J. Hyg., Camb. (1982), **88**, 95
Printed in Great Britain

Observations on the epidemiology of lumpy skin disease in Kenya

By F. G. DAVIES
Veterinary Research Laboratory, P.O. Kabete, Kenya

(*Received* 5 *March* 1981)

SUMMARY

Lumpy skin disease virus strains isolated in Kenya over a period of some 20 years have proved to be serologically identical. They were indistinguishable by indirect fluorescent antibody and serum neutralization tests from the South African Neethling and West African serotypes. These two serological methods proved of value in studying the antibody responses to infection.

While epizootic spread of LSD has occurred in Kenya, most cases are of a sporadic nature and are thought to be the result of accidental contacts with a maintenance cycle. There is evidence of antibody to LSD in the African buffalo (*Syncerus caffer*) in those areas where LSD is considered to be enzootic in Kenya, and also in small numbers of domestic cattle. No buffalo or bovine sera contained antibody to cowpox virus. An area enzootic for LSD is proposed and it is suggested that the maintenance cycle involves the buffalo. No antibody was found in the other wild ruminant species examined.

INTRODUCTION

Lumpy skin disease (LSD) was first recognised in Kenya in 1957 (MacOwan, 1959) when it appeared as an epizootic. There was a relatively low morbidity however when compared with the epizootic in South Africa in 1944. Whereas in Kenya, the morbidity was 1–2% (Ayres Smith, 1960) it approached 90% on occasion in South Africa (von Backstrom, 1945; Diesel, 1949) and in the Sudan (Ali & Obeid, 1977). There may have been different vector species transmitting the disease in the different situations. Subsequently the disease has occurred in Kenya in a sporadic manner with occasional cases in certain parts of the country. The possibility of epizootic spread of the disease in 1968 may have been prevented by the use of a vaccine developed by Coakley & Capstick (1961).

Epizootics of LSD have been seen in South Africa in 1953–4, 1959, 1963, 1967, 1976–8, and more recently the disease has been recognized in several West African countries (Woods, 1974, OIE statistics, 1978), and in the Sudan in epizootic form in 1974 and 1980 (Ali & Obeid, 1977).

The Capripox viruses have a narrow host range compared with those in the Orthopox group. It was considered likely that the maintenance hosts for the virus in the interepizootic periods must be found amongst the wild or domestic ruminant populations. The behaviour of the disease in Kenya has led to a hypothesis that the virus persists in areas of high altitude indigenous forest. This study outlines

the basis for the hypothesis and attempts to define the maintenance hosts by examining sera for antibody to LSD virus from wild and domestic ruminants in these areas.

MATERIALS AND METHODS

Virus isolation

The virus was isolated from full skin thickness biopsies of the lesions of lumpy skin disease, following the methods described by Davies *et al.* (1971).

Virus strains

The LSD/2490 strain of lumpy skin disease virus isolated in Kenya has been shown by cross immunity and by serological methods to be identical with the Neethling strain from South Africa. The LSD/2490 strain has been used as the reference strain in this work. The CP-LB-P strain of cowpox virus was used in the fluorescent antibody and serum neutralization tests. The strain of Kenya sheep and goat pox virus KSGPV/240 (Davies, 1976), was used as the reference virus for comparison with LSD.

Preparation of immune sera

(*a*) *Rabbit antisera*

Antisera to LSD/2490 and KSGPV/240 were prepared by the weekly inoculation of rabbits with equal volumes of a fluorocarbon extract of bovine fetal muscle cell culture harvests emulsified with Freunds incomplete adjuvant, repeated 5–6 times. Then a final intravenous inoculation of 1 ml of the purified virus suspension was given and the rabbit bled out ten days later. Intradermal lesions were produced in rabbits with the cowpox virus, and these were hyperimmunized following the above schedule.

(*b*) *Cattle*

Sera were available from cattle used in the experiment described by Davies *et al.* (1971). Direct conjugates were prepared from these sera, specific to the 2490 strain of lumpy skin disease virus. A further two 18-month old Hereford cross steers were given multiple inoculations intradermally with 0·1 ml aliquots of a suspension of LSD/2490 strain containing $10^{5\cdot2}$ $TCID_{50}$ per 0·1 ml. Pre-inoculation sera were taken and then serial samples obtained for a 2 month period at the end of which the animals were slaughtered.

Cell cultures

The cell cultures used in the work were lamb testis and BHK21 C 13 cells, grown and maintained as described by Davies *et al.* (1971). A further cell culture extensively used was the bovine fetal muscle cell described by Davies & Atema (1978). This has been used for much of the serological work carried out with lumpy skin disease, Kenya sheep and goat pox and Orthopox viruses.

Serological methods

The microserum neutralization tests (MSNT) were carried out as described for the Kenya sheep and goat pox virus (KSGPV) by Davies & Atema (1978). This used the bovine fetal muscle cell seeded in microtitre plates, and all virus

titrations, antibody assays and strain comparisons were carried out in this system. The constant virus-varying serum dilution method was employed.

The indirect fluorescent antibody method (IFAT) was used following the principles described by Davies & Atema (1978). Flying coverslip preparations of the different virus strains were prepared in cell cultures and fixed when some 15–25 % of the cells contained the virus. This was determined by direct fluorescent staining at various times after inoculation. The virus strains were compared with rabbit immune sera to the LSD/2490 strain, cowpox and KSGPV/240 viruses.

Serum sampling methods

(1) Where possible, following the identification of a clinical case of lumpy skin disease, the remaining animals in the herd were bled for serum to estimate the level of challenge and presence of a subclinical component of the disease.

(2) To attempt to define the enzootic areas, sera were obtained from the FAO/Ken 70/522 project which had sampled most of the country in clusters of 25 cattle. Those from the postulated enzootic areas were examined.

(3) Sera from wild ruminant species were obtained from a wide variety of sources, notably the FAO/Ken 68/013 and CIDA wildlife project at the Veterinary Research Laboratory, Kabete. The Uganda samples were obtained from the East African Veterinary Research Organization and the Zambian samples from Dr Rottcher.

(4) Some sera were received at the laboratory from suspected clinical cases of LSD in Nigeria and Ghana.

(5) Some 200 cattle sera from areas of Kenya of similar altitude to the proposed enzootic areas, were also tested. They were from areas where LSD has not occurred.

RESULTS

Virus isolation

The sites at which clinical cases of lumpy skin disease occurred between 1968–80, and from which the virus has been isolated are shown in Fig. 1. The clustering of these cases around the large indigenous forest mass of the Mau Forest (indicated on the figure) is apparent. The distribution of the 1957–59 epizootic may be an extension from this zone. The sporadic occurrence of the disease is apparent from this map.

Comparisons of the different strains of lumpy skin disease

The Kenyan strains of virus together with some from Uganda isolated at the laboratory, have been compared with the LSD/2490 strain. The results are shown in Table 1. All strains of LSD isolated in Kenya and Uganda appear to be identical and indistinguishable from the KSGPV/240 strain.

The interrelationships of these strains were not quantitated by the fluorescent antibody method but all showed a similar intensity and distribution of fluorescence when stained with the LSD/2490 direct conjugate. There was however, a low titre cross fluorescence with cowpox. There was no cross neutralization of lumpy skin disease nor KSGPV viruses by the cowpox rabbit immune serum, nor of cowpox virus by LSD or KSGPV immune sera.

https://doi.org/10.1017/S002217240006993X Published online by Cambridge University Press

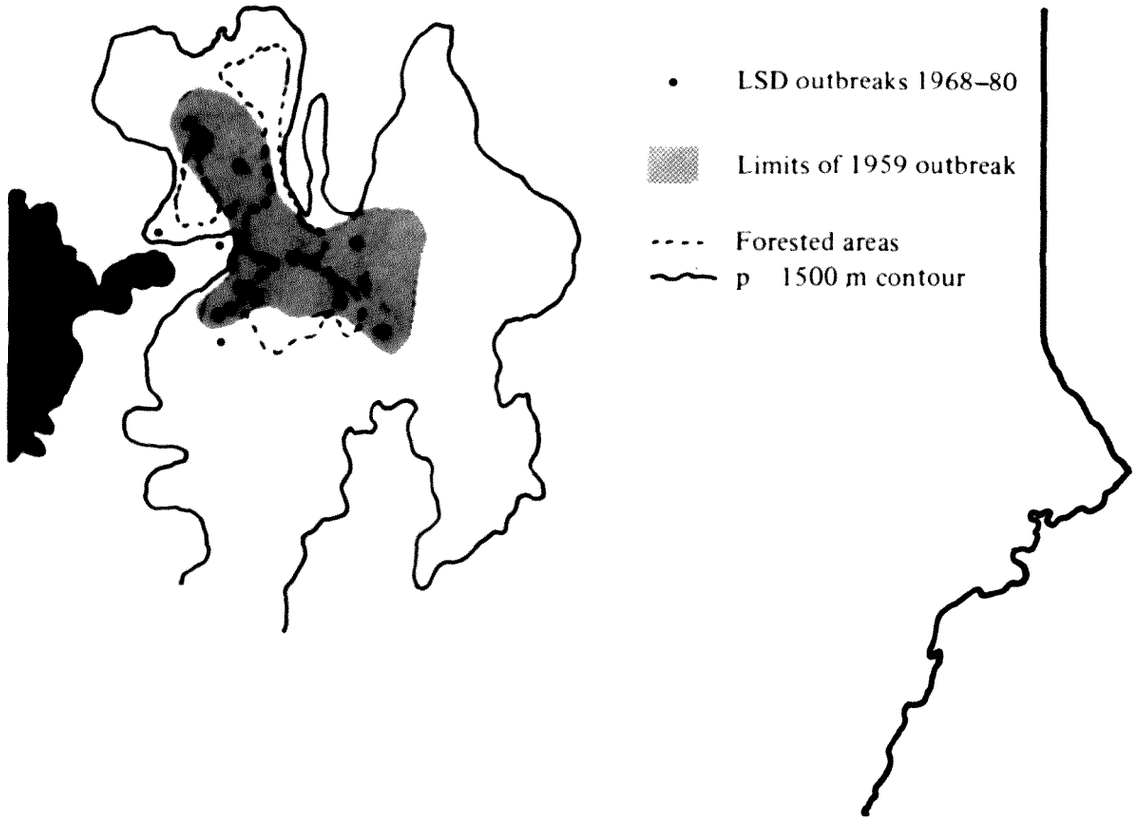

Fig. 1. A map of Kenya showing the areas affected in the 1957–9 outbreak of LSD and the subsequent sporadic cases. The outlines of the forested zone above the 1500 m contour are also shown.

Serological response to LSD virus

The serological responses of two cattle inoculated with LSD virus showed differences which were related to the number of skin lesions developing. One formed severe lesions at the 25 intradermal inoculation sites, while the other developed smaller lesions at 8. Peak titres on the MSNT were 4096 for the former and 128 for the latter; in the IFAT the figures were 1280 and 80 respectively. These had decreased by at least two dilutions at the time of slaughter some 10 weeks after inoculation.

The antibody titres in sera from cattle in Nigeria which had been infected with LSD were from 256 to 2048 against 100 $TCID_{50}$ of LSD/2490 virus. Similar titres were obtained with sera from Ghanaian cattle recovering from the disease. The West African strains appear to be serologically identical with LSD/2490.

Investigation of clinical cases of LSD

(*a*) Two clinical cases occurred in cattle kept on the floor of the Rift Valley near Lake Nakuru, the site of the original 1957 epizootic. The remainder of the herd were bled for serum and screened against LSD virus in the indirect fluorescent antibody test. None of the 37 cattle had any antibody to LSD detectable by this method. The two clinically affected animals were the only ones positive on this test.

Table 1. *A serological comparison of strains of LSD virus isolated in Kenya, with LSD, cowpox and Kenya sheep and goat pox hyperimmune rabbit sera, by microserum neutralization tests (MSNT) and fluorescent antibody test (FAT)*

	MSNT		Direct FAT	
Virus strain/year	LSD/2490	KSGPV/240	Cowpox	LSD/2490
2490/58	10*	10*	3*	8*
4/57	8	7	—	—
1581/59	8	8	—	—
5994/61	9	8	—	—
1538/58	7	8	—	—
4986/59	8	8	—	—
3906/59	10	9	—	—
Londiani/59	8	8	—	—
2968/68	10	10	—	—
2390/68	11	11	—	—
3238/68	7	7	—	—
2956/68	8	8	—	—
2773/68	10	10	—	—
3480/68	10	10	—	—
330/71	—	—	—	5†
762/71	—	—	—	5†
872/71	—	—	—	5†
632/74	—	—	—	5†
19/76	8	8	2	7
0–240/KSGPV	10	10	3	8
Cowpox CP LB-P strain	0	0	8	3
Neethling–LSD (South African)	9	8	2	8

* Geometric mean titres, $\log_2$ (assayed against 60–100 $TCID_{50}$ of virus in the MSNT).
† Bright specific fluoresence at this dilution, not titrated.
— Denotes not done.

(*b*) A single clinical case in an animal of Channel Island breed occurred in the middle of a tea estate cut out of the Mau Forest. There were very few cattle kept within five miles of this case, these were bled for serum and tested by IFAT and MSNT. Two others were found which were IFA and MSNT positive; the titres on neutralization were low, 27 and 81, but indicate some previous challenge with LSD virus.

Attempts to discover a vertebrate maintenance host for LSD virus

Wild ruminant sera were obtained from areas covered by the 1957–9 Kenya epizootic. Eight common species were examined for antibody to capripox virus by the IFAT. Only African buffalo (*Syncerus caffer*) proved positive, some 150/254 being positive at the 1/20 screening dilution. To examine this further and to eliminate the possibility that the antibody detected may be to a virus related to cowpox, these sera were tested by MSNT against both LSD/2490 and cowpox CP-LB-P. The results are shown in Table 2. Three groups of buffalo sera contained a significant number which neutralized the LSD/2490 strain of virus. There was no neutralization of cowpox virus by any of these positive sera, which

https://doi.org/10.1017/S002217240006993X Published online by Cambridge University Press

Table 2. *The neutralization of LSD and cowpox virus by buffalo sera collected in different parts of East Africa*

Origin of buffalo	LSD epizootic area	Virus: Cowpox	Virus: LSD 0	LSD 1/9*	LSD 1/27	LSD 1/81	LSD 1/243
Mara, Kenya	Probable	0	4	3†	5	5	2
Rift Valley, Kenya	Epizootic	0	4	2	7	3	1
Kabete, Kenya	Non epizootic	0	2	—	—	—	—
Timau, Kenya	Non epizootic	0	2	—	—	—	—
Galana, Kenya	Non epizootic	0	3	1	—	—	—
Kiboko, Kenya	Non epizootic	0	2	—	—	—	—
Rumuruti, Kenya	Non epizootic	0	21	—	—	—	—
Zambia	Unknown	0	32	—	—	—	—
Queen Elizabeth Park, Uganda	Probable	0	15	13	13	11	6

* Titre of serum suppressing 100 $TCID_{50}$ of cowpox or lumpy skin disease viruses, assayed in a microtitre system.

† Number of sera with this titre.

Table 3. *Antibody to LSD virus in sera from cattle living in those areas postulated as enzootic for LSD in Kenya*

District	Altitude (ft)	Buffalo present	Forest edge	No positive/ tested
Kericho	6050	Close	Yes and riverine	3/25*
Kericho	6200	No	No	0/25
Nandi	5900	No	Yes and riverine	22/25
Kericho	6400	Yes	Yes	14/21
Kericho	6000	Yes	Yes	6/25
Kericho	7200	Occasional	Yes	3/25
Kericho	7400	Yes	Yes	5/25
Koyet	6500	Yes	Yes	4/25
Kericho	7100	Yes	Yes	8/25
Kericho	6700	No	No	0/25
Kericho	6100	No	Riverine	1/25
Kericho	6300	No	No	4/25
Kericho	6000	No	No	1/25
Kericho	5800	No	No	1/25
Kericho	6000	No	No	1/25
Nanyuki (non LSD area)	6300	Yes	Yes	0/200

* Indirect fluorescent antibody test, $\frac{1}{40}$ dilution of serum.

increases the likelihood that the neutralization of LSD is due to specific antibody, and not due to non specific neutralizing properties of the sera.

Domestic cattle populations

Clusters of cattle kept adjacent to the Mau Forest were examined by the IFA test at 1/20 dilutions of sera. The results of these tests are shown in Table 3 which also shows the relative proximity of buffalo to the cattle grazing areas. All sites

were close to the Mau Forest and were in those areas where the sporadic cases of LSD have occurred since 1959. There are some positive to the LSD antigen on IFA tests which may indicate either that clinical disease occurred and was not recognized or that there was subclinical disease. Unfortunately it was not possible to test these sera by the MSNT, for they had been treated with sodium azide and were toxic for cell cultures.

Cattle sera from non enzootic areas

Two hundred cattle sera were obtained from farms at a similar altitude near Mount Kenya where LSD has not been clinically identified. None contained antibody to Capripox virus when screened by the IFA test.

DISCUSSION

These results support a conclusion that the virus of LSD is maintained in forest or at forest edge situations in Kenya in a fairly high rainfall zone at 1000–2500 m. The vertebrate hosts which are found in these areas and which might support this Capripox virus are the wild buffalo and domestic cattle populations. Buffalo living in or close to the postulated enzootic areas were found to contain both fluorescent and neutralizing antibody to Capripox virus in their sera. There was also evidence to suggest that there might be subclinical infections of cattle kept adjacent to the forest areas. The sporadic clinical cases which occur in most years are thought to be accidental involvement in the maintenance cycle, in which it might not be necessary for any arthropod vector to be involved.

Epizootic spread of LSD has occurred in Kenya and elsewhere with considerable economic losses. The circumstantial evidence has led to conclusions that an arthropod vector must be involved in the rapid spread of the disease (Diesel, 1949; MacOwan, 1959). It has not proved possible to study this aspect of the problem. The strains of LSD isolated during the first Kenyan outbreak and in subsequent years have proved to be serologically identical. No differences were detected between these and the South African Neethling, or the West African strains. All have some relationship with cowpox virus which can be demonstrated by direct and indirect FAT but not by MSNT. These tests which have been used for the KSGPV have also proved suitable for studies with LSD. The two viruses are indistinguishable from one another serologically.

This paper is published by kind permission of the Director of Veterinary Services, Kenya.

REFERENCES

Ali, B. H. & Obeid, H. M. (1977). Investigation of the first outbreak of lumpy skin disease in the Sudan. *British Veterinary Journal* **133**, 184–189.

Ayres-Smith, R. A. (1960). The symptoms and clinical diagnosis of lumpy skin diease. *Veterinary Record* **72**, 469–472.

Coakley, W. & Capstick, P. B. (1961). Protection of cattle against lumpy skin disease, factors affecting small scale production of a tissue culture propagated virus vaccine. *Research in Veterinary Science* **2**, 369–371.

DAVIES, F. G., KRAUSS, H., LUND, L. J. & TAYLOR, M. (1971). The laboratory diagnosis of lumpy skin disease. *Research in Veterinary Science* **12**, 123–127.

DAVIES, F. G. (1976). Characteristics of a pox virus causing a pox disease of sheep and goats in Kenya with observations on the epidemiology and control. *Journal of Hygiene* **76**, 163–171.

DAVIES, F. G. & ATEMA, C. (1978). The antibody response in sheep to infection with a Kenyan sheep and goat pox virus. *Journal of Comparative Pathology* **88**, 205–210.

DIESEL, A. M. (1949). The epizootiology of lumpy skin disease in South Africa. Proceedings of the 14th International Veterinary Congress London, vol. 2, pp. 492–500.

MACOWAN, K. D. S. (1959). Observations on the epizootiology of lumpy skin disease in Kenya during the first year of its occurrence in Kenya. *Bulletin of Epizootic Disease of Africa* **7**, 7–20.

OFFICE INTERNATIONAL DES EPIZOOTICS (1959–78). Statistiques.

VON BACKSTROM, U. (1945). Ngamiland cattle disease, a preliminary report on a new disease, the aetiological agent being probably of an infectious nature. *Journal of the South African Veterinary Medical Association* **16**, 29–35.

WOODS, J. A. (1974). A skin condition of cattle. *Veterinary Record* **95**, 326.