/8/35,5
		48 hours	Anterior site identified by ill-defined areas of necrosis corresponding to needle tracks without peripheral discolouration. Turgid multilocular vesicle of 3 × 2 cm with pale white surface raised 2 to 3 mm above epithelium on posterior site (Fig. 1). 0,2 ml clear fluid aspirated with epithelium readily detachable leaving raw bleeding surface. Dejected appearance and not feeding	64/12/36,5
		72 hours	Dull, not feeding, head pushed against wall, biting metal stanchions.	
		4 days	Posterior lesion enlarged to 8 × 5 cm with central pseudomembranous areas surrounded by lobulated vesicles 5 mm above the surface and 2,5 cm wide. Surface pale white and turgid (Fig. 2). Very little food taken . . .	60/8/36,5
		7 days	Epithelium over lesion absent with surface smooth and glistening. Secondary visicles on tongue and left commisure of mouth, ruptured with necrotic centres. Small palpable swellings adjacent to toe nails of right hind foot .	60/9/36,5
		8 days	Lameness of left hind leg.	
		10 days	All buccal lesions healing. Vesicles of 3,5 × 2,5 cm posterior to 1st and 3rd toe nails of right and left fore feet	68/12/36,5
		12 days	Large vesicle posterior to first toe nail of right hind foot.	
		13 days	Vesicle enlarged to 14 cm on right hind foot.	
		17 days	Numerous vesicles appearing on feet some up to 14 cm in length with areas in which skin has ruptured. Sole of left hind foot detaching	72/12/36,5
		20 days	Buccal lesions reduced in size but scars clearly visible. Feet show no further tissue damage and virtually no fluid is exuding from ruptured vesicle.	
		22 days	Posterior half of sole on left fore foot detached	52/12/36,5
		24 days	Feet lesions healing.	60/8/35,5
2×10^6	6 Age 3 years Sex male Condition fair	0	Scar of incised wound with pale periphery 14 cm from tip of tongue. Bruise with blue discolouration on right side of tongue 17 cm from tip	64/8/37
		24 hours	Anterior site indicated by small 3 mm × 3 mm necrotic focus. Posterior site with small vesicle of 1 cm × 1 cm surrounded by blanched epithelium and one necrotic needle track	68/12/36,5
		48 hours	Indefinite and indistinct marks at anterior site of inoculation. On posterior site there are three distinct needle tracks, pale pink in colour but with no evidence of vesiculation	72/8/36,5
		4 days	Both inoculation sites show only pale indistinct needle tracks	68/8/36,5
		7 days	Virtually no evidence of inoculation sites	72/12/37
		8 days	Suggestion of pain in feet.	
		11 days	Hind feet swollen and lameness evident.	
		12 days	Lame with swelling extending 8 cm from soles of all four feet.	
		19 days	Inoculation sites almost undetectable. Feet and gait appear normal . .	68/8/36,5
	10 Age 2 years Sex female Condition fair	0	Few ill-defined scars over dorsum of tongue	68/8/37
		25 hours	Anterior site shows two linear lesions of 2 × 0,5 cm with broken epithelium surrounded by pale area with distinct boundary. Posterior site one small necrotic focus	72/8/37
		72 hours	Anterior lesions healing. Posterior lesions showing little tendency to enlarge	72/12/37
		6 days	Pseudomembranous area of 1 cm diameter at anterior inoculation site with development of clear vesicle up to 1 cm wide on periphery, raised 2 mm above epithelium. Posterior lesion now measuring 5 × 5 cm with necrotic centre. Irregularly shaped visicles on periphery. Small secondary vesicles appearing on the tongue	64/8/37
		7 days	Feet appear painful	
		8 days	Slight lameness.	
		9 days	Buccal lesions healing. Lameness not detectable	72/12/36,5
		12 days	Swelling of hind feet evident without detectable vesicles.	
		20 days	Ruptured vesicles between 1st and 2nd and 3rd and 4th toes of left fore foot. Indefinite vesicle-like swelling between 2nd and 3rd toe of right fore feet	80/8/37
		24 days	No further development of lesion on feet	64/11/36,5
	11 Age 3 years Sex female Condition fair	25 hours	Anterior site. Three distinct slightly raised linear necrotic scars surrounded by blanched area of 1 cm. Posterior site. Three focal lesions 2 cm in diameter, prominently raised above the epithelium and surrounded by blanched area .	64/16/37
		48 hours	Buccal discomforture, biting stanchions and frequently placing trunk in mouth without introducting food	60/15/37
		72 hours	Inoculation sites with areas of necrosis coalescing and enlarging to form central diphtheritic membrane and peripheral vesicles.	
		5 days	Alternately shifting mass on feet and leaning against wall, limb muscles trembling. Feet probed frequently with trunk.	

APPENDIX TABLE 1 (continued) Summary of observations recorded during examination of experimentally inoculated elephants during the acute stages of the disease

Dose of virus (Cattle ID 50/ml)	Elephant No.	Period after infection	Clinical observations	Pulse/min. Resp./min. Temp. °C
		6 days	Buccal lesions healing and covered by (Fig. 5) dark brown tissue on posterior dorsum of tongue 7 × 5 cm in area. One secondary vesicle ruptured. Feet very painful with reflex retraction when handled under narcosis. Feet warm on palpation with soft swellings on medial aspect of left fore foot (Fig. 7). Skin between majority of toe nails turgid with loss of elasticity	60/9/37
		7 days	Feet very painful.	
		8 days	All four feet generally swollen but lameness not marked. Marked loss in condition and mass.	
		9 days	Regeneration of epithelium and healing of primary and secondary vesicles in mouth. Lame. Right fore foot swollen over ¾ of circumference, withdraw 10 ml clear red brown fluid (Fig. 9). Entire perimenter of left fore foot 2,5 cm above sole swollen and flaccid. Left hind limb also swollen and fluid withdrawn. Discrete vesicles between individual toes of right hind foot . .	60/12/36,5
		11 days	Lameness continuing, mild diarrhoea.	
		12 days	Blanched vesicles appearing between toes of left fore and hind feet.	
		13 days	White protruding necrotic tissue lining perimeter of ruptured vesicles between toes	64/8/37
		15 days	Easier gait with noticeable loss of mass. Soles of right fore and left hind feet detached exposing yellow subcutis. Severing of skin over ruptured vesicles extending around ¾ of left and right fore feet. Pulse weak and irregular.	
		17 days	Linear scars of buccal lesions still visible with no necrotic tissue. Pulse slow and irregular. Detachment of soles from subcutis progressing further on both fore and left hind feet	60/13/37,5
		20 days	Buccal lesions still visible. Separated soles necrotic with suppurative process extending to remaining attached portions of sole	80/16/37
		22 days	Heart weak and sounds indistinct. Separation of soles continuing . . .	52/12/36,5