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Investigations of Allerton-type herpes virus infection in East African game animals and cattle

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SUMMARY

Neutralization tests with a strain (BA) of Allerton-type herpes virus, derived from a buffalo (*Syncerus caffer*) were carried out on 924 sera from 17 species of E. African game animals and on cattle sera from Tanzania (2001), Kenya (792) and Uganda (410).

Buffalo populations throughout E. Africa showed a very high rate of infection, with all animals over 2 years of age serologically positive. Antibody was present in some giraffe, waterbuck and hippopotamus sera and, less frequently, in impala, eland, bushbuck and oryx. Data are provided on the titres of positive samples; the mean titre of buffalo sera increased with age.

Cattle in many localities of N. Tanzania and S. Kenya showed a very high rate of infection, 85–95 % of sera from animals more than 2-years old containing antibody; the titres recorded were lower than those in buffaloes. Very high infection rates were also found in Karamoja and Teso (Uganda) and also in some other areas of Kenya, whilst a considerably lower incidence of infection was detected in W. Nile Province of Uganda and in central Tanzania. Differences in infection rates may have been related to herd size and husbandry practices.

It was shown that a wave of infection was probably spreading through cattle in N. Tanzania at about the same time as an outbreak of disease occurred in buffaloes and it is suggested that virus transmission may have been by biting flies.

No clinical signs attributable to the virus were reported in cattle but mouth lesions similar to those recorded in buffaloes, or nasal lesions, could have passed undetected. Allerton-type virus probably produces a range of clinical syndromes in cattle, closely resembling those associated with some herpes viruses in primates but infection is seldom related in the field to either pseudo-lumpy skin disease, mammillitis or stomatitis.

INTRODUCTION

In December 1969 an outbreak of disease, associated with an appreciable mortality, occurred in buffaloes (*Syncerus caffer* Sparrman) of the Serengeti National Park, Tanzania and in the contiguous Mara Game Reserve of Kenya. The disease affected predominantly young animals, 6–12 months of age, and was associated with the presence of well-defined areas of necrosis and ulceration in the

mucosa of the upper alimentary tract, including the tongue, oral cavity, oesophagus and rumen (Schiemann, Plowright & Jessett, to be published).

From a tongue ulcer in one yearling animal a virus (BA) was isolated in calf kidney and testis cultures which had the characteristic cytopathology of the Allerton-type of pseudo-lumpy skin disease virus (Alexander, Plowright & Haig, 1957). The quantity of virus recovered from diseased tongue tissue, $10^{4\cdot2}$ TCD 50/g., left little doubt that it was the cause of the lesion and diagnostic cytopathological changes were seen in stained sections of the same ulcerations. Neutralizing antibody to the BA virus was present in 11 of 13 buffalo sera collected during and immediately after the outbreak.

The present paper describes extensive serological investigations, designed to establish the prevalence of infection with Allerton-type virus in East African game animal and domestic ruminant populations. A future communication will describe the identification of the buffalo strain as closely related to or identical with the prototype South African Allerton virus and record observations on its pathogenicity for experimental cattle.

MATERIALS AND METHODS

Virus strains

The origin of the buffalo Allerton-type virus (BA) is described above (Schiemann *et al.*, to be published).

Propagation of virus

Virus was grown in monolayers of primary bovine kidney (BK) cells produced by methods already described (Plowright, Herniman & Rampton, 1969). Before inoculation with virus, cultures were washed twice with phosphate buffered saline (PBS of Dulbecco & Vogt, 1954) to remove bovine serum incorporated in the growth medium; the maintenance medium contained 2% unheated horse serum and was changed completely every 2 days. For stock virus preparation fluids were harvested when at least 80–90% of the cell sheet exhibited cytopathic effects; small volumes of clarified culture fluids were stored at $-70°$ C. and thawed rapidly at 37° C. when required.

Virus assay

Virus preparations were diluted in 1·0 or 0·5 $\log_{10}$ steps, using culture maintenance medium as a diluent. Each of a suitable range of dilutions was inoculated into five BK tube cultures in a dose of 0·2 ml. and the tubes were afterwards rotated in roller drums at $36\cdot5 \pm 0\cdot5°$ C. Microscopic examination for the typical syncytial foci produced by Allerton-type virus was carried out before medium changes and finally on the 5th and 6th days. Titres were calculated by the method of Spearmann–Kärber (Dougherty, 1964). The means and standard deviations for two virus stocks which were titrated 10 and 20 times were $10^{5\cdot78\pm0\cdot20}$ and $10^{5\cdot67\pm0\cdot15}$ respectively. The virus was stable over periods of at least 4 months at $-70°$ C.

Sera

Cattle sera from Tanzania and Kenya had been collected as part of a large-scale serological survey of rinderpest immunity, resulting from annual vaccination campaigns. Animals had been assigned to age groups on a basis of dentition, horn growth, body size and information provided by the owners, who were predominantly semi-nomadic pastoralists. Sera from small domestic ruminants were collected during a survey of rinderpest epizootiology in N. Tanzania.

Some game animal sera were obtained in the course of studies on the epizootiology of rinderpest, malignant catarrhal fever and African swine fever; others became available as the result of controlled game 'cropping' operations. Information on age has been included where adequate observations had been made on the dentition and other biometrical data; otherwise animals were simply regarded as of unknown age, although descriptions such as 'mature', 'immature' or 'young' may have been given.

Most of the sera were separated within 24 hr. of collection and stored, either immediately or after short periods on ice, at $-20°$ C.

Neutralizing antibody detection and titration

Sera were inactivated at 56° C. for 30 min. and mixed with an equal volume of virus diluted to give an estimated $10^{2·0}$ TCD 50 per 0·1 ml. The mixtures were placed overnight in the refrigerator (about 4° C.) and inoculated on the following morning, in a dose of 0·2 ml., into primary BK cultures. In screening tests three to five tubes were used per serum sample and the complete protection of one or more cultures by undiluted serum was regarded as evidence of past exposure to Allerton or a closely related virus. This deduction was justified by the fact that frankly negative sera, e.g. from 'insusceptible' species or from uninfected cattle herds, failed to suppress the cytopathic effects of the virus for longer than 2–3 days, whereas sera which had a marginal activity often showed complete protection at this time with virus 'breaking through' later. Occasionally sera which were assessed as positive on screening test were completely negative on titration, although the same stock virus at the same dilution had been employed; again a late virus breakthrough was characteristic of these sera.

Representative samples of positive bovine and game animal sera were titrated for neutralizing activity using four-fold serum dilutions in maintenance medium. The results were expressed as $\log_{10}$ SN 50 titres calculated after 5 days of incubation.

RESULTS

Neutralizing antibody to BA virus in East African buffaloes (Syncerus caffer)

Table 1 summarizes the results of screening tests for neutralizing antibody carried out on buffalo sera from N. Tanzania, S. Kenya and W. Uganda. Infection with BA virus was evidently extremely common in these widespread areas, there being only a few young animals in the yearling and 2-year age groups, which were devoid of antibody. In the case of the Serengeti National Park (SNP) it was clear

that infection had existed there since at least late 1967, although no disease episodes attributable to the virus were reported until December 1969. No disease which could be associated with BA virus has ever been reported from the Queen Elizabeth National Park (QENP), Uganda (M. H. Woodford, personal communication).

Table 1. *Neutralizing antibody to BA virus in E. African buffaloes* (Syncerus caffer)

	Serengeti: Tanzania				S. Kenya	W. Uganda (Q.E. Park)
Age group	x. 67	iii. 69 to viii. 69	xii. 69 to ii. 70	Total	1969 and 1970	i. 69 to viii. 69
⩽6 months	1/1*	—	3/3	4/4	1/2	1/1
7–18 months	3/3	—	6/6	9/9	0/1	1/3
2–4 years	2/2	3/3	0/2	5/7	0/1	5/5
5–7 years	5/5	5/5	4/4	14/14	—	8/8
⩾ 8 years	7/7	3/3	1/1	11/11	—	43/43
Unknown	—	1/1	—	1/1	3/3 (adults)	—
Totals	18/18	12/12	14/16	44/46	4/7	58/60

* The figures represent no. positive/no. tested.

Table 2. *The titre of neutralizing antibody in Serengeti buffalo sera*

Age group	No. titrated	Mean titre*	Standard deviation	Range
⩽ 6 months	4	1·35	0·58	0·6–1·9
7–18 months	9	1·25	0·57	0·6–2·2
2–4 years	5	1·34	0·50	0·6–1·8
5–7 years	14	1·47	0·26	1·2–1·8
⩽ 8 years	11	1·72	0·35	1·0–2·4
Totals	43	1·46	—	0·6–2·4

* Log_{10} SN 50.

Titrations were carried out on 43 positive buffalo sera from the SNP and the figures obtained are given in Table 2. Titres were, generally speaking, higher than in cattle (*vide infra*) and probably increased with age, as well as showing less variation; the high titres recorded in three of four animals about 6 months old ($10^{1·2}$ to $10^{1·9}$) were almost certainly due to active infection, as mouth lesions were still present in them and passively acquired antibody should have declined to negligible titres at this age (see Rweyemamu, Johnson & Laurillard, 1969, for cattle data).

Kenya buffaloes were found to possess antibodies in three widely separated areas, the titres varying between $10^{1·2}$ and $10^{1·4}$; young animals between 6 months and 2 years of age were devoid of neutralizing antibody in three of four cases.

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Neutralizing antibody to BA virus in other wild ungulates

The occurrence of neutralizing antibody in 811 serum samples from 16 species is summarized in Table 3; all sera were derived from areas where infection was shown to occur in cattle or buffaloes. It can be seen that infection with BA virus, or a closely related agent, probably occurs in giraffe (5/33 sera positive); waterbuck

Table 3. *Neutralizing antibody to buffalo Allerton virus in E. African game animals*

Region	Species (common name)	Scientific name	Date of collection	No. positive/ no. tested	Totals
N. Tanzania	Wildebeest	*Connochaetes taurinus*	1965–68	0/113	
Kenya	Wildebeest	*C. taurinus*	1969–70	2/49	2/162
N. Tanzania	Eland	*Taurotragus oryx*	1963–68	0/7	
Kenya	Eland	*T. oryx*	1969–70	1/12	1/19
N. Tanzania	Kongoni	*Alcelaphus buselaphus cokii*	1969	0/1	
Kenya	Kongoni	*A. buselaphus cokii*	1969–70	0/63	0/64
N. Tanzania	Giraffe	*Giraffa camelopardalis*	1969	3/7	
Kenya	Giraffe	*G. camelopardalis*	1969–70	2/26	5/33
N. Tanzania	Impala	*Aepyceros melampus*	1968–70	1/51	
Kenya	Impala	*A. melampus*	1969–70	1/16	2/67
N. Tanzania	Topi	*Damaliscus korrigum*	1969	0/1	
W. Uganda	Topi	*D. korrigum*	1970	0/20	0/21
N. Tanzania	Grant's gazelle	*Gazella granti*	1963–68	0/6	
Kenya	Grant's gazelle	*G. granti*	1969–70	0/14	0/20
N. Tanzania	Thompson's gazelle	*G. thompsonii*	1968	0/35	
Kenya	Thompson's gazelle	*G. thompsonii*	1969–70	0/70	0/105
S. Kenya	Oryx	*Oryx beisa callotis*	1969	2/2	2/3
S. Kenya	Bushbuck	*Tragelaphus scriptus*	1970	0/2	
W. Uganda	Bushbuck	*T. scriptus*	1970	1/6	1/8
S. Kenya	Waterbuck	*Kobus ellipsiprymnus*	1969	3/9	
W. Uganda	Waterbuck	*K. defassa*	1970	1/5	4/14
S. Kenya	Reedbuck	*Redunca redunca*	1969	0/3	
W. Uganda	Reedbuck	*R. redunca*	1970	0/11	0/14
W. Uganda	Uganda kob	*Adenota kob*	1962–70	0/22	0/22
W. Uganda	Hippopotamus	*Hippopotamus amphibius*	1963	20/199	20/199
N. Tanzania	Warthog	*Phacochoerus aethiopicus*	1969–70	0/24	
W. Uganda	Warthog	*P. aethiopicus*	1968–70	0/36	0/60

Table 4. *The distribution of positive sera in hippopotamuses from the Queen Elizabeth National Park, Uganda,* 1963

Age group (years)	No. of animals positive/ no. tested
1–4	1/13
7–8	0/15
10–13	5/43
15–20	1/25
21–25	3/24
28–38	9/78
Total	19/198*

* One positive animal included in Table 3 was of unknown age.

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(4/14 sera positive) and hippopotamuses (20/199 positive). There was some evidence for infrequent infection of eland with 1/19 positive; impala (2/67 positive); bushbuck (1/8 positive) and wildebeest (2/163 positive). Two of three oryx also possessed antibody.

The hippopotamuses had been accurately aged (Plowright, Laws & Rampton, 1964) and a breakdown of positive sera according to age groups is given in Table 4; there was no obvious correlation between age and the presence of neutralizing activity. The majority of positive sera were titrated and found to have SN50 end-points in the range $10^{0.5}$ to $10^{1.4}$. Giraffe had low titres, varying from a trace to $10^{0.9}$, waterbuck $10^{0.7}$ to $10^{1.3}$ and one impala $10^{1.5}$.

Neutralizing antibody to BA virus in Tanzanian cattle

Sera from 1098 cattle derived from four to twelve herds in each of six localities situated in the Northern and Lake Provinces of Tanzania (Fig. 1) were screened for the presence of neutralizing antibody to BA virus and the results are shown in Table 5. In July and November 1969 it was apparent that there had already been widespread infection of animals in the 0–6 months and 7–12 months age groups in

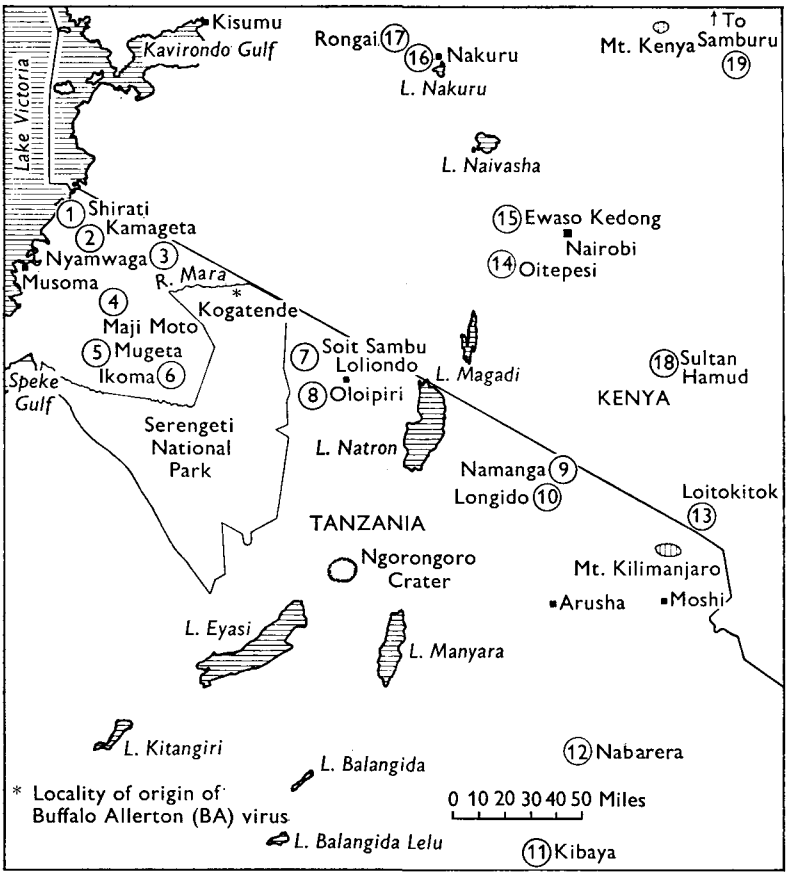

Fig. 1

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Table 5. *The distribution of antibody to BA virus in Tanzanian cattle*

Locality*	Date of collection	No. of sera positive/no. tested in age group: ⩽ 3 mo	4–6 mo	7–12 mo	13–18 mo	17–24 mo	25–42 mo	4–5 yr	⩾ 6 yr	Totals
Shirati (1)	15 xii. 69	0/1	4/17	4/19	3/6	2/2	6/8	2/2	5/5	26/60
Kamageta (2)	18 xii. 69	2/2	3/4	16/24	2/4	4/5	7/7	1/1	3/3	38/50
Nyamwaga (3)	22 xii. 69	1/12	1/5	8/21	4/9	1/1	4/4	2/2	3/4	24/58
Maji Moto (4)	13 xii. 69	1/4	3/7	20/26	23/27	4/4	18/18	2/2	25/25	96/113
Mugeta (5)	3 xxi. 69	5/8	0/15	1/23	3/22	3/9	16/23	4/5	27/28	59/133
Ikoma (6)	8 xii. 69	1/1	2/13	12/23	10/18	7/8	23/27	4/4	19/19	78/113
Totals		10/28	13/61	61/136	45/86	21/29	74/87	15/16	82/84	321/527
(Lake Province)		35·7%	21·3%	44·9%	52·3%	72·4%	85·1%	93·7%	97·6%	60·9%
Soit Sambu (7)	xi. 69	26/36		6/7	7/9	1/3	9/10	47/49		96/114
Oloipiri (8)	xi. 69	21/25		20/24	7/7	11/12	25/25	23/24		107/117
Namanga (9)	vii. 69	1/2		11/13	5/7	—	20/21	7/7		44/50
Longido (10)	vii. 69	3/4		17/30	1/3	6/7	8/10	10/11		45/65
Kibaya (11)	i. 70	13/19		12/21	9/11	2/5	9/9	46/50		91/115
Nabarera (12)	i. 70	5/8		8/10	11/15	6/7	19/20	46/50		95/110
Totals		69/94		74/105	40/52	26/34	90/95	179/191		478/571
(Northern Province)		73·4%		70·5%	76·9%	76·5%	94·7%	98·9%		83·7%
Kondoa: Singida	vi. 70	6/48		7/60	13/60			16/60		42/228
Kiomboi	vi. 70	4/18		6/59	5/32			18/39		33/148
Totals		10/66		13/119	18/92			34/99		75/376
(Central Province)		15·1%		10·9%	19·6%			34·3%		19·9%

* The numbers in brackets are those used in Fig. 1.

N.B. The cattle in localities (1) to (12) above were derived from the herds of 4 to 12 owners.

N. Province; thus more than 70% of these groups possessed antibody in localities 7–12 inclusive. That the antibody demonstrated was actively acquired is strongly suggested by the considerably lower incidence in cattle of comparable age groups from the Lake Province; thus only 26 and 45% were positive in the 0–6 months and 7–12 months groups, respectively. The infection rates in adult animals, 4 years or more old, was comparable in both Provinces (97 and 99% respectively) and hence the proportion of calves acquiring colostral antibody should have been similar.

Table 6. *The titre of neutralizing antibody to BA virus in the serum of Tanzanian cattle*

Age group	No. titrated	Range of titres*	Mean titre*
0– 6 mo	11	0·4–1·6	0·90
7–12 mo	13	0·5–1·8	0·77
13–18 mo	7	0·4–1·1	0·64
19–24 mo	14	0·5–1·2	0·69
25–42 mo	11	0·2–1·2	0·74
4–5 yr	8	0·4–1·2	0·64
⩾ 6 yr	27	0·4–1·8	0·89

* Log_{10} SN 50.

In the Lake Province there were clear indications that the infection during December had reached various stages of completion in different localities. Thus at Mugeta (5) only 4 of 60 and at Shirati (1) only 11 of 42 animals between the ages of 4 and 18 months possessed antibody; the corresponding figures for Kamageta (2) and Maji Moto (4) were 21 of 32 and 46 of 60 respectively. As the proportion of positive cattle in age groups of 2 years or more was consistently very high it appeared reasonable to assume that BA virus infection was passing through the younger cattle of this extensive Province during December 1969, at the same time as buffaloes in the Kogatende area (Fig. 1) were suffering from a mortality in which this agent was involved.

Sera collected in June 1970 in the Kondoa: Singida area, immediately to the south of the zone already described, showed a very much lower rate of infection with only 34% of positives in the ⩾ 25 months age group (Table 5). In August 1970 a further 527 from 2 to 3 years old cattle in the same area were examined and only 85 (16·1%) possessed antibody.

A total of 91 positive cattle sera from the Lake Province, collected during December 1969, were titrated for antibody to BA virus, the results being given in Table 6. It is probable that much of the antibody in calves up to 6 months of age, including six which were aged 3 months or less, was passively acquired; the mean titre was approximately the same as for older cows (⩾ 6 years), whereas intermediate groups showed a moderate decline. The higher titres in older cattle suggested that they may have resulted from frequent reinfection or perhaps chronic infection, although there is as yet no firm evidence for either of these suggestions. The pattern was similar to that in buffaloes (Table 2) but the mean titres recorded were consistently lower.

Neutralizing antibody in sheep and goats in Tanzania

Between December 1967 and February 1968 sera were collected from sheep and goats of various ages in the Loliondo District of N. Tanzania (Fig. 1).

There was only one positive sample among 44 sheep sera and one more among 148 goat sera; the titres of these were $10^{0.7}$ and $10^{1.1}$ respectively. If a comparison is made with cattle in the same district (localities 7 and 8, Table 5) it is evident that small domestic ruminants are not important hosts of Allerton-type virus, even when exposed to heavy infection. Capstick (1959) found that Allerton-type virus caused reactions to high titre when inoculated intradermally in these species and a Kenya strain induced local skin lesions on inoculation of sheep, followed by the development of neutralizing antibody (MacOwan, 1962).

Neutralizing antibody in cattle in Kenya and Uganda

In the Masailands (Rift Valley Province) of S. Kenya infection with Allerton-type virus was approximately as frequent as in the W. Province of Tanzania. Thus 85 % of cattle which were ⩾ 25 months of age possessed antibody, the proportion declining to 30 % in the first 6 months of life with evidence of active infection in the two intermediate groups (Table 7).

Table 7. *The distribution of antibody to BA virus in Kenyan cattle*

Locality*	Date of collection	No. positive/no. tested in age group				
		0–6 mo	7–12 mo	13–24 mo	⩾ 25 mo	
Loitokitok (13)	i. 70	0/6	5/16	4/13	7/11	16/46
Oitepesi (14)	i. 70	5/26	17/33	9/19	14/19	45/97
Ewaso Kedong (15)	i. 70	10/20	15/26	15/20	40/44	80/110
Sultan Hamud (18)	ii. 70	3/7	21/29	5/8	24/26	53/70
Total for Rift Valley Province		18/59 30·5%	58/104 55·8%	33/60 55·0%	85/100 85·0%	194/323 60·1%
Nakuru: Rongai (16:17) A†	vii./xi. 1969	— —	— —	48/70 68·6%	— —	48/70 68·6%
Nakuru: Rongai (16:17) B‡	vii. 69–viii. 70	— —	— —	0/289 —	— —	0/289 0·0%
Samburu (19)	vii. 70	16/32 50·0%	19/24 79·2%	10/14 71·4%	35/40 87·5%	80/110 72·7%

* The numbers in brackets are those used in Fig. 1.
† Sum of two positive groups bought from cattle dealer.
‡ Sum of eight negative groups bought from cattle dealer.

In the Samburu District of N. Kenya, cattle owned by a semi-nomadic pastoral people related to the Masai showed a very high rate of infection with 87·5 % of cattle positive in the age group ⩾ 25 months (Table 7).

In the central, highly developed, part of the Rift Valley Province of Kenya (Nakuru–Rongai in Fig. 1) the incidence of infection was estimated by the examination of sera from groups of experimental cattle, about 18–24 months old, which were collected by a dealer over a period of about 1 year. Eight of ten groups were

completely negative and the other two showed a high proportion of positives (69%), comparable to that in less-developed regions (see Table 7). It was not possible to trace the farms of origin of any of these cattle but the results were highly suggestive of an irregular distribution of virus.

In Uganda, relatively small numbers of samples from the northern provinces were examined. There was a considerably higher incidence of infection in the Karamoja and Teso districts, where infection rates were comparable to those in the western Lake Province of Tanzania (Table 8). In the W. Nile district, however, the infection rate was lower, not exceeding 43% and not apparently increasing materially after the age of 7–12 months.

Table 8. *The distribution of antibody to BA virus in Ugandan cattle*

	No. of sera positive/no. tested in age group				
Province	0–6 mo	7–12 mo	13–24 mo	⩾ 25 mo	Totals
Karamoja	—	1/1	26/38	100/111	126/149
	—	—	71·0%	83·0%	80·0%
Teso	4/4	7/10	7/10	105/128	123/152
		70·0%	70·0%	82·0%	80·9%
W. Nile	0/1	2/8	24/53	16/48	42/109
	—	25·0%	43·0%	32·0%	37·4%

DISCUSSION

The seriological results presented in this paper show that infection with Allerton-type herpes virus occurs regularly in all the E. African buffalo populations which were sampled; every animal estimated to be more than 2 years of age possessed neutralizing antibody and a large proportion had probably been infected by the age of 18 months. In spite of the presence of distinctive ulcerations in the mouth of sick buffaloes in the 1969 incident (Schiemann *et al.*, to be published) no other indications have been reported that the virus causes overt disease in buffaloes. This is particularly significant in the case of the population in the Queen Elizabeth National Park, Uganda, where the species was under close veterinary observation during the period when sera were being collected (M. H. Woodford, personal communication). It therefore seemed to be highly unlikely that the virus was a primary cause of the severe morbidity and mortality observed in 1969 in the Serengeti region of Tanzania (Schiemann *et al.*, to be published).

The occurrence of significant titres of neutralizing antibody in a smaller proportion of some other game animal species, exposed to more or less close contact with buffaloes or cattle, suggests that the same or a related virus may infect giraffe, oryx, waterbuck and the hippopotamus, possibly also infrequently eland, impala and wildebeest. It could be that some of these species are not susceptible to infection with Allerton virus but are commonly infected with similar herpes viruses, which only induce cross-neutralizing antibody in a few individuals.

There is no doubt that, among primates, several herpes viruses occur in Old World monkeys, including B virus (Keeble, Christofinis & Wood, 1958); SA8

(Malherbe & Harwin, 1958; Malherbe, Harwin & Ulrich, 1963); a cytomegalovirus (Black, Hartley & Rowe, 1963) and LV virus (Clarkson, Thorpe & McCarthy, 1967). In New World monkeys and marmosets another agent, herpes-T or marmoset herpes virus, has been shown to cause severe, generalized infection in some species but an inapparent or mild infection in others (Holmes, Devine, Nowkowski & Deinhardt, 1966; Daniel *et al.* 1967). It seems highly probable that many herpes viruses, sometimes closely related antigenically, will eventually be found in the richly varied ungulate fauna of Africa; one of these, the cause of malignant catarrhal fever in cattle, has already been shown to be universal in E. African wildebeest populations (Plowright, 1965; 1967) and neutralizing antibody to this or a closely related agent is also present in two other members (topi and kongoni) of the same family, the Alcelaphinae (Plowright, unpublished work).

The apparent limitation of lesions caused by BA virus to the upper alimentary tract of buffaloes (Schiemann *et al.*, to be published) is in distinct contrast to the original association of this virus in cattle in South Africa, Ruanda Urundi and Kenya with a mild form of 'lumpy skin disease' (Alexander *et al.* 1957; Huygelen, Thienpont & Vandervelden, 1960; MacOwan, 1962). Huygelen *et al.* (1960) did, however, mention the occurrence of erosions of the buccal mucosa and described the transmission of infection by scarification of the tongue mucosa of experimental cattle. Such lesions have not been observed by other investigators whether in Africa (Capstick, 1959; Weiss, 1963) or in Britain (Lepper, Haig & Wilcox, 1969; Martin *et al.* 1969).

The high rate of infection in E. African cattle was not entirely unexpected, since the examination of limited numbers of sera had already shown that infection with Allerton-type virus was not uncommon in Kenya and South Africa (Weiss, 1963; Martin & Gwynne, 1968). Figures provided by the latter authors were conservative in that sera were only screened at a dilution of 1/4, a procedure which would almost certainly have missed many animals with previous exposure to the virus. Their figures, like our own and those of Rweyemamu *et al.* 1969, show a definite tendency for antibody to increase in frequency with age. Nevertheless, it could hardly have been foreseen that about 85–95 % of cattle more than 2 years of age would show evidence of past infection in vast areas of E. Africa including the Masailands, Sukumaland (Lake Province, Tanzania), Samburu and Karamoja, and that the infection was never absent in any locality tested.

All the areas of higher incidence are characterized by big cattle populations, with semi-nomadic pastoralists owning large herds which are frequently brought to communal watering points and herded at night into crowded enclosures for protection against predators. All of them possess, incidentally, relatively large herds of buffaloes but there is no reason, at present, to suppose that this species is essential for the initiation or maintenance of infection, though they may undoubtedly contribute to the latter. The widespread inapparent infection of buffaloes probably means that Allerton-type virus has been present in this species for a very long time and is not a relatively recent introduction to Africa as suggested by Rweyemamu (1969).

Outside the areas of very high incidence, as in the Central Province of Tanzania

and the W. Nile District of Uganda, there was some evidence, as also in the Nakuru–Rongai area of Kenya, that the frequency of antibody varied considerably from herd to herd, some showing a moderate to high infection rate, others a very low or nil rate. This possibly reflects infrequent inter-herd contacts and a more static animal husbandry with smaller individual holdings.

The variable antibody rates in young cattle sampled during December 1969 in different localities of the Lake Province of Tanzania suggests that a wave of infection was passing through the area at that time and raises the question of the usual method of transmission of the virus. Weiss (1963) reported that, when animals infected with Allerton virus were kept in close contact with susceptible cattle, in an insect-proof stable, transmission did not occur even when attempts were made to favour virus transfer by handling susceptible animals after sick ones. In this laboratory we have also failed on several occasions to demonstrate contact infection of susceptible cattle which were housed together with excretor animals and drank from the same water-bowls. It seems possible that a flying vector is involved since Allerton virus was isolated from *Biomyia fasciata* caught on infected cattle and persisted for 6 days in flies which had fed on infected cell-culture fluids; transmission experiments using infected flies failed, however (du Toit & Weiss, 1960). No observations on possible vectors were made in the present instance but ticks and lice were observed in large numbers on sick buffaloes (Schiemann *et al.*, to be published). Rweyemamu *et al.* (1969) have suggested that biting flies may be involved in Britain in the transmission of bovine herpes mammillitis virus (BHMV), which is immunologically indistinguishable from Allerton virus (Martin, Hay, Crawford, le Bouvier & Crawford, 1966).

No clinical signs attributable to Allerton virus have been reported in E. African cattle in the areas of highest incidence. It remains to be determined whether the usual manifestation is a necrotic and ulcerative stomatitis, as described in sick buffaloes. In this connexion it is interesting to note that Lepper *et al.* (1969) and Martin *et al.* (1969) noted lesions of the rhinarium and nostrils, similar to those produced by us in experimental cattle with BA virus inoculated intravenously (Kalunda & Plowright, to be published). Such necrotic and eroded areas would not by themselves be expected to attract attention in most parts of E. Africa. There is no evidence that lesions comparable to those caused by BHMV in Britain occur in E. African cattle; Martin & Gwynne (1968) failed to detect such cases in Kenya and the vast majority of serological conversions occur before breeding age (Tables 5 and 7).

The cumulative evidence lends considerable support to the hypothesis, first put forward by Martin, Martin, Hay & Lauder (1966) that Allerton virus, *alias* BHMV, behaves epidemiologically in *Bovinae* like herpes simplex in man. Thus it is capable of causing a number of clinical syndromes, including generalized pseudo-lumpy skin disease, mammillitis and gangrene of the skin of the udder (Martin, Martin & Lauder, 1964; Rweyemamu, Johnson & Tutt, 1966; Derbyshire & Haig, 1969) and necrotic and ulcerative lesions of the upper alimentary and respiratory tracts. It has not, so far, been reported as causing genital lesions or systemic infections of the newborn but these are obvious possibilities. In the meantime, is there any real

justification for adopting different names for indistinguishable strains of virus derived from diverse clinical syndromes?

The demonstration of a primary association of some strains of Allerton virus with the oral cavity of cattle would provide a very close analogy with herpes simplex in man or with some of the simian viruses such as herpes B or T, both of which naturally cause ulcerative lesions of the mouth mucosae (Keeble *et al.* 1958; Daniel *et al.* 1967). Allerton virus and the primate agents mentioned all fall into the subgroup A of Melnick *et al.* (1964) characterized by ready release from cultured cells and they all produce large syncytia in monolayers. A surprising feature for a herpes virus would be the common involvement of an insect vector but contact transmission of herpes T virus from sick squirrel monkeys to marmosets has already been shown to occur with difficulty if at all (Daniel *et al.* 1967); perhaps biting flies would also be able to effect virus transmission in this case.

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