Lumpy skin disease in cattle in central Ethiopia: outbreak investigation and isolation and molecular detection of lumpy skin disease virus

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Summary

The study was a combination of two investigations into active outbreaks of lumpy skin disease (LSD) in cattle in central Ethiopia and a retrospective analysis of outbreak reports between January 2007 and December 2011 covering the entire country. Active outbreaks were investigated in four districts of central Ethiopia: Adama, Wenji, Mojo and Welenchiti. A semi-structured questionnaire was used to acquire data at individual and herd levels, and tissue samples were collected for viral isolation and characterisation. The retrospective analyses showed that, during the five-year period, a total of 1,675
outbreaks were reported, with 62,176 cases and 4,372 deaths. The highest numbers of outbreaks were reported in Oromia (1,066), followed by Amhara (365) and the Southern Nations, Nationalities and People’s Region (123). Outbreaks were more frequently observed between September and December and the highest number of outbreaks was reported in 2010. During the period studied, a total of 2,174 local zebu cattle were clinically examined and morbidity and mortality rates of 13.61% (296) and 4.97% (108) were recorded, respectively. Analysis of the active outbreaks revealed a relatively consistent morbidity rate, with the highest observed in Adama (15.38%), followed by Wenji (10.26%). The highest mortality rates were also observed in Adama (5.89%) and Wenji (3.42%). The LSD virus was isolated from 22 samples and all tested positive in polymerase chain reaction analysis. The disease was observed in the cattle regardless of previous vaccination with Kenyan sheep- and goat-pox vaccine; thus, vaccine efficacy was assessed under field conditions and the authors’ findings, together with a possible remedy, are presented in this paper.

Keywords

Introduction

Lumpy skin disease (LSD) is a pox disease of cattle and is characterised by fever, nodules on the skin, lesions in the mouth, pharynx and respiratory tract, emaciation, enlarged lymph nodes, oedema of the skin, and sometimes death (1, 2, 3). The disease is one of the most important viral diseases of cattle, causing loss of condition in infected animals and permanent damage to hides. The most effective route of transmission is mechanical via biting flies. The incidence of LSD is high during wet seasons when populations of the flies are abundant; the incidence decreases or ceases during the dry season (4, 5, 6).

Lumpy skin disease has a different geographical distribution from that of sheep- and goat-pox, suggesting that cattle strains of capripoxvirus
do not infect or transmit between sheep and goats (3, 7). The disease was first observed in 1929 in northern Rhodesia (currently Zambia) and rapidly spread north and south. It now occurs in most of Africa (except Libya, Algeria, Morocco and Tunisia) and much of the Middle East (8).

The World Organisation for Animal Health (OIE) categorises LSD as notifiable because of the substantial economic impact of an outbreak. The disease is more severe in cows at peak lactation and causes a sharp drop in milk yield, often leading to secondary bacterial mastitis. Temporary or permanent infertility may occur in cows and bulls. The emaciation of infected animals and a convalescence period lasting for several months causes a decreased growth rate in beef cattle (8, 9). The morbidity and mortality of the disease vary considerably, depending on the breed and immunological status of the cattle population and the insect vectors involved in transmission, but morbidity rates are generally between 1% and 20%. In a few outbreaks morbidity has been reported as more than 50%, although the mortality rates were usually less than 10%. The abortion rate in pregnant cows may range from 1% to 7% (10, 11).

In Ethiopia, LSD was first observed in 1983 in the north-western part of the country (south-west of Lake Tana) (12). The disease has now spread to almost all regions and agro-ecological zones of the country. Because of the wide distribution of the disease and the size and structure of the cattle population in Ethiopia, it is likely that LSD is one of the most economically important livestock diseases in the country (4, 5, 6).

The control of LSD can be achieved through vaccination, restriction of animal movement and eradication of infected and exposed animals. However, this requires adequate financial, infrastructural and human resources, and information systems. Under the prevailing conditions in Ethiopia it has not been possible to implement all these strategies and thus vaccination has been adopted as the most important practical approach to LSD control for many years. The Kenyan sheep- and goat-pox vaccine strain KS-1 has been in use because of its advantage
of conferring cross-protection to LSD, in accordance with OIE recommendations (13). However, reports from field veterinarians of LSD in vaccinated animals have been increasing during the past few years (field feedback report of the Ethiopian National Veterinary Institute [NVI]). The present study was therefore undertaken to investigate outbreaks of LSD, using both active disease follow-ups and analysis of retrospective data. In addition, the level of protection provided by the currently used vaccine was assessed in field conditions.

**Materials and methods**

**Study area**

Ethiopia has nine administrative regional states and two city administrations that are further divided into zonal administration areas and districts. The diverse topographical structure of the country forms the basis for the three major agro-climatic zones (Fig. 1): highland agro-ecology occurs 2,300 metres above sea level (m.a.s.l.); areas between 2,300 and 1,500 m.a.s.l. are known as the midlands; areas below 1,500 m.a.s.l. are classified as lowlands (14). The five-year retrospective data covering the entire country were acquired from the Animal and Plant Health Regulatory Directorate (APHRD) of the federal Ministry of Agriculture (MOA). Active outbreaks were investigated in four districts of central Ethiopia: Adama, Mojo, Welenchiti and Wengi (Fig. 1). Central Ethiopia has a bimodal rainfall season: the long rainfall season from late June to late September and the short season from February to April, with a mean annual rainfall range of 450 mm to 1,000 mm and temperature range of 17°C to 30°C (Eastern Shewa Agricultural Bureau report).

**Study population**

Active outbreaks were investigated on nine feedlot farms with 200 to 300 cattle per farm and on 40 smallholder farms in the vicinity of the investigated districts. The farms maintained herds of local zebu cattle, many of which were acquired for fattening purposes from Borana rangeland and other pastoral areas. The investigations were in
response to LSD outbreaks following a vaccination campaign using KS-1 vaccine produced by the NVI. As these animals were brought onto the farms from different parts of the country, mainly from pastoral regions, vaccination against LSD and other diseases was mandatory on arrival. The cattle were also treated with acaricides and dewormed as routine practice during the first week of arrival. Unlike the situation on feedlot farms, most of the animals kept by smallholder farmers in the same vicinity were not vaccinated against LSD.

**Study design and sampling**

**Active disease investigation**

In response to feedback from cattle owners and animal health professionals on LSD vaccination failure, the NVI launched a preliminary field investigation on feedlot and surrounding extension farms. The farms under investigation all suffered LSD outbreaks and were selected on the basis of reports from the district Animal Health Services Departments. Nine fattening and 40 smallholder farms with a total of 2,174 cattle were enrolled in the study; 1,992 animals came from feedlots and the remaining 182 were from smallholdings. Because of the nature of the production system, the population was largely skewed towards males (bulls) (98.8%). All 49 cattle owners took part in semi-structured questionnaire interviews on LSD occurrence and its associated impacts. Questions included the vaccination status of the animals, when and by whom the cattle were vaccinated, and the batch number of the vaccine used. In addition, farm management and environmental factors were recorded, together with indicators for income losses resulting from the outbreak. Animals were assessed for characteristic clinical signs of LSD, such as visible skin lesions, enlarged lymph nodes and fever. Nodule skin biopsies were collected aseptically from 22 acutely sick cattle. The tissue samples were placed in sterile universal bottles, transported on ice to the virology laboratory at the NVI within 4 h to 8 h of collection, and kept at –20°C until processing (3).
Retrospective data

Outbreak data for the entire country in the period January 2007 to December 2011 were retrieved from the MOA.

Laboratory diagnosis

Virus isolation

The biopsy samples were thawed at room temperature and washed three times in sterile phosphate-buffered saline (PBS, pH 7.2). Approximately 1 g washed tissue sample was mixed with 9 ml sterile PBS containing antibiotic (0.1% gentamicin, Sigma-Aldrich, Germany) and ground using a sterile mortar and pestle. The tissue suspension was centrifuged at 600 g for 15 min and the supernatant filtered through a membrane of pore size 0.45 µm (Millipore, United States of America [USA]). Approximately 1 ml filtered supernatant was inoculated onto a monolayer of Vero cells in 25 cm² tissue culture flasks, incubated at 37°C for an hour for adsorption, and then 9 ml Glasgow modified minimum essential medium (GMEM, Sigma-Aldrich), containing 0.1% gentamicin and 2% fetal calf serum (Sigma-Aldrich), was added. The inoculated flasks were incubated at 37°C in a humidified incubator with 5% CO₂. Cells were monitored daily for 14 days, using an inverted microscope, for evidence of virus-induced cytopathic effects (CPEs); cells were frozen at –20°C when CPEs were present.

Polymerase chain reaction

DNA was extracted using a DNeasy kit (Qiagen, USA), according to the manufacturer’s instructions. Polymerase chain reaction (PCR) assay was used to detect the virus with capripoxvirus-specific primers: forward primer (5’-TCTATGTCTTGATATGTGGTG GTAG-3’), reverse primer (5’-AGTGATTAGGTGGTGTATTATTTTCC-3’) (15). DNA was amplified in a final volume of 50 µl containing the following: 5 µl PCR buffer (10 mM), 1.5 µl MgCl₂ (25 mM), 1 µl dNTP mixture (10 mM), 1 µl forward primer (50 mM), 1 µl reverse primer (50 mM), 5 µl DNA template, 0.5 µl Taq DNA polymerase
(5 U/µl) (Invitrogen) and 35 µl of RNase-free water. The PCR was run in a thermocycler (Applied Biosystems® 2720, USA) using the following amplification programme: initial denaturation at 95°C for 1 min, followed by 40 cycles at 95°C for 30 s, 55°C for 30 s and 72°C for 1 min. Additional elongation was at 72°C for 5 min. Amplified products were analysed using 1.5% gel electrophoresis and positive results were confirmed based on the size (172 base pairs [bp]) of the bands.

Data management

The collected data were recorded on Excel (Microsoft Corp., USA) spreadsheets. Stata software version 9 (StataCorp, College Station, Texas, USA) was used for descriptive statistical analysis. Morbidity, mortality and case fatality rates were estimated in accordance with different variable categories. Outbreak frequencies in relation to geography and season were depicted graphically. Maps were generated using ArcGIS version 9.0 software (ESRI, Redlands, California, USA).

Results

Retrospective data analysis

Analysis of retrospective data between January 2007 and December 2011 indicated that a total of 1,675 outbreaks with 62,176 cases and 4,372 deaths were reported to the Ethiopian MOA. The highest number of outbreaks was recorded in 2010 (447) followed by 2009 (339). The frequency of reported outbreaks was higher between September and December, with the highest numbers in October (266) and November (287); the lowest number was reported in May (53) (Fig. 2). The analysis showed that LSD was reported from all regions of the country except Harari and Dire Dawa. Outbreaks were frequently reported from Oromia (1,066), Amhara (365) and the Southern Nations, Nationalities and People’s Region (SNNPR) (123) (Fig. 3). The majority of the outbreak reports were from the central and south-western parts of the country; Illubabor, Jimma, South-West Shoa, and Arsi were the most frequently affected zones (Fig. 4).
Outbreak investigations

Two active outbreaks were investigated between September 2011 and April 2012: the first in September 2011 at Wenji and Adama, the second between December 2011 and January 2012 in the Mojo and Welenchiti areas. During the two outbreaks, 296 cattle were affected (13.61% of all cattle in the regions) and 108 (4.97%) died. The disease was observed on all visited farms, regardless of the KS-1 vaccination status. The most commonly observed clinical signs of LSD were fever, different sizes of skin nodules including necrotic nodules and deep scab formation (Fig. 5), enlarged peripheral lymph nodes, dullness, lameness and lacrimation.

Morbidity was highest at Adama (15.38%), followed by Wenji (10.3%), Welenchiti (8.8%) and Mojo (7.0%) (Table I). The highest mortality rate was observed in Adama (5.89%), followed by Wenji (3.42%), Welenchiti (2.4%) and Mojo (0%). The case fatality rate was highest in Adama (38.2%); no deaths from LSD were reported in Mojo.

Affected animals were divided into two age groups: young (<2 years) and adult (≥2 years). Morbidity rates were higher in young animals (18.0%) than in the adults (11.9%), as were the age-specific mortality and case fatality rates (9.8% and 54.6%, respectively). The morbidity rate was higher in female animals (23.07%) than in the males (13.5%), whereas the mortality and case fatality rates were higher in male animals at 4.98% and 36.89%, respectively. The highest morbidity (15.1%) and mortality rates (5.37%) were observed in vaccinated animals. Morbidity (14.1%) and mortality (5.26%) rates were higher in feedlot cattle than in those extensively managed (Table II).

Virus isolation

Cytopathic effects began on incubation days 5 to 11 and were observed in all 22 cell cultures that were inoculated. The CPEs were characterised by rounding of single cells, aggregation of dead cells and destruction of monolayers. None of the negative control cultures showed any CPE.
Polymerase chain reaction

DNA amplified from 22 CPE-positive tissue culture samples using LSDV-specific primers produced DNA fragments of 172 bp, the expected amplicon size for the LSDV genomic region targeted (Fig. 6).

Questionnaire survey

The questionnaire survey among the 49 cattle owners found that the local name for LSD is *fentata*. The respondents indicated that before 2009 the disease had occurred sporadically but since June 2009 it had become endemic to the area. They also reported that the incidence of the disease increased during the rainy season. Concerning the possible source of infection, the cattle owners responded as follows: contact with sick animals within the herd (19/49) or introduction of sick animals to the herd (7/49); the other 23 cattle owners did not know of any source. The records of the feedlot farms, most of them having their own veterinarian on call, indicated that most animals were vaccinated against LSD upon arrival. Clinical disease symptoms from as early as 15 days after arrival, and up to two months, were reported, regardless of the vaccination status of the herd.

The survey enabled an estimation of the direct economic losses resulting from animals dying from LSD. Production losses were estimated from the weighted average price of each animal that died. Thus, the average cost of a single ox dying from LSD was calculated as 9,000 Ethiopian birr (ETB), equivalent to US$477.7 (US$1 = 18.84 ETB). For the two active outbreaks investigated, the total economic loss from the deaths of 108 cattle was therefore 972,000 ETB (US$51,590). An additional average cost of 16.50 ETB per animal was incurred for supportive veterinary treatment of LSD. High economic losses were also incurred by feedlot owners for extra feed bought to assist sick animals during their recovery and the lengthened period required for fattening. Further, animals that recovered were no longer fit for export purposes and were therefore sold at local markets at a lower price. Lastly, the survey found that
animals that had recovered from LSD produced less milk and suffered a loss in draught power.

**Discussion**

The retrospective analysis of data from January 2007 to December 2011 showed that 1,675 outbreaks of LSD were reported to the Ethiopian MOA. These outbreaks and associated losses were reported from nine administrative regions of the country. No outbreaks were reported from Dire Dawa and Harari. In Oromia Regional State, the most frequently affected areas with the highest numbers of LSD outbreaks were Illubabor, Jimma, Arsi and South-West Shewa. The majority of outbreaks were reported from the midland agro-climate zone, which is known to be favourable for the breeding of the blood-feeding insect vectors of LSD and has the highest population density of livestock in Ethiopia (6). This observation is in agreement with previous study findings of a high seroprevalence (5) and occurrence of clinical LSD in the midland agro-ecological zone. During the present study, the highest frequency of LSD outbreaks was reported between September and December, which is the end of the main rainy season in most parts of the midland and highland agro-ecological zones. The seasonality of the outbreaks was also substantiated by questionnaire respondents who provided information on active disease surveillance. The temporal pattern of outbreaks is in accordance with previous reports indicating that the disease is more common after the rainy season (4, 10, 16). It is likely that the prevailing humidity and ambient temperature support the development and abundance of the vector populations.

The morbidity and mortality rates observed during the outbreaks are in agreement with findings of other authors who have reported that LSD has high rates of morbidity (up to 50%) and low mortality (<10%) (7, 9, 16). These figures are highly variable according to geographic location and climate; the management conditions, breed, immune status and overall condition of the animals; virus virulence; and the population levels and types of putative insect vectors (7, 9, 17). High morbidity and mortality rates were observed on intensive fattening
farms and could be associated with the presence of high densities of biting insects around the feedlot areas, a consequence of improper management of farm waste. Moreover, feedlot farmers do not practise an ‘all-in all-out’ strategy that would prevent virus-incubating or sick animals from serving as a source of infection to new arrivals before vaccination or the development of protective immunity following vaccination. According to the OIE (3), protective immunity to LSD is expected to develop between 10 and 21 days post vaccination.

In the present study, the morbidity, mortality and case fatality rates of LSD indicated that calves were more susceptible to infection than adult cattle. This finding is in agreement with the reports of Ahmed and Kawther (2008) and Vorster and Mapham (2008) (7, 11). Fever, skin nodules, enlarged lymph nodes, lacrimation and salivation were also documented as characteristic clinical features of LSD (1, 3, 10). Host susceptibility in relation to age, immunological status, and dose and route of virus inoculation also affect disease severity.

Lumpy skin disease causes severe economic losses as a result of the prolonged debilitating clinical course of the illness, reduced weight gain, temporary or permanent loss of milk production, infertility problems or even sterility in bulls, abortions in pregnant cows (4, 10, 11) and permanent damage to hides. Although a clear and quantifiable economic data analysis is lacking in this study, the findings associated with LSD mortality and veterinary expenses for treating sick animals are suggestive of heavy losses in the sector.

At least four live attenuated strains of capripoxvirus are currently used as vaccines to control LSD (3); these include the Kenyan sheep- and goat-pox strain (KS-1), the Yugoslavian RM 65 sheep-pox strain, the Romanian sheep-pox strain and the South African Onderstepoort LSDV strain (1, 3, 9, 18). All strains of capripoxvirus that have been examined share a major neutralising epitope; therefore, it was predicted that cattle could be protected against LSDV infection by using strains of capripoxvirus derived from sheep or goats (9, 18). Moreover, it is claimed that lifelong protection is achieved after natural infection and that vaccinated animals will develop protective
immunity from 10 to 21 days post vaccination, and then require an annual booster dose (3). However, the authors’ findings suggest that KS-1 is an ineffective vaccine. A similar phenomenon has been reported in Egypt and Israel (13, 16) and vaccine failure on dairy farms in Ethiopia was also reported recently (19). This apparent emerging vaccine failure is a serious problem for efficient control of LSD, as the disease has been manifested by high morbidity and mortality rates, regardless of vaccination status. The observed vaccine failure may be due to a lack of cross-protection of the KS-1 vaccine strain against circulating virulent field strains. However, the lack of an ‘all-in all-out’ system in feedlots, together with the purchase of animals from different parts of the country without adequate knowledge of their history, may possibly influence vaccine efficacy through the introduction of animals already incubating the virus or newly vaccinated animals becoming infected before they develop protective immunity.

In conclusion, the present study has shown that LSD is causing considerable economic losses in livestock sectors across Ethiopia. Outbreaks of the disease reach a peak after the annual wet season and occur throughout the country, with the highest prevalence in the midland agro-ecological zone. Although the economic impact survey and analysis in this study was partial, it is indicative of the potential direct and indirect losses associated with the disease. The occurrence of LSD on an outbreak scale, despite the use of a vaccination regime, is suggestive of vaccine failure. The possible causes reported must be addressed through the use of a laboratory efficacy trial for all vaccine strains, including the one derived from the South African Neethling strain of LSDV. The cellular and humoral immune responses elicited need to be measured and complemented with experimental challenge.

References


Table I
Morbidity, mortality and case fatality rates in outbreaks of lumpy skin disease in four study areas of central Ethiopia

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of susceptible cattle</th>
<th>No. of affected cattle (%)</th>
<th>No. of deaths (%)</th>
<th>Case fatality rate (%)</th>
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</thead>
<tbody>
<tr>
<td>Adama</td>
<td>1,495</td>
<td>230 (15.38)</td>
<td>88 (5.89)</td>
<td>38.26</td>
</tr>
<tr>
<td>Wenji</td>
<td>497</td>
<td>51 (10.26)</td>
<td>17 (3.42)</td>
<td>33.33</td>
</tr>
<tr>
<td>Welenchiti</td>
<td>125</td>
<td>11 (8.8)</td>
<td>3 (2.40)</td>
<td>27.27</td>
</tr>
<tr>
<td>Mojo</td>
<td>57</td>
<td>4 (7.01)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2,174</td>
<td>296 (13.61)</td>
<td>108 (4.97)</td>
<td>36.49</td>
</tr>
</tbody>
</table>
Table II
Morbidity, mortality and case fatality rates of lumpy skin disease
based on the sex, age and vaccination status of the cattle

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of susceptible cattle</th>
<th>No. of affected cattle</th>
<th>Morbidity rate (%)</th>
<th>No. of deaths</th>
<th>Mortality rate (%)</th>
<th>Case fatality rate (%)</th>
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</thead>
<tbody>
<tr>
<td>Sex</td>
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</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>6</td>
<td>23.07</td>
<td>1</td>
<td>3.85</td>
<td>16.67</td>
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<tr>
<td>Male</td>
<td>2,148</td>
<td>290</td>
<td>13.5</td>
<td>107</td>
<td>4.98</td>
<td>36.89</td>
</tr>
<tr>
<td>Age (years)</td>
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<td></td>
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</tr>
<tr>
<td>&lt;2</td>
<td>599</td>
<td>108</td>
<td>18.03</td>
<td>59</td>
<td>9.84</td>
<td>54.62</td>
</tr>
<tr>
<td>≥2</td>
<td>1,575</td>
<td>188</td>
<td>11.9</td>
<td>49</td>
<td>3.1</td>
<td>26.1</td>
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<td>Vaccination status</td>
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<tr>
<td>Vaccinated</td>
<td>1,601</td>
<td>242</td>
<td>15.1</td>
<td>86</td>
<td>5.37</td>
<td>35.5</td>
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<tr>
<td>Unvaccinated</td>
<td>573</td>
<td>54</td>
<td>9.4</td>
<td>22</td>
<td>3.84</td>
<td>40.7</td>
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<td>Management</td>
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<td></td>
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<tr>
<td>Feedlot</td>
<td>1,992</td>
<td>281</td>
<td>14.1</td>
<td>105</td>
<td>5.26</td>
<td>37.4</td>
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<tr>
<td>Extensive farm</td>
<td>182</td>
<td>15</td>
<td>8.2</td>
<td>3</td>
<td>1.65</td>
<td>20.0</td>
</tr>
</tbody>
</table>
SNNPR: Southern Nations, Nationalities and People’s Region

**Fig. 1**

Study areas of lumpy skin disease outbreaks in Ethiopia

The study areas are in close proximity to each other and lie at the centre of Ethiopia
Fig. 2
Occurrence and seasonality of lumpy skin disease outbreaks in Ethiopia, 2007–2011
The high number of outbreaks from September to December is evident
SNNPR: Southern Nations, Nationalities and People’s Region

**Fig. 3**

**Numbers of lumpy skin disease outbreaks reported in different regions of Ethiopia, 2007–2011**

The greatest number of outbreaks were reported from Oromia
Fig. 4
Map of Ethiopia showing the distribution of lumpy skin disease outbreaks, 2007–2011
Lumpy skin disease (LSD) outbreaks were reported from most parts of the country, with the highest number in the midland agro-ecological zone.
Fig. 5
Clinical signs of lumpy skin disease: necrotic nodules and deep scabs may be observed all over the body
**Fig. 6**
Polymerase chain reaction-based detection of lumpy skin disease virus
Lane M: 100 bp DNA ladder
Lane NTC: negative template control
Lanes 1, 2: positive samples from Adama
Lanes 3, 4: positive samples from Wenji
Lane 5: positive sample from Mojo
Lane 6: positive sample from Welenchiti