An investigation of possible routes of transmission of lumpy skin disease virus (Neethling)

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(Accepted 3 October 1994)

SUMMARY

British cattle were infected with the South African (Neethling) strain of lumpy skin disease virus (LSDV) and their clinical signs monitored over a 3-week period. Different routes of infection were assessed for effect on the clinical characteristics of the disease by using a clinical scoring system. Neither of 2 animals inoculated onto the conjunctival sac showed clinical signs or seroconverted. The intradermal route produced local lesions in 21 of 25 animals, and generalized infection in 4. In contrast the intravenous route produced generalized lesions in 8 of 11 animals. Seven uninfected animals were housed in contact with infected animals for 1 month. None developed clinical signs or produced detectable serum neutralizing antibodies. Six of seven of these animals were then challenged and were fully susceptible to infection. The results suggest that the transmission of LSDV between animals by contagion is extremely inefficient, and that parenteral inoculation of virus is required to establish infection. The high proportion of animals with generalized disease following intravenous inoculation implies that naturally occurring cases of generalized LSD may follow spread by intravenously feeding arthropods.

INTRODUCTION

Lumpy skin disease (LSD) is a malignant pox disease of cattle caused by strains of capripoxvirus. The disease was first recognized in Northern Rhodesia (Zambia) in 1929 [1], and is now endemic in most of sub-Saharan Africa, parts of North Africa and has been reported from the Middle East [2]. Live attenuated vaccines are available for control of the disease [3], but, due to problems with local reactions following vaccination, and difficulties with vaccine supply, owners have been either unable or reluctant to vaccinate and many animals remain unprotected. LSD is the cause of major economic loss through decreased milk yield, poor growth, hide damage and infertility [4]. The characteristics of the disease, and its varied clinical manifestations, have been described previously [2, 4–6] but information about the transmission and pathogenesis of the disease is lacking. Epidemiological evidence suggests a strong association between outbreaks of disease and the wet season and the presence of numerous biting arthropods [4, 7–11].

There is no experimental or epidemiological evidence to suggest biological transmission of poxviruses by arthropod vectors, but mechanical transmission of the viruses of fowl pox [12], myxomatosis [13] and swine pox [14] has been shown. Squirrel pox virus and Shope fibroma virus [15] have been transmitted experimentally by mosquitoes, and mosquitoes are implicated in the transmission of Tana pox virus [14]. Experimentally, capripoxvirus has been transmitted between goats by *Stomoxys calcitrans* (stable fly) [16], although, in contrast to LSD virus (LSDV), sheep and goat pox virus are spread predominantly through contact [17]. No spread of LSDV between cattle housed in contact in the absence of arthropods has been reported [7, 18], although saliva and shared water troughs have been implicated in transmission under the same conditions [19].

This paper describes the attempted transmission of LSDV from infected to susceptible cattle housed in contact, in order to establish the potential for LSDV to spread in the absence of arthropods. Arthropod transmission of LSDV was investigated by inoculating susceptible cattle by three routes consistent with mechanical transmission by arthropods: onto the conjunctival sac, intradermally and intravenously.

MATERIALS AND METHODS

Virus strains

A virulent South African Neethling strain of capripoxvirus, originally recovered from a cow with LSD and then passaged in cattle at the Institute for Animal Health (IAH), Pirbright [20], was used to inoculate experimental animals. Primary lamb testis cells, prepared from prepubertal lambs [21] were cultured in 175 cm² tissue culture flasks in Glasgow modified Eagle's medium supplemented with glutamine (GMEM) and 5% foetal calf serum. Lacrimal fluid from a heifer infected with LSDV, or skin biopsy material as a 10% suspension in phosphate buffered saline (PBS) was used to infect a 90% confluent cell culture. After 1 h at 37 °C the cell culture was washed with PBS and overlaid with GMEM. Virus was harvested when the characteristic cytopathic effect [21] was seen in 90% of the cells. The flask and contents were frozen at -20 °C, thawed, and the cell debris pelleted at 500 g for 20 min. The supernatant was removed, titrated as described below, and used to inoculate experimental animals.

Virus titration

Lamb testis cells were added to all wells of microtitre plates (50 µl GMEM with 6×10^6 cells/ml). Fifty µl of decimal dilutions (10^{-1} – 10^{-7}) of the virus suspension were used to infect rows A–G of the lamb testis cells. Fifty µl of GMEM was added to row H, the cell controls. Plates were examined for cytopathic effect on day nine [21]. The virus titre was then calculated from the number of wells infected on day nine [22].

Virus neutralization tests

Virus neutralization tests were carried out using a constant serum varying virus method [23], following the protocol described by Carn and colleagues [24]. Briefly, inactivated serum samples, diluted 1/5 (v:v) in GMEM were added to all rows in

Table 1. *Scale of clinical response following infection of cattle with lumpy skin disease virus (Neethling)*

Reaction score	Severity of clinical response
10	Severe generalization leading to culling. Numerous secondary nodules, 0.5–5 cm diameter, with oedema, hyperaemia and pain. Severe lymphadenopathy, conjunctivitis, rhinitis, severe debility and inappetence.
9	Severe generalization with depression. Numerous secondary nodules, severe lymphadenopathy, conjunctivitis and rhinitis.
8	Generalization with many secondary lesions and severe lymphadenopathy. No systemic disturbance.
7	Generalization with few secondary nodules, severe lymphadenopathy, no systemic disturbance.
6	Severe local reaction at inoculation site: heat, pain, oedema, lesion > 6 cm diameter. Severe lymphadenopathy.
5	Severe local reaction: heat, pain, oedema, lesion > 6 cm diameter. Prescapular lymph node twice normal size.
4	Local reaction moderately severe: > 6 cm diameter, some heat, pain and oedema. Mild lymphadenopathy.
3	Mild local reaction (< 6 cm diameter) and lymphadenopathy.
2	Mild local reaction: < 5 cm diameter. No lymphadenopathy.
1	Transient local reaction.
0	No detectable reaction.

duplicate columns on microtitre plates. Fifty μ l of decimal dilutions (10^{-1} – 10^{-7}) of virus suspension were added to rows A–G. Fifty μ l of GMEM was added to row H, the cell controls, and the plates incubated at 37° for 1 h. Lamb testis cells were then added to each well (50 μ l GMEM with 6×10^6 cells/ml). The cells were examined on day 9 for cytopathic effect and the titre of virus in the presence of test serum calculated [22]. Neutralization indices were expressed as the $\log_{10}$ difference between the calculated virus titre for the day 0 serum samples (pre-infection) and the test serum samples for each animal [23].

Experimental animals

Friesian-cross cattle were kept in the high security facilities at IAH, Pirbright. Twenty-five animals were inoculated intradermally using 0.5 inch 25 gauge needles, into the clipped skin on the neck, 20–25 cm cranial to the scapula. Eleven animals were inoculated intravenously via the left jugular vein. Each animal received between 10^2 and $10^{6.7}$ TCID₅₀ of LSDV (Table 2). Two animals were inoculated with 0.2 ml tissue culture supernatant, containing 10^3 TCID₅₀ of LSDV, by instillation onto the conjunctiva of the right eye.

In 7 separate experiments 1 susceptible animal was housed in contact with 2 animals which had been inoculated intradermally with LSDV. Observations were made of clinical signs in all 3 animals in each of the 7 experiments. In 6 of the 7 experiments the animal in contact was inoculated intradermally with $> 1 \times 10^4$ TCID₅₀ LSDV, into the skin of the neck, 28 days after the start of the experiment. Susceptibility to LSD was measured by examination of regional lymph nodes, and of the inoculation site for a delayed type hypersensitivity response (DTH), which in immune animals can be detected as an indurated swelling at the site between 24–48 h post inoculation (PI) [25].

The body temperature of each animal was recorded daily. Animals were examined regularly for clinical signs, and observation of changes at the inoculation sites were recorded including size of lesion, degree of hyperaemia, pain and oedema. The 7 animals in contact were not restrained and did not undergo a full clinical examination for the first 28 days of the experiment. Blood samples were collected by jugular venepuncture at the beginning of each experiment, and at varying intervals thereafter, with the exception of those animals placed in contact with infected animals, which were not bled during the first 28 days of the experiment.

Scoring of the clinical reaction

A clinical reaction score was calculated for each animal following a detailed clinical examination (Table 1). Scores of 7–10 indicated generalized disease of varying severity, and scores of 1–6 the severity of the local reaction at the inoculation site and associated lymphadenopathy.

RESULTS

In 2 of the 7 contact experiments both the animals inoculated with LSDV at the beginning of the experiment showed generalized lesions; in one experiment one of these animals showed generalized lesions, and in the other four experiments none of the infected cattle developed generalized lesions although at least one animal in each group had a severe local lesion and clinical lymphadenopathy. None of the seven animals housed in contact with the animals described above showed any clinical signs of disease, nor produced a detectable serum neutralizing antibody response. Six of the 7 in contact animals showed no delayed type hypersensitivity reaction to intradermal challenge at 28 days, and were fully susceptible to subsequent challenge.

Neither of the two animals inoculated onto the right conjunctival sac developed clinical signs of LSD, pyrexia, or produced detectable virus neutralizing antibody by day 20 pi.

Twenty-one of the 25 animals inoculated intradermally had local reactions with clinical scores of 2–6. The remaining four animals developing generalized clinical signs (clinical reaction scores of 9 and 10). The results are summarized in Table 2.

Eight of the 11 animals inoculated intravenously had a reaction score of seven or more. The remainder had reaction scores of five and six (Table 2).

Statistical Analysis

Fisher's Exact Test was carried out on a 2×2 table of numbers of generalized cases following intravenous (8/11) or intradermal (4/25) inoculation. The significance of the differences was shown by the two tailed P value (0.0018) and an odds ratio of 14.

DISCUSSION

The hypothesis that the transmission of LSDV is inefficient in the absence of arthropods is in agreement with the experimental results of Alexander [7] and Weiss [18], who both reported lack of spread between animals housed together in

Table 2. *Clinical reaction score of cattle inoculated intradermally or intravenously with different doses of lumpy skin disease virus (Neethling)*

Intradermal inoculation			Intravenous inoculation		
No. of animals	Dose TCID ₅₀	Reaction score	No. of animals	Dose TCID ₅₀	Reaction score
2	2.0	9	1	3.3	8
2	4.3	4	1	3.3	10
1	4.6	2	1	4.3	5
1	4.6	3	1	4.3	6
3	4.6	4	1	4.3	7
1	4.6	5	2	4.3	8
1	4.7	5	1	4.3	9
2	4.7	6	1	5.3	9
1	5.0	4	1	5.3	8
1	5.0	5	1	6.0	5
1	6.1	4			
1	6.1	10			
6	6.3	6			
1	6.7	6			
1	6.7	9			

the absence of arthropods. It is also consistent with reports from the field, where outbreaks in the absence of significant populations of biting flies have remained contained [26], and disease has diminished with the onset of the dry season and the reduction in the number of biting flies [10, 11].

Previous observations that LSDV does not spread by contact are supported by the fact that despite close contact between the inoculated and uninoculated animals, who shared feeding and watering utensils and had the opportunity for mutual grooming, there was no transmission of LSDV in the experiments described above. None of the in contact animals either seroconverted or developed a DTH reaction to challenge. They remained fully susceptible and developed typical post-inoculation lesions, and some peripheral lymph node enlargement, within 7 days. Animals infected with LSDV within the previous 28 days produce a DTH reaction within 24 h of intradermal inoculation of LSDV, and susceptible animals have no local lesion until at least 3 days following challenge [25].

Muscid flies feed on ocular secretions, and *Musca sorbens* and *M. biseta* are known vectors of ophthalmic diseases [27], probably due to their sponge-like feeding apparatus, feeding habits and frequent vomiting during feeding. However, Du Toit and Weiss [28] did not isolate virus from various *Musca* spp. after feeding on LSDV infected cattle. Conjunctival instillation of LSDV failed to elicit a detectable neutralizing antibody response in the two animals inoculated in this experiment.

In contrast to both direct contact and conjunctival inoculation, intradermal inoculation invariably produced infection, although only 1 in 6 animals developed generalized disease. Experimental transmission of capripoxvirus between sheep has been shown using *Stomoxys calcitrans* [16], and virus has been isolated from *Hydrotea irritans* fed on infected sheep [16], and from *Biomyia fasciata* fed on infected blood [28]. *Stomoxys calcitrans* has been implicated in the first LSD

outbreak in Israel in 1993, when morbidity was low (10%), and there were no deaths [26]. Large biting Diptera have been implicated in the mechanical transmission of Rift Valley fever virus (*Glossina* spp.), Myxoma virus (Tabanidae), equine infectious anaemia virus (Tabanidae) and African swine fever virus (*Stomoxys calcitrans*) [29], amongst others. These flies excoriate the epidermis and feed on the blood pool that develops. Virus transmitted mechanically by these species is therefore deposited intradermally. Field evidence supports the hypothesis that these species are associated with LSD outbreaks where the proportion of animals affected with generalized disease, and accompanying mortality, are low [26].

While intradermal inoculation of virus appears to be associated with a local lesion, and a low likelihood of generalized disease, the intravenous route is highly likely to produce generalized disease. Fenner and colleagues [13] reported that, in myxomatosis, rabbit with generalized lesions were the most important in the transmission of the disease. Rao and colleagues [30] concluded that the risk of the spread of smallpox virus was greater when there was generalized disease, and Kitching and Taylor [17] similarly implicated animals with secondary lesions as the predominant source of infectious virus in the spread of capripoxvirus to other sheep and goats. Generalized capripox and LSD produce multiple lesions, which are particularly attractive to feeding flies, and in which high titres of virus (10^4 – 10^6 TCID₅₀/gm) may be present. Cattle with generalized lesions can therefore be assumed to be the most important with respect to the transmission of LSD. As the intravenous route of infection with LSDV predisposes to generalization it can be postulated that intra-venously feeding insects are most likely to be associated with outbreaks of LSD characterized by severe clinical disease and hence rapid spread. Mosquitoes and sandflies feed intravenously, and are both invariably found in large numbers during the wet season in the areas of endemic LSD. The potential for high mortality following iatrogenic intravenous inoculation was demonstrated in South Africa during 1945, following the intravenous use of an anaplasmosis vaccine derived from the blood of donor cattle which were subsequently found to be infected with LSDV [4]. Of the cattle on the farms that were issued with vaccine, and on which deaths occurred, 39% of inoculated cattle became infected and 23% of these died.

That LSDV is transmitted very inefficiently in the absence of an arthropod vector but causes severe disease when inoculated intravenously has implications for control. Historically, it has been noted that livestock movement restrictions were inadequate to control disease [19], whereas in other outbreaks spread was limited [26]. If an intravenously-feeding insect vector is required to produce generalized disease, it follows that control of these vectors, in the absence of vaccination, is a priority for reducing the consequences of an outbreak. This work describes experimental studies on routes of infection and implicates certain species of arthropod in the epidemiology of LSD. The results are consistent with field observations that there are large variations in morbidity and mortality in naturally occurring cases of LSD, and suggest that the important determinants for transmission of LSD are the presence of intravenous feeding arthropod vectors, which predispose to generalized infection and increase the opportunity for further transmission of the virus by arthropod vectors.

ACKNOWLEDGMENTS

VMC is supported by a grant from the Overseas Development Administration, 94 Victoria Street, London SW1E 5JL.

The authors would like to thank L. Hoinville for assistance with the statistical analysis.

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