REVIEW

Review: Lumpy Skin Disease: An Emerging Threat to Europe, the Middle East and Asia

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Clinical Signs and Economic Impact of LSD

Lumpy skin disease (LSD) is an acute or inapparent cattle disease caused by lumpy skin disease virus (LSDV). The virus is classified in the genus *Capripoxvirus* within the subfamily *Chordopoxvirinae* of the family *Poxviridae* (Bulker et al., 2005). The characteristic clinical signs have been described in detail by several authors (Thomas and Mare, 1945; Haig, 1957; Weiss, 1968; Prozesky and Barnard, 1982; Coetzer, 2004; Babiuk et al., 2008a). Briefly, in the majority of cases, the initial evidence of infection is lachrymation and fever (40–41°C), but some cases are non-febrile. Subcapsular and pre-cural lymph nodes become noticeably enlarged. Shortly after the onset of fever, skin nodules (1–5 cm in diameter) become apparent, in varying numbers, from only a few to multiple lesions covering the entire animal (Fig. 1). In severely affected animals, ulcerative lesions appear in the mucous membranes of eye and oral/nasal cavities causing excessive salivation, lachrymation and nasal discharge. All these secretions may contain LSDV (Babiuk et al., 2008b; OIE, 2008). Pox lesions may also be present in the pharynx, larynx, trachea, lungs and throughout the alimentary tract. In post-mortem examination of severe cases, pox lesions may be present on the surface of almost any organ (Babiuk et al., 2008b). Some infected animals develop oedematous swelling of one or more legs and lameness. The incubation period in natural outbreaks is estimated to be 1–4 weeks (Haig, 1957; Coetzer, 2004). In outbreaks of the disease, the morbidity rate varies widely depending on the immune status of the hosts and the abundance of mechanical arthropod vectors and usually ranges from 3% to 85% (Thomas and Mare, 1945). During the outbreak in Israel in 2006, up to 41.3% morbidity rates were reported (Brenner et al., 2009). The mortality rate is generally low (1–3%) but may sometimes reach 40% (Coetzer, 2004).

The World Organization for Animal Health (OIE) categorizes LSD as a notifiable disease because of the substantial economic impact of an outbreak. The disease is more severe in cows in the peak of lactation and causes a sharp drop in milk yield because of high fever caused by the viral infection itself and secondary bacterial mastitis. Temporary or permanent infertility may occur in cows and bulls. Emaciation of infected animals and a convalescence period lasting for several months may cause decreased growth rate in beef cattle (Weiss, 1968). Deep skin lesions leave permanent scars and decrease the value of hides (Green, 1959). Restrictions to the global trade of live animals and animal products, costly control and eradication measures such as vaccination campaigns as well as the indirect costs because of the compulsory limitations in animal movements cause...
significant financial losses on a national level. The disease is listed as notifiable throughout the European Community (82/894/EEC, 89/162/EEC), and should an outbreak occurs, regulation would require the slaughter of affected and in-contact animals, and the implementation of a 3-km protection zone and a 10-km surveillance zone around the infected premises (Council directive 92/119/EEC). These restrictions at a farm level would remain in force for at least 28 days or until permission had been obtained from veterinary authorities to remove them. Three disease-free years are required for a country to regain an official disease-free status that underlines the economic severity of an outbreak. Capripoxviruses are classified by the government of the United States as potential agents for agriterrorism. In intensive cattle farming units, direct and indirect production losses caused by LSD have been estimated to be as high as 45–65%. In developing countries, the poorest small-scale farmers and rural communities, whose livelihood is totally dependent on cattle, bear the heaviest burden during outbreaks.

**Geographical Distribution**

A new skin disease, referred as ‘pseudo urticaria’, of cattle was first reported in 1929 in Northern Rhodesia (now Zambia) (MacDonald, 1931) from where the disease spreads to other southern African countries by the 1940s. During the following decades, LSD spreads slowly northwards and is currently present virtually throughout the entire continent of Africa, including Madagascar (World Animal Health Information database, OIE WAHID Interface). The only African countries still considered free of the disease are Libya, Algeria, Morocco and Tunisia. It has been suggested that, while extending its geographical distribution, the virus has increased in pathogenicity causing extensive epidemics and pandemics on the African continent with sporadic cases occurring during inter-epidemic years (Rweyemamu et al., 2000).

The first LSD outbreak to occur in Egypt was reported in May 1988 (Ali et al., 1990). The Egyptian veterinary authorities were not able to trace the origin of the outbreak with certainty. A higher rate of disease incidence in some parts of the country was associated with greater insect population densities (Ali et al., 1990). In August 1989, the disease spreads for the first time out of Africa into Israel (Yeruham et al., 1994). A wind-borne mode of transmission via the stable fly (*Stomoxys calcitrans*) from disease foci in Egypt was suspected. This assumption was based on the fact that no new animals were introduced into the infected herds, LSDV had previously been isolated from stable flies caught after feeding on infected animals (Weiss, 1968), stable flies had been shown to be able to transmit capripoxvirus between infected and susceptible animals (Mellor et al., 1986, 1987) and *Stomoxys* spp. predominated over other blood-feeding insects in Israel (Yeruham et al., 1994).

After an apparent absence of 17 years, LSD reoccurred in Egypt in 2006, being introduced into the country by infected cattle imported from the African Horn countries (El-Kholy et al., 2008). The disease spreads surprisingly swiftly throughout the country in spite of an extensive vaccination campaign. In June 2006, cases of LSD were again reported in Israel, and the Israeli authorities speculated that LSDV may have already been circulating in other Middle Eastern countries (Brenner et al., 2009).

LSD outbreaks have been reported in the Middle Eastern region since 1990 (Fig. 2). According to the OIE, LSD has been reported in Kuwait in 1991, Lebanon in 1993, Yemen in 1995, United Arab Emirates in 2000, Bahrain in 2003, Israel in 2006–2007 and Oman in 2010. The presence of LSDV in Saudi Arabia (reported in 1992) was never confirmed with certainty. To feed a rapidly growing population, the Middle East has become a substantial importer of live cattle, frozen meat and animal feed from Europe, Asia and Africa (Shimshony and Economides, 2006). Without strict testing regimes of imported live animals and sufficient control during the quarantine period, the large-scale importation of live animals and animal products allows for an easy entry of infectious diseases. The cattle farming industry in the region is limited by a general lack of suitable fertile land, which leads to the maintenance of dense cattle populations in relatively small areas located mainly on river deltas and basins. High temperatures and heavy seasonal rains, and the presence of water courses, lead to an increase in populations of blood-feeding arthropods, which enhance the likelihood of the transmission of vector-borne diseases, such as LSD. The spread of infectious animal diseases

Fig. 1. A lumpy skin diseased cow showing skin nodules covering the entire body.
within the region is compounded by uncontrolled animal movements, communal grazing and nomadism. The poor animal health situation in the politically unstable Middle East region, the lack of early laboratory detection, inefficient control and eradication measures in combination with inadequate communication between countries and under-reporting to international organizations, such as OIE, increase the hazard of the disease spreading to neighbouring countries (Shimshony and Economides, 2006), especially if the outbreaks cannot be effectively controlled by vaccination.

Control of LSD through Vaccination

Only live attenuated vaccines against LSD are currently commercially available. Because of antigenic homology and cross-protection between sheep pox, goat pox and LSD viruses, any of these viruses can be used as a vaccine strain to protect cattle against LSD (Kitching, 1983). Experimentally, LSDV has been successfully used as a vector for recombinant subunit vaccines, such as rabies (Aspden et al., 2002), rinderpest (Ngichabe et al., 1997) (Ngichabe et al., 2002) and Rift Valley fever (Wallace et al., 2007).

Because of potential safety issues with the live attenuated LSDV vaccine, its use is not recommended in countries previously free of the disease, such as Egypt and Israel. Instead, in these countries during LSD outbreaks, cattle were vaccinated with sheep pox vaccine, as the same vaccine was already being used in the area to protect sheep against sheep pox. During the 2006 outbreak of LSD in Egypt, it was reported that the live attenuated sheep pox vaccine (comprising Kenyan sheep and goat pox virus, O-240 strain) did not provide cattle with complete protection against LSD (Marshall, 2006). Additionally, incomplete protection was observed when the Yugoslavian RM 65 (Ramyar) sheep pox vaccine was used to vaccinate cattle against LSD in Israel from 2006 to 2007 (Brenner et al., 2009). A retrospective study carried out by Brenner et al. (2009) involving 4607 vaccinated cattle showed that the number of clinical LSD cases was five times greater in unvaccinated compared to vaccinated herds, which demonstrated that increased levels of protection were indeed achieved in the vaccinated animals as compared to those not vaccinated. However, 11.1% of the vaccinated animals developed cutaneous lesions after exposure to virus in the field. Skin nodules collected from these animals tested LSDV positive using a polymerase chain reaction (PCR)
test which enabled differentiation between sheep pox virus and LSDV (Stram et al., 2008). Therefore, the authors were able to exclude the possibility that the vaccine virus was responsible for inducing the skin lesions.

It is known during vaccination campaigns that not all animals develop absolute protective immunity against LSDV. Several factors have been reported to contribute to real or apparent vaccine breakdown (Carn, 1993; Kitching, 2003). Cattle may be incubating the disease when vaccinated, or some animals may be ‘missed’ during a vaccination campaign. If proper needle hygiene is not practised, needles or diluents contaminated with virulent LSDV during the actual vaccination procedure may transmit the virus. Inappropriate storage of vaccine or a failure in one or more steps of the cold chain may occur, or vaccine may be inactivated because of exposure to direct sunlight or high environmental temperatures during the vaccination process. In some cases, vaccine may be poorly administrated or an incorrect dosage used. Also, maternally derived antibodies are known to cause interference in the development of active immunity in calves up to 6 months of age, so calves vaccinated before 6 months of age, which were born to naturally infected or vaccinated dams, may not be protected.

Typically, LSD outbreaks occur in cycles with quiescent periods lasting several years. In South Africa, during such periods, vaccine sales drop because of complacency and reluctance amongst farmers to vaccinate owing to adverse vaccine reactions in some animals, leading to a low overall immunity to LSD in cattle herds across the country and the risk of a large outbreak (Hunter and Wallace, 2001). It is likely that during the LSD outbreak in Egypt in 2006, optimal conditions for the spread of LSDV were created through the presence of high numbers of susceptible animals in combination with the uncontrolled movements of infected animals and the high abundance of insect vectors.

Although vaccination does not result in a complete protection against the disease in each vaccinated animal, it is currently the only effective way to control the spread of LSDV. In non-endemic areas, the use of live attenuated vaccines could, however, compromise the disease-free status of the country, and it would be highly questionable on grounds of safety. In addition, the use of genetically modified recombinant live vaccines may not be permitted. The use of inactivated vaccines could be considered as a short-term solution in an emergency; however, the protection provided by inactivated vaccines is not solid and is only short lived (Kitching, 1983). Because of the limited commercial market for LSDV vaccines, suppliers may not be able to provide a sufficient amount of vaccine at short notice to non-endemic countries. It is not possible to differentiate infected from vaccinated animals using currently available tests. The heightened risk of LSD spreading from the Middle East to the rest of Asia or to Europe underlines the need for the development of a differentiating infected from vaccinated animals (DIVA) vaccine and associated diagnostic tests for all capripoxviruses.

**Transmission of LSDV**

The transmission of LSDV is believed to occur mainly by blood-feeding arthropods (Weiss, 1968; Kitching and Mellor, 1986; Chihota et al., 2001). During the first LSD outbreaks in southern Africa, it was observed that isolated outbreaks occurred in widely scattered herds in the absence of cattle movements. These outbreaks were associated with wet and warm weather conditions with an abundance of blood-feeding arthropod populations, and it was not possible to control the spread of the disease effectively by quarantine measures (Thomas and Mare, 1945; Weiss, 1968). Currently, it is widely agreed that LSDV is transmitted mechanically via arthropod vectors. Female *Aedes aegypti* mosquitoes were shown to transmit LSDV from infected to susceptible cattle for 2–6 days post-feeding on experimentally infected animals (Chihota et al., 2001). Experimentally, stable flies (*Stomoxys* sp.) are able to mechanically transmit capripoxvirus between sheep (Mellor et al., 1987), and live LSDV has been isolated from stable flies after feeding on infected cattle (Weiss, 1968). However, attempts to transmit LSDV between experimentally infected and susceptible cattle by *Stomoxys calcitrans* have failed (Chihota et al., 2003), as did the transmission of LSDV by two species of mosquito (*Anopheles stephensi* and *Culex quinquefasciatus*) and the biting midge (* Culicoides nubeculosus*) (Chihota et al., 2003). Recently, new evidence has been published reporting a possible role for hard ticks in the transmission of LSDV (Tuppurainen et al., 2011). The study showed molecular evidence of transstadial and transovarial transmission of LSDV by *R. (B.) decoloratus* ticks and mechanical or intrastadial transmission by *R. appendiculatus* and *A. hebraeum* ticks.

A cross-sectional, questionnaire-based study investigating the risk factors associated with the spread of LSD in Ethiopia has been carried out (Gari et al., 2010). A warm and humid agroclimate was associated with a higher prevalence of LSD, and the authors concluded that these conditions were associated with high levels of vector populations. Communal grazing and watering points were found to be associated with the occurrence of LSD. They also reported that the introduction of new animals to a herd had a strong association with an increased risk of disease in the herd. Surprisingly, no association was found between cattle movements and the prevalence of disease (Gari et al., 2010).

Deliberate attempts to transmit LSDV via the manual handling of infected animals immediately prior to contact
of the handler with susceptible cattle, or keeping naïve and infected animals in the same pen, failed. Therefore, it was concluded that direct or indirect contact between infected and susceptible animals is an inefficient method of transmission (Weiss, 1968; Carn and Kitching, 1995). However, successful transmission was achieved when naïve animals were allowed to share a drinking trough with severely infected animals (Haig, 1957). Transmission studies are further complicated by recent observations from experiments which demonstrated that, although only approximately 50% of infected animals are likely to show clinical signs, the majority of experimentally infected animals become viraemic (Weiss, 1968; Tuppurainen et al., 2005; Osuagwu et al., 2007; Annandale et al., 2010). Molecular diagnostic tools such as PCR methods were not developed when these earlier transmission experiments were conducted, and thus, further studies using current diagnostic techniques are required to fully understand the complexity of the transmission mechanisms of LSDV.

Transmission of LSDV through semen (natural mating or artificial insemination) has not been experimentally demonstrated, but LSDV has been isolated in the semen of experimentally infected bulls for 22 days post-infection (dpi) (Weiss, 1968). A more recent study demonstrated the persistence of live virus in bovine semen for up to 42 dpi, and viral DNA was detected until 159 dpi (Irons et al., 2005). In both studies, the virus was isolated from the semen of bulls with inapparent disease. Using both PCR and virus isolation, the epididymis and testis were identified as the sites of persistence of LSDV, and viral DNA was detected in all fractions of semen (Annandale et al., 2010). Vaccination of the bulls with the South African live attenuated Neethling strain prevented shedding of LSDV in the semen in animals challenged with LSDV after vaccination, and vaccinated animals did not shed vaccine virus in the semen (Osuagwu et al., 2007). During the natural outbreak of LSD in Egypt in 2006–2007, the ovarian activity in 640 cows was examined on a regular basis by gynaecological examination and ultrasonography. Of these cows, 25% were infected with LSDV, and a high percentage of the infected cows (93%) suffered from ovarian inactivity and showed no signs of oestrus. In the infected cows, the ovaries were smaller than average, and no activity was detected on the ovarian surface. In addition, lower progesterone and decreased albumin, copper and iron levels were detected in their blood (Ahmed and Zaher, 2008).

**A Potential Role for Wildlife in the Spread of LSDV**

In general, capripoxviruses are highly host specific, with only a few known exceptions. Very little data are available on the susceptibility of wild ruminants to LSD or on the role of wildlife as potential reservoirs of the virus. Natural infections were reported in five Asian water buffalo (*Bubalus bubalis*) during the LSD outbreak in Egypt in 1988, but the morbidity was significantly lower in buffalo (1.6%) than in cattle (30.8%) (Ali et al., 1990). Clinical signs of LSD have been demonstrated in impala (*Aepyceros melampus*) and giraffe (*Giraffa camelopardalis*) after experimental inoculation with LSDV (Young et al., 1970). LSD was reported in an Arabian oryx (*Oryx leucoryx*) in Saudi Arabia (Greth et al., 1992). Capripoxvirus was detected using electron microscopy in skin nodules of the oryx, and raised antibody levels against capripoxvirus were detected in paired serum samples tested using a neutralization test. However, whether the disease was actually caused by LSDV or sheep pox virus was never confirmed. Recently, the persistence of LSDV nucleic acid was reported in skin samples collected from springbok (*Antidorcas marsupialis*) in South Africa (Lamien et al., 2011).

The presence of antibodies in an animal species indicates its susceptibility to the virus and its potential involvement in the epidemiology of the disease (Barnard, 1997). However, antibody-positive animals do not necessarily produce a productive infection and may not be able to transmit virus. Antibodies against sheep pox virus, goat pox virus and LSDV cannot be differentiated from each other by using a neutralization test. Antibodies against capripoxvirus have been detected in blue wildebeest (*Connochaetes taurinus*), black wildebeest (*Connochaetes gnou*), springbok, eland (*Taurotragus oryx*) and impala (Barnard, 1997). The seroprevalence varied from 10% to 27%, averaging 17% in a grassland and 33% in a forest transition environment (Barnard, 1997). Antibodies were also detected in serum samples collected from African buffalo (*Syncerus caffer*) in Kenya (Davies, 1982). In another study, low levels of antibodies were detected in kudu (*Tragelaphus strepsiceros*), two waterbuck species (*Kobus ellipsiprymnus* and *Kobus defassa*), red buck (*Redunca arundinum*), impala, springbok and giraffe, leading to the conclusion that the samples may have contained non-specific virus inhibitors (Hedger and Hamblin, 1983). However, the antibody titres in the giraffe and red buck samples were as high as in convalescent cattle, which was assumed to be indicative of past infection (Hedger and Hamblin, 1983).

Animals with mild or inapparent infection with LSDV do not always show antibody levels detectable with a neutralization assay. Therefore, it is possible that the actual number of LSDV-infected wild ruminants may be considerably higher than that revealed by this test. Wild animals showing clinical signs of LSD are likely to be more susceptible to predators, which could explain the lack of reports of clinical disease in wildlife species. Also, the presence of clinical signs of LSD in wildlife is easily...
missed as the monitoring of the skin lesions is difficult or impossible, especially in mild cases (Barnard, 1997).

### Novel Diagnostic Tests

The tentative diagnosis of LSD is usually based on characteristic clinical signs, and the clinical diagnosis is confirmed by using conventional PCR (Ireland and Binepal, 1998; Heine et al., 1999; Mangana-Vougiouka et al., 1999; Tuppurainen et al., 2005; Orlova et al., 2006; Zheng et al., 2007) or real-time PCR methods (Balinsky et al., 2008; Bowden et al., 2008). Electron microscopy examination and serum/virus neutralization tests are also still widely used as gold standard methods for the detection of capripoxviral antigen and antibody. Although gel-based PCR is more time- and labour consuming than real-time PCR, it is a cheap and reliable method and is therefore useful in countries with limited resources. Recently, a capripoxvirus real-time PCR method using primers and a probe previously described (Bowden et al., 2008) has been validated (Stubbs et al., submitted). Sequence analysis of the P32 gene has revealed differences between sheep pox and goat pox viruses (Hosamani et al., 2004), and further sequence analysis of a putative gene encoding a homologue G-protein-coupled chemokine receptor (Q2/3L) (Cao et al., 1995) from 26 capripoxvirus isolates revealed three distinct clusters that consisted of sheep pox virus, goat pox virus and LSDV (Le Goff et al., 2005). To carry out a wider phylogenetic analysis, the same gene from another 58 capripoxvirus isolates was sequenced (Le Goff et al., 2009). Based on these studies, a species-specific, real-time PCR method using dual hybridization probes has been developed (Lamien et al., 2011). This assay can be used for the rapid genotyping of capripoxvirus strains and may provide a valuable tool for the differentiation of capripoxviruses in situations where characteristic clinical signs are detected in wild ruminants or viral persistence in arthropod vectors is investigated (Lamien et al., 2011).

The immunity against LSDV is thought to be predominantly cell mediated, and therefore, serological testing may not be sufficiently sensitive to detect mild and longstanding LSDV infections or antibodies in vaccinated animals (Kitching et al., 1987). In addition, poxviruses are known to modulate host’s innate and acquired immune responses in both intra- and extracellular environment (Seet et al., 2003). The neutralization test is not sufficiently sensitive to detect the true immunological status of the target population, so the development of alternative diagnostic tests such as a cell-based assay is required.

Attempts to develop an enzyme-linked immunosorbent assay (ELISA) for the detection of capripoxviral antibodies have been hampered both by difficulties in producing inactivated whole virus in sufficient volumes and by the instability of recombinant antigens (Bowden et al., 2009). All serological assays face the same problem that different surface proteins of non-enveloped intracellular mature virions and intracellular and extracellular enveloped virions induce the formation of different antibodies in the host, and their relative proportions may vary during different stages of infection.

Various antibody ELISAs have been developed in the past with limited success. The earliest ELISA developed for capripoxviruses utilized a protein encoded by P32 (vaccinia H3L homologue) as an antigen (Carn et al., 1994; Heine et al., 1999). More recently, an indirect ELISA was developed based on whole heat-inactivated sheep pox virus as an antigen (Babiuk et al., 2009). When 276 cattle serum samples were tested, the diagnostic sensitivity and specificity of this assay were 88% and 97%, respectively. Unfortunately, because of difficulties in producing the inactivated antigen in sufficient quantities, this assay is currently not available for use. In another study, 42 open reading frames (ORF) of the capripoxvirus genome were evaluated for their antigenic potential, and two ORFs encoding virion core proteins were selected as the best candidate antigens for use in an ELISA. These proteins were then expressed in Escherichia coli and used as antigens for an indirect ELISA (Bowden et al., 2009). However, only nine serum samples collected from two experimentally infected calves were available for evaluating the performance of the test. Recently, an ELISA based on a synthetic peptide targeting the major antigen P32 has been described for the detection of sheep pox and goat pox antibodies (Tian et al., 2010). Unfortunately, the performance of this ELISA has not been evaluated using LSD cattle sera. An indirect fluorescent antibody test (IFAT) was demonstrated to be suitable for use in retrospective serological surveys in a study carried out in Ethiopia (Gari et al., 2008).

### Conclusions

Lumpy skin disease has now spread out of the African continent into the Middle East region (Fig. 2). There are no geographical or epidemiological reasons why the disease cannot spread further north into Turkey and Europe, or further east into Asia. It is also difficult to predict with accuracy how global climate change may affect the abundance and distribution of mechanical vector populations, which may have a direct effect on the spread of this disease. As currently available live attenuated vaccine does not provide each individual with solid protection, vaccinated animals can develop skin lesions that contain high virus titres, resulting in the possible dissemination of the virus by blood-feeding arthropods. No alternative safe and effective vaccines, suitable for use in countries free of
LSD, sheep pox and goat pox, are currently available. The eradication of the disease is further hampered by a lack of epidemiological data about how long the virus can survive in the environment and whether wildlife plays a role as a reservoir for LSDV between outbreaks.

These observations underline the need for the development of improved vaccines that can be used in non-endemic countries and can be combined with sensitive serological assays capable of differentiating vaccinated from infected animals. Further work is also required to improve the sensitivity and specificity of capripox antibody ELISAs, especially for LSD, and, as rapid recognition of the disease is essential for its successful control and eradication, there is also a need to develop pen-side tests for the swift detection of LSDV at a farm level.

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