CHAPTER- I: INTRODUCTION

Lumpy skin disease (LSD) is a viral disease caused by lumpy skin disease virus (LSDV) that belongs to the family *Poxviridae* under the genus *Capripoxvirus*. The disease affects a wide range of domestic animals including cattle, buffalo, sheep and goat and is clinically manifested mainly by fever and nodular lesions on the skin, mucous membrane of respiratory and digestive tracts (Alkhamis and VanderWaal, 2016; El-Nahas et al., 2011; Coetzer and Tuppurainen, 2004). The World Organization for Animal Health (OIE) included the disease in notifiable transboundary disease list due to its substantial economic losses in terms of reduced productivity, poor hide quality, poor growth rate, infertility and even death of the animal (Tuppurainen et al., 2017; Tuppurainen and Oura, 2012).

LSDV is believed to be transmitted mainly through the bites of insects such as mosquitoes, flies, ticks (Magori-Cohen et al., 2012). Higher incidence of this disease is observed in crossbred young animals with communal grazing and in the wet season when the activities of arthropods vectors are abundant. Introduction of new animals is another important risk factor associated with the occurrence of this disease in the herds (Ochwo et al., 2019; Rammahi and Jassim, 2015; El-khabaz, 2014).

Zambia is the first country where LSD was identified in 1929 followed by many African and Middle Eastern countries (Kasem et al., 2018). Although many countries have experienced several outbreaks including Egypt, Israel, Iraq, Turkey, Azerbaijan, Saudi Arabia, Armenia, Greece, Bulgaria etc., most of those outbreaks experienced approximately 5-35% morbidity and 1-3% mortality. Usually the very first suspicion of LSD is raised when several febrile animals with highly characteristic skin nodules, eye discharge and enlarged lymph nodes are detected by cattle owners. Dairy cattle are daily monitored, but the virus may sometimes circulate for weeks in free-ranging beef cattle herds before detected, allowing plenty of time for vectors to become infected and spread the virus to within the currently affected regions. Livelihoods of poor smallholders and backyard farmers are most severely affected and mass vaccination with sufficient coverage is fundamental for halting the spread of a vector-borne LSDV supported by the other control measures (Mercier et al., 2018; Mafirakureva et al., 2017a; El-khabaz, 2014; Magori-Cohen et al., 2012). To date, none of the affected countries has been able to permanently eradicate the disease, once it has got a foothold in their territories. However, the effectiveness of the total stamping-out measure is likely to vary depending on the region and cattle farming practices. In case outbreaks are detected in a very early stage, epidemiological unit sizes are small and cattle movements can be properly controlled, a

total stamping-out measure would probably stop the spread of LSD without vaccination (Tuppurainen and Oura, 2012).

In Bangladesh, outbreak of an unknown disease with nodular skin lesions was reported by local veterinary services authority in mid-2019 in commercial and backyard cattle population in some upazilla (Anwara, Karnaphuli and Patiya) of Chattogram district (Anonymous, 2019a). Same pattern of clinical onset was reported later on in different districts of the country. The outbreak was primarily confirmed based on clinical signs and later using the RT-PCR test by the Department of Livestock Services (DLS), Bangladesh and notified the disease as LSD to World Organization for Animal Health (OIE) in August, 2019. According to OIE, India and China also confronted by LSD in the same time (Anonymous, 2019b). It clearly point out that there might be a cause connected between the countries for the upsurge of the disease. The possible modes of transmission might have happened between the countries where the animal movement may play a significant role. According to government livestock authority of Bangladesh LSD spread alike as bushfire in the country.

Very little scientific research initiated for better understanding of LSD in the area of Chattogram or elsewhere. There are no previous data on prevalence or risk factors of this disease or molecular identification of circulating isolates. Therefore a cross sectional surveillance study was undertaken on clinically suspected LSD cases prevalent in south-eastern part of Bangladesh.

As this disease was newly introduced in the study area this investigation undertaken to -

- Assessment of the prevalence of LSD in the study area
- Explore the plausible risk factors of LSD
- Characterization of the virus using histopathology and molecular techniques
- Phylogenetic analysis of the circulating LSDV strains for detecting probable geographical origin of the virus strain

CHAPTER- II: REVIEW OF LITERATURE

2.1 Overview of study area

2.1.1 Topography and climate

Chattogram, the port city of Bangladesh located in between 21°54' and 22°59' north latitudes and in between 91°17' and 92°13' east longitudes. It is bounded by Khagrachori and Rangamati districts and Tripura state of India on the north, Cox's Bazar district on the south, Bandarban, Rangamati and Khagrachhari Districts on the east and Noakhali district and the Bay of Bengal on the west. Chattogram District is quite different from other Districts for its unique natural beauty characterized by hills, rivers, sea, forests and valleys. The average elevation of Chattogram, Bangladesh is 15 meters having tropical monsoon climate. Administratively, the district is divided into 14 upazilla (sub-district).

2.1.2 Cattle population and management practice in Bangladesh

Livestock population in Bangladesh is currently estimated as 24.3 million cattle, 1.49 million buffaloes, 26.4 million goats, 3.6 million sheep, 296.6 million chicken and 59.7 million ducks (DLS, 2019a). This density has been increasing every year in the country. The country has a relative density of livestock population well above the averages of many other countries of the world. In spite of a high density of livestock population, the country suffers from an acute shortage of livestock products. The shortage is approximately 45 lakh Metric Ton milk, 5 lakh Metric Ton meats according to the Department of Livestock services (DLS), Bangladesh. In Bangladesh, almost 85% of total households own livestock (animals or poultry or both as commercial or backyard). About 45% of the households possess bovine stack, and 75 percent possess poultry. On average, each household owns 1.52 bovine animals, 0.9 goat and sheep and 6.8 chicken and ducks (Banglapedia, 2019). Cattle reared in Bangladesh are mainly indigenous zebu, some exotic breeds and their crosses predominantly Holstein-Friesian, Jersey, Sahiwal and Sindhi. Indigenous cattle are relatively small and give less milk as compared to crossbred cattle. To improve milk production of the native cow, crossbreeding of indigenous cattle with Holstein-Friesian and Sahiwal is common. Commercial goats, beef and dairy farms usually meet the local demands of meat and milk. Small farms raise cattle, buffalo, sheep and goats with smaller numbers of animals in contrast with the large herds of commercial farms. Livestock in the rural areas are maintained on communal grazing land. They are allowed to graze during the day on natural pasture, homestead forest and fallow land. Men play a major role in raising large animals, while women play a vital role in sheep and goat

production activities. Furthermore, teenagers also play a significant role in raising livestock in Bangladesh. Chattogram Metropolitan Area (CMA) is situated at the center of Chattogram Division. The animals mainly reared intensively in urban area where they are kept on concentrate feeding and fewer amounts of green grass. Infectious diseases like Foot and Mouth Disease (FMD), Bovine Ephemeral Fever (BEF) brucellosis, anaplasmosis, black quarter along with parasitic diseases found prevalent in the location.

2.2 What is LSDV?

Lumpy skin disease virus (LSDV) is double-stranded DNA virus. It is a member of the capripox virus genus of *Poxviridae*. Capripoxviruses (CaPV) represent one of eight genera within the Chordopoxvirus subfamily (Alexander et al., 1957). The *Capripoxvirus* genus consists of LSDV, as well as sheep pox virus, and goat pox virus given in Figure 1. CaPV infections are usually host specific within specific geographic distributions even though they are serologically indistinguishable from one another (Fauquet et al., 2005).

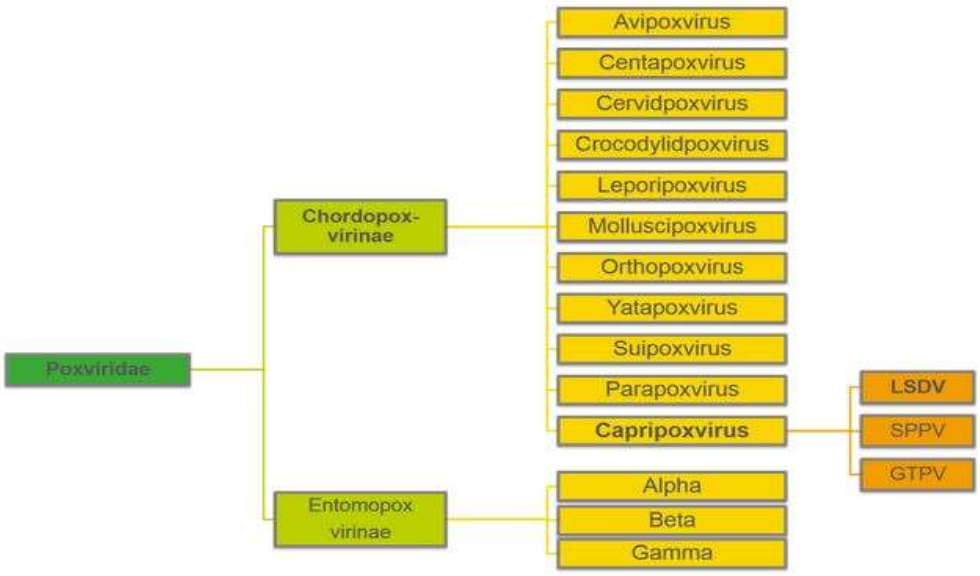

Figure 1: Classification of Lumpy skin disease virus

2.3 What is Lumpy Skin Disease?

2.3.1 History of Lumpy skin disease

The clinical syndrome of lumpy skin disease (LSD) was first described in Zambia (formerly Northern Rhodesia) in 1929. Initially, it was considered to be the result either of poisoning or a hypersensitivity to insect bites. Between 1943 and 1945, cases occurred in Botswana, Zimbabwe and the Republic of South Africa (Alemayehu et al., 2013). The

infectious nature of the disease was recognized at this time. A panzootic in South Africa, which lasted until 1949, affected some eight million cattle and consequently incurred enormous economic losses. LSD was first identified in East Africa in Kenya in 1957 and the Sudan in 1972 and in West Africa in 1974, spreading into Somalia in 1983. From 1929 to 1986 the disease was restricted to countries in sub-Saharan Africa, although its potential to extend beyond this range had been suggested. In May 1988, LSD was recognized clinically in the Suez Governorate of Egypt, where it was thought to have arrived at the local quarantine station with cattle imported from Africa (House et al., 1990a; Peck, 2017). The disease spread locally in the summer of 1988 and apparently overwintered with little or no manifestation of clinical disease. It reappeared in the summer of 1989 and, in a period of 5 to 6 months, spread to 22 of the 26 governorates of Egypt. A rapid reaction to the problem led to the vaccination of nearly two million cattle with a sheep pox vaccine. Morbidity in this epizootic was low, being 2 percent of the whole cattle population. In 1989, an outbreak of LSD was identified in Israel and subsequently eliminated by slaughtering all infected cattle as well as contacts. Ring vaccination with a sheep pox strain was carried out around the focus area and no further clinical cases have occurred. In sub-Saharan Africa, LSD is now enzootic in all the countries in which it has occurred and has proved impossible to eradicate (Ali, 1977). Restrictions on cattle movements have not prevented its spread within countries and today LSD is liable to extend its range eastward from northeastern Africa and Egypt into the highly receptive Tigris-Euphrates delta (Agianniotaki et al., 2018).

2.3.2 Response to physical and chemical action

Temperature: The virus is susceptible to 55°C/2 hours, 65°C/30 minutes. It can be recovered from skin nodules kept at –80°C for 10 years and infected tissue culture fluid stored at 4°C for 6 months (EFSA, 2015).

pH: LSDV is susceptible to alkaline or acidic pH. No significant reduction in titre was found when held at pH 6.6–8.6 for 5 days at 37°C (EFSA, 2015; Tuppurainen, 2018).

Chemicals/Disinfectants: LSDV is susceptible to ether (20%), chloroform, formalin (1%), and some detergents, e.g. sodium dodecyl sulphate. Susceptible to phenol (2%/15 minutes), sodium hypochlorite (2–3%), iodine compounds (1:33 dilution), Virkon® (2%), quaternary ammonium compounds (0.5%) (EFSA, 2015).

Survival: LSDV is remarkably stable, survive for long periods at ambient temperature, especially in dried scabs. LSDV is very resistant to inactivation, surviving in necrotic skin nodules for up to 33 days or longer, desiccated crusts for up to 35days, and at least 18 days in air-dried hides. It can remain viable for long periods in the environment. The virus is susceptible to sunlight and detergents containing lipid solvents, but in dark environmental conditions, such as contaminated animal sheds, it can persist for many months (EFSA, 2015).

2.4 Genome

Capripox viruses are double-stranded DNA viruses that have hairpin loop ends which are similar to other poxviruses. The genomes of members of the Capripox virus genus are approximately 150 Kbp in length (Kara et al., 2003). Capripox viruses are similar to other poxviruses, with the genome being complex and encoding many genes. Before full-genome sequencing was available, restriction fragment analysis was used to compare and classify the relationship between capripox viruses and to some orthopox virus members (Gershon et al., 1989). These studies demonstrated the relationship between capripox viruses and other poxviruses. The full-genome sequencing of lumpy skin disease virus together with bioinformatics revealed that lumpy skin disease virus encodes 156 putative genes (Tulman et al., 2002). Lumpy skin disease contains many genes which are similar to other poxviruses which have been studied in greater detail (Gershoni and Black, 1989).

2.5 Pathogenesis and pathology of LSD

Lumpy skin disease is a systemic disease, with cell-associated viremia preceding the appearance of lesions and marked lymphadenopathy. It is likely that blood monocytes are important in spreading virus to secondary sites of infection (Prozesky and Barnard, 1982). Like most members of the subfamily *Chordopoxviridae*, capripox viruses exhibit a distinct tropism for keratinocytes. Skin lesions are characterized by hyperplasia and ballooning degeneration of keratinocytes of the stratum spinosum, formation of epidermal microvesicles, and infiltration of inflammatory cells into the dermis (Young et al., 1970). In lumpy skin disease, epidermal microvesicles coalesce into large vesicles that quickly ulcerate. LSD virus in experimentally infected cattle was demonstrated in saliva 11 days after the development of fever, in semen after 22 days, and in skin nodules after 33 days, while the virus not found in urine or faeces. Viremia occurred after the initial febrile reaction and persisted for at least 4 days (Möller et al., 2019). Various types of cells such

as pericytes, fibroblasts, epithelial and endothelial cells can be infected by the virus. Viral replication in pericytes, endothelial cells and probably some cells in blood vessel and lymph vessel wall results in severe vasculitis and lymphangitis in affected areas (Davies, 1991). The disease is characterized first by fever ranging from 40°C to 41.5°C with lachrymation, inappetence, depression and unwillingness to move. Fever occurs around 5 days following experimental inoculation and remains elevated over several days. In the next few days of the onset of fever, eruption of skin lesions, so-called nodules occurs (Kiplagat et al., 2020). These nodules range in size from 5 to 50 mm and are circular, raised, firm and well-circumscribed. Large irregular circumscribed plaques can occur from fused nodules. The deep nodules are present throughout all layers of the skin, including the epidermis, dermis and adjacent subcutaneous layers and sometimes even the adjacent musculature (Mafirakureva et al., 2017a).

The clinical presentation of the skin lesions can vary dramatically in cattle with respect to numbers and size. These nodules may be painful and usually appear first around the head, including the mouth, nose and eyes, followed by the neck, body, udder, genitals, legs and tail. The number of nodules in an infected animal can range from a single nodule to over a thousand in severely affected cattle (Milovanović et al., 2019). Later, the skin lesions often become necrotic plugs or so-called sit fast which then slough off, leaving large ulcers in the skin. These necrotic cores are very susceptible for secondary bacterial infections and are attractive for flies. When skin nodules heal, they leave permanent scars on the hides. Infected cattle can have increased levels in serum alanine aminotransferase, aspartate aminotransferase activities, creatinine phosphokinase and creatinine level in their blood (Saegerman et al., 2018). Rhinitis and nasal discharge starts as serous but later becomes mucopurulent. Conjunctivitis and ocular discharge can occur and sometimes keratitis is observed. In addition, excessive salivation, a loss of appetite leading to weight loss and depression may also occur (Gubbins et al., 2020). Characteristic pox lesions can develop in the mucous membranes of the mouth including the inside of the lips, gingivae and dental pads, tongue, soft palate, pharynx, epiglottis as well as the digestive tract. In addition, pox lesions can be found in the mucous membranes of the nasal cavities, turbinate, trachea and lungs. Infection in the lung can lead to primary or secondary pneumonia and respiratory distress. Even though the case fatality of lumpy skin disease virus is low, the affected cattle become debilitated and can remain in poor condition for many months following infection (Davies, 1991). The scars destroy the value of the hide for use in the leather industry. Milk yield is reduced in lactating cattle and mastitis can

occur. Abortions can occur in pregnant cattle and there have been reports of aborted fetuses having multiple skin lesions as well as calves born with extensive skin lesions (Rouby and Aboulsoud, 2016). A recent report describes a premature 1-day-old calf which was delivered by a cow that had lumpy skin disease in the seventh month of pregnancy. The calf died 36 hours after birth and was weak, immature with a low body weight, ill-defined teeth, hyperemic oral mucosa and respiratory distress. The calf had hard nodules on the skin and "sitfasts". Necropsy revealed nodules in the lungs, liver and ruminal pillars as well as enlarged lymph nodes (Vidanović et al., 2016).

2.6 *Capripoxvirus*:

Nodular proliferative lesions can occur internally in severe sheep pox and goat pox, most notably in the lungs but also in the fore stomachs and less frequently in the liver, tongue, and kidneys. Lung lesions are markedly proliferative in nature, involving hyperplasia of type II pneumocytes and the bronchiolar epithelium. The presence of mature viral particles within these lesions by electron microscopy confirms that they are sites of productive viral replication (Bedeković et al., 2018). The histologic lesions of sheep pox and goat pox typically include cells with vacuolated nuclei, marginated chromatin, and eosinophilic intracytoplasmic inclusion bodies referred to as sheep pox cells, which represent virus-infected mononuclear phagocytes and fibroblasts. In the lymph nodes and spleen, the essential histological lesion is necrosis and lymphoid depletion (Annandale et al., 2010).

2.7 Mode of transmission

The transmission of LSDV is believed to occur mainly by blood-feeding arthropods (Chihota et al., 2001; Sprygin et al., 2019). During the first LSD out-breaks 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 (Lubinga et al., 2015). 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.) were able to mechanically transmit capripox virus between sheep (Aleksandr et al., 2020) 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*) (Sameea et al., 2017). Recently, new evidence has been published reporting a possible role of hard ticks in the transmission of LSDV (Tuppurainen et al., 2017). The study showed molecular evidence of transstadial and transovarial transmission of LSDV by ticks and mechanical. 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 agro climate was associated with a higher prevalence of LSD, and the authors concluded that these conditions were associated with high levels of vector populations (Babiuk et al., 2008b). 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 (Alkhamis and VanderWaal, 2016b). Surprisingly, no association was found between cattle movements and the prevalence of the disease (Gari et al., 2015). Deliberate attempts to transmit LSDV via the manual handling of infected animals immediately prior to contact of the handler with susceptible cattle, or keeping naive 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 (Abdulqa et al., 2016). However, successful transmission was achieved when naive animals were allowed to share a drinking trough with severely infected animals. 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 (Weiss, 1968). A more recent study demonstrated the persistence of live virus in bovine semen for up to 42dpi, and viral DNA was detected until 159 dpi (Tulman et al., 2002). In both studies, the virus was isolated from these men 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 (Mathijs et al., 2016). During the natural out-break of LSD in

Egypt in 2006–2007, the ovarian activity in 640 cows was examined on a regular basis by gynecological 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 estrus. 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).

2.8 Vectors of Lumpy skin disease virus (LSDV)

The indirect transmission of LSDV is assumed to be mechanically vectored by arthropods, in which the virus does not replicate or circulate. The virus' high stability makes it possible to survive in many different vectors (Allepuz et al., 2019). Mechanical virus transmission rate is inversely proportional to the virus survival in the interval between the vector blood meals. True flies (Dipteran) with an interrupted or repeated feeding pattern can thus be efficient vectors of viruses (Ardestani and Mokhtari, 2020). This feeding pattern, where blood meal taken after one bite is not sufficient, due to interruption by the host reaction force the vector to visit the same or different host in a short time is sufficient for the virus survival (Machado et al., 2019). The role of an arthropod as a vector of LSDV should be demonstrated both on its competence and capacity. Vector competence is usually studied under controlled conditions and defines its ability to infect a susceptible animal after feeding on an infectious animal (Macauley et al., 1999). Vector capacity summarizes quantitatively the basic biological and ecological attributes of the vector which are associates with viral transmission. These include traits like biting rate, feeding preference and frequency and the size of vector population (Mercier et al., 2018b). To date there is no particular arthropod for which both competence and capacity were demonstrated. Vector competence of several arthropods was tested in the laboratory. *Aedes aegypti* female mosquitoes that had fed upon lesions of LSDV infected cattle were able to transmit virus to susceptible cattle over a period of 2–6 days post-infective feeding. The virus was isolated from all the recipient steers, though only five of them developed disease which was usually mild (Chihota et al., 2001). *Aedes aegypti (A. Aegypti)* is therefore a competent vector of LSDV. However, outbreaks of LSDV occurred in several European and Middle East countries, in which this vector is not abundant (Kahana-sutin et al., 2017). In the same genus, *Aedes albopictus* (*A. albopictus*) is also known as a competent vector of many viruses and although it is more widespread than *A. aegypti*, its presence in several affected countries was anecdotal during the eruption of LSD epidemics

(Saegerman et al., 2018). These mosquitoes prefer human blood over blood of other mammals (Lounibos and Kramer 2016), further reducing their capacity as vectors of LSDV. The competence of the mosquitoes *Anopheles stephensi* and *Culex quinquefasciatus*, the stable fly *Stomoxys calcitrans* and the biting midge *Culicoides nubeculosus* were assessed as well. None of these blood-feeding dipterans were able to infect susceptible cattle 24 hours after feeding on blood infected by LSDV (Swiswa et al., 2017). LSDV was identified from all of the dipteran species tested in excess of the minimum infectious dose for cattle via the intradermal and intravenous routes. However, while the mosquitoes were culture test positive for LSDV up to 4 days after feeding, *S. calcitrans* and *C. nubeculosus* were positive only on the feeding day (Taylor et al., 2019). Despite the failure of *S. calcitrans* to transmit LSDV in the above study, there are several evidences to support its vectoring potential. *S. calcitrans* was shown to transmit several animal pathogens including viruses (Molla et al., 2017) and most importantly it was shown to transmit the Yemen capripox strain to a susceptible goat. It is an interrupted feeder which is mostly abundant near the legs of cattle and horses and can take 2–3 blood meals every day (Prozesky and Barnard, 1982). A study performed to reveal the seasonal pattern of potential dipteran vectors of LSDV in dairy farms; the relative abundance of *S. calcitrans* in affected dairy farms was highest in December, January and April and was highly correlated with the occurrence of LSDV outbreaks. The abundance of other blood-feeding dipterans (e.g. biting midges and mosquitoes), however, was poorly associated with the timing of the outbreaks (Osman, 2011). Grazing beef cattle, during these outbreaks, was mostly affected during the summer months. It was therefore suggested that different flies might serve as vectors in grazing and zero-grazing herds (Sanz-Bernardo et al., 2020). As the abundance of the horn fly *Haematobia irritans* was reported to be high in beef herds during the outbreaks, it was suggested as the potential vector in these settings (Awad et al., 2010). The circumstantial nature of this evidence, as well as the lack of successful transmission of other viruses by this fly, suggest that further studies are necessary before incriminating the horn fly as a potential vector of LSDV (Babiuk, 2018). Several studies have also demonstrated the competence of ticks as vectors of LSDV. Transstadial and mechanical transmission of LSDV was demonstrated in males of *Rhipicephalus appendiculatus* and *Amblyomma hebraeum* (Annandale et al., 2010). This was demonstrated by both virus isolation from the saliva of fed adults, or from adults fed as nymphs on infected cattle, and by transmission of the virus by these ticks to susceptible animals (Issimov et al., 2020; Lubinga et al., 2015). Transovarial transmission of LSDV was shown in *R. decoloratus* ticks. The adults which developed from eggs laid by infected

ticks infected susceptible cattle and caused viremia and mild clinical disease (Sprygin et al., 2019). These evidences suggest that ticks may play an important role as reservoirs of LSDV. Their role in transmission of the virus in large outbreaks of cattle awaits capacity studies and is probably less important as large outbreaks have occurred in zero-grazing cattle where ticks are mostly rare (Gelaye and Lamien, 2019) and as the spread velocity of LSDV epidemics cannot be explained by tick borne transmission (Babiuk et al., 2008a).

2.9 Risk Factors in the Herd Level

The protection conferred by herd vaccination is discussed in detail in other parts of this book. The influence of other factors at the herd level was examined in several studies, which were mainly performed in Africa. The caveat of most of these studies is the poor control for various confounders, such as region, climatic factors and vaccination. Hence the results are quite inconsistent. In a study performed in Ethiopia, herd size was found to be positively associated with the risk for LSD (Abera et al., 2015). The same association was found in Turkey (Şevik and Doğan, 2017a). In another study performed in Ethiopia, feedlot cattle was found to be in higher risk for LSD infection compared to extensively managed herds (Ayelet et al., n.d.) . In Turkey, the incidence in beef herds was higher than in dairy herds, though this difference was not statistically significant (Sevik and Dogan 2016). In Zimbabwe, LSD morbidity was highest in resettlement farms, though the authors explain this finding by higher accessibility of veterinary service in these regions (Gomo et al., 2017).

2.10 Risk factors in the host level

Breeds of zebu type indigenous to Africa are generally less susceptible to infection by LSD and may develop extensive skin lesions but have less severe clinical disease and lower mortality rates than cattle exotic to Africa (Abutarbush, 2017). Similar findings were reported in studies conducted in Ethiopia, Turkey and the Sultanate of Oman (Gari et al., 2010). In these studies, a more severe disease and a higher mortality were observed in European cross breeds, as compared to local breeds. Interestingly, in a study conducted in Ethiopia, similar morbidity rates were observed in zebu cattle and zebu-Holstein cross breeds (Ambilo and Melaku, 2013). However, in the zebu cattle morbidity rate among vaccinated cattle was more than four times higher than among non-vaccinated, while in the cross breeds, vaccine did not show any protective effect. These findings might be the result of non-standardized definition of morbidity and lack of control for various

confounding effects (Ayelet et al., 2014). The factors affecting the wide range of disease are likely to be complex and multifactorial, including the dose of virus inoculate, genetic factors of the host and the virus as well as the immune competence and possibly the age of the host with some studies demonstrating younger animals being more susceptible. With sheep pox and goat pox, younger animals are more susceptible (Ardestani and Mokhtari, 2020). Clinical signs caused by LSDV were demonstrated to be much more severe in high-producing dairy breeds such as Holstein Friesian cattle compared to indigenous breeds (Tageldin et al., 2014).

2.11 Diagnosis of LSD in cattle

Capripoxvirus genus have a general host tropism for their host species, before the molecular diagnostic methods become available, the virus was always classified according to the host it was isolated from. For example, if a Capripox virus was isolated from a sheep, the virus would be called a sheep pox virus; if isolated from a goat, it would be goat pox; and if isolated from cattle, the virus would be classified as a LSDV. This has generally been useful although, it has caused some confusion with certain viruses. Generally, LSDV does not cause disease in sheep and goats. However, there has been one instance where LSDV caused disease in sheep in Kenya (Menasherow et al., 2014). This virus was assumed to be a sheep pox virus; however, genetic sequencing of the Kenyan virus isolated has revealed that was LSDV. Therefore, it must be kept in mind that the general rule of classifying capripox viruses based on the host species where the virus was isolated is not perfect and additional molecular approaches should be used to confirm the virus identity (Aiel, 2009). Several different methods can be used for diagnosis. These include classical methods such as electron microscopy and virus isolation as well as more modern molecular methods including various PCR and real-time PCR, loop-mediated isothermal amplification (LAMP) and DNA sequencing protocols (Gari Jimolu, 2011).

Three conventional PCR assays were developed using the P32 gene as a target (Zeedan et al., 2019). A multiplex PCR-based species-specific primer to differentiate between capripox virus species has been developed (Orlova et al., 2006). A duplex PCR assay was developed to detect both capripox virus and Orf virus using the A29L gene region of capripox virus (Gharban et al., 2019). Although this assay was only evaluated on sheep pox and goat pox viruses, the capripox virus primers in the assay will also amplify LSDV based on sequence homology.

To ensure the proper identity of the capripox virus species, full-length genomic sequencing is the most appropriate method. However due to the cost of full-length sequencing, it is not routinely performed. For this reason molecular epidemiology for capripox viruses is not as advanced compared to many other viruses of veterinary importance

2.12 Differential Diagnosis:

Although the clinical disease presentation and the visceral pox lesions in cattle caused by lumpy skin disease virus (LSDV) are strongly indicative of lumpy skin disease (LSD), a definitive diagnosis requires laboratory confirmation. Milder forms of LSD can be confused with many different agents or diseases (Shalaby et al., 2016). Allergic reactions and physical trauma to the skin caused by insect and/or tick bites as well as urticaria and photosensitization also need to be ruled out. These include differentials of several agents that cause skin lesions including viral agents such as parapox viruses, bovine papular stomatitis virus and pseudo cowpox, orthopoxviruses such as vaccinia and cowpox and bovine herpesvirus 2 causing pseudo lumpy skin disease (Davies et al., 1971). Since rinderpest has been eradicated, it is no longer a differential. Adverse reactions to LSDV vaccines can also occur (so-called Neethling disease) and is characterized by the appearance of skin nodules which are smaller than those caused by virulent LSDV field strain (Erster et al., 2019). Other skin diseases in cattle caused by bacterial agents such as *Hypoderma bovis* infection, cutaneous tuberculosis, dermatophilosis and *Corynebacterium pseudotuberculosis* are also differentials. Additional differentials comprise demodicosis or mange caused by *Demodex bovis* as well as other skin lesions caused by parasites such as onchocercosis caused by *Onchocerca ochengi* or besnoitiosis caused by the protozoa *Besnoitia besnoiti* (Brenner et al., 2009).

2.13 Epidemiology

2.13.1 Spatial epidemiology

Spatial epidemiology is the sub-discipline of epidemiology where the geographical location of the events is the fundamental component (Saez et al., 2007) with the primary purpose is to describe and explain spatial pattern of diseases. Up to 1980s, it was difficult to find examples of spatial epidemiology in the veterinary literature; the exceptions are works undertaken by parasitologists, interested in the interaction between

climate and disease via its effect on vectors and intermediate hosts. One of the first works was conducted by Robson et al. (1961) who showed that the East Coast fever in Tanzania was confined to areas where tsetse flies were absent and cattle was present. Also, in Tanzania (Lake Victoria) by carefully mapping disease outbreaks in relation to the cattle population, a separating line of enzootic and epizootic areas was identified (Kaiser et al., 1988). In human medicine, there are studies dating from the beginning of the 1800s in which maps were employed to demonstrate the distribution of disease (Lawson and Williams, 2001). Possibly the most famous use of mapping in epidemiology in this period were the studies by John Snow of the cholera epidemics in London in 1854 through observation of the addresses of the people who die. Snow was among the first to show clearly that cholera could be spread through a contaminated water supply (Robson and Chapman, 1961) (Figure 2).

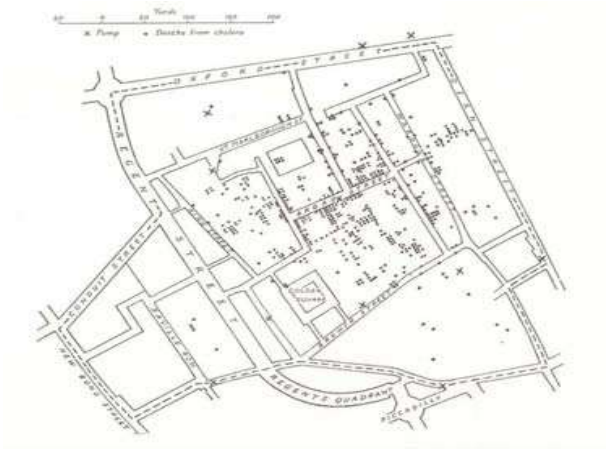

Figure 2: John Snow map of cholera deaths and water supply in London

Advances in geographic information systems (GIS), statistical epidemiology and availability of high-resolution, geographically referenced health and environment data, created new opportunities to investigate geographic variations in disease occurrence (Foody, 2006). GIS, spatial analysis and remote sensing are the main tools employed in spatial epidemiology (Dürr et al., 2013).

2.13.2 Application of spatial epidemiology in veterinary field of Bangladesh

In Bangladesh very few previous reports on spatial epidemiology of large and small animal disease was observed. In case of poultry, first spatial epidemiology of avian influenza outbreak in poultry was described by (Ahmed et al., 2010) in January 2010. In the same year (Loth et al., 2010) applied spatial epidemiology technique to identify the cluster of avian influenza outbreak cluster in Bangladesh was published. Spatial analysis

deals with the exploration, description and analysis of data taking into account their geographical distribution. Spatial data are defined as geographical features and the attributes of these features, each feature will often have multiple attributes (Saez et al., 2007).

2.13.3 Disease mapping

Disease mapping is an approach to summarize spatial variation in disease risk, in order to assess and quantify the amount of true spatial heterogeneity and the associated patterns, to highlight areas of elevated or lowered risk and to obtain clues as to the disease etiology (Best et al., 2005). The detection of disease clusters has typically been come up to as a hypothesis testing problem; whether the geographical distribution of disease or any event is random or not, adjusting for the geographical distribution of the population (Ugarte et al., 2005). Disease mapping methods are most useful for apprehending gradual regional changes in disease rates, and are less useful in detecting abrupt localized changes indicative of clustering (Gangnon and Clayton, 2000). The objectives of presenting the data in map are to identify locations with unusually high or low disease levels, a communal parameter represented is the ratio between the observed and expected cases (Elliott and Wartenberg, 2004).

2.13.4 Data visualization

The results of the statistical procedures are represented visually in mapped form. Hence, some consideration must be given to the purely cartographic issues that affect the representation of geographical information. The type of map presentation depends on the type of data available, either the actual event locations (such as the x-y coordinates) or aggregate data (Pfeiffer et al., 2002).

2.13.5 Point data

To visualize point data**,** the oldest and most frequently-used method is to plot the locations of the study subjects using their Cartesian coordinates. Whereas plots of point events provide a general impression of the spatial characteristics of the process under investigation, they present problems where there are multiple events at the same location since no indication of event density can be appreciated. Because of this, point maps are best suited for displaying location information for small number of events

(Lawson and Williams, 2001). If a continuous surface is to be mapped based on a discrete set of observation points, then interpolation techniques, based on geostatistical methods, must be used (Lawson et al., 2003). Interpolation techniques enable the construction of isopleth maps. These maps show the distribution of spatially continuous phenomena by a logical sequence of tones color that symbolizes equal values. Isolines are often overlaid on top of an isopleth map to indicate threshold value. The point distribution of farms in the study is shown in Figure 3 (Islam et al., 2020)

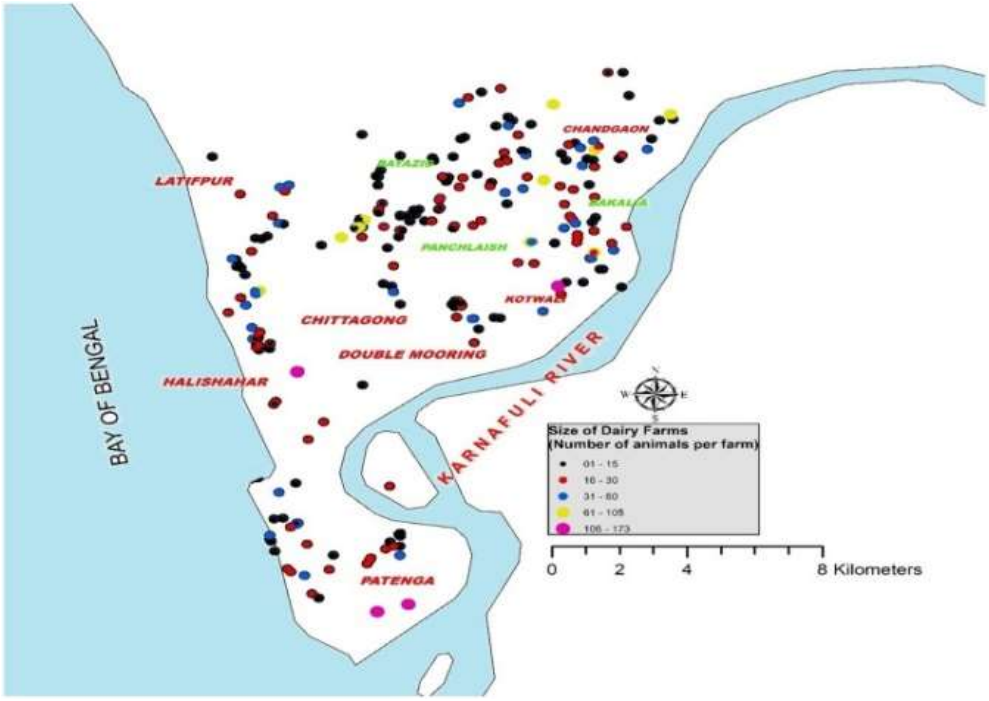

Figure 3: Spatial distribution of the farms inside the study area by point data

CHAPTER-III: MATERIALS AND METHODS

3.1 Ethical approval

Ethical approval was obtained from the institutional ethical approval committee of Chattogram Veterinary and Animal Sciences University (CVASU) [CVASU/Dir (R&E) EC/2019/126(13)].

3.2 Study design

The study was conducted over a period of five months (August to December 2019) in Chattogram district at the onset of the outbreak. A cross-sectional study was designed to collect the samples and individual animal was considered as the sampling unit. A standard questionnaire was used to collect demographic data such as breed, age, sex and other epidemiological data (e.g., introduction of new animals, source of water supply in the farm, etc.). Selected animals were categorized as Holstein Friesian crossbred (*Bos taurus* X *Bos indicus*) and indigenous cattle (*Bos indicus*). Age of the animals was categorized as calf: ≤ 1 year; heifer: >1-≤ 2.5 years for crossbred and >1 - ≤ 3.5 years for indigenous cattle; cow: >2.5 years for crossbred and >3.5 years for indigenous cattle and bull (≥ 1 year) (Alim et al., 2012). Selection of study area and animals were based on the suspected cases reported by the local veterinarians and physical visit to the farms. A total of 19 commercial farms from Chattogram district (6 farms from Pahartali area, 3 farms from Sitakunda and 2 farms from each of Chattogram port, Double Mooring, Hathazari, Panchlaish and Chadgaon area) were selected (Figure. 2). Farms that comprises at least 15 cattle were included into the study. Sample from affected animals were collected randomly from the individual farms with simple randomization techniques. Moreover, a farm was considered positive for ectoparasites (flies, ticks, lice) when an individual animal was infested with any of those parasites.

3.3 Sample collection and preservation

A total of 19 farms having 3327 animals were considered where there were 669 calves, 281 heifers, 2272 cows and 105 bulls. Data were collected by face to face interview of the animal attendants of the particular farm and physical examination of the cattle. Among the clinically ill or suspected cattle (Figure. 4 A & B), a total of 120 skin biopsy from nodular lesions were collected aseptically using punch biopsy techniques (Kasem et al., 2018). Briefly, the biopsy site was shaved by the sterile instruments and followed by a small punch was taken deeply in the skin so that all layers along with the subcutaneous tissue

were detached. Half of the skin biopsy specimen was kept in neutral buffered formalin (10%) for histological examination following routine Hematoxylin and Eosin (H&E) staining (Luna, 1968). Rest half of the skin biopsy samples were subsequently preserved in -20^{0}C for molecular confirmation of the virus.

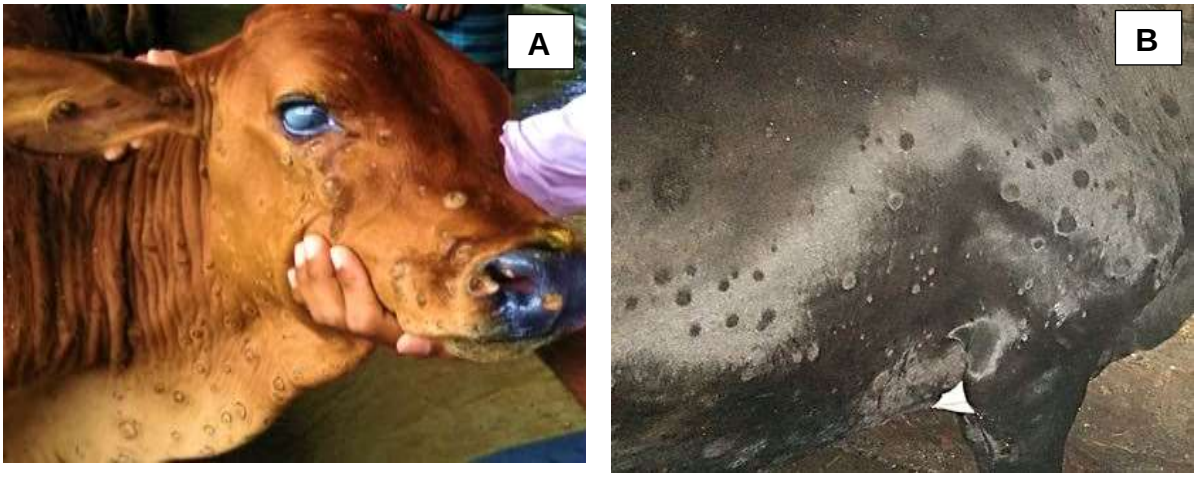

Figure 4A & B: Nodular lesions all over the body of LSD affected cattle.

3.4 DNA extraction and PCR confirmation of Lumpy skin disease virus

Total genomic DNA was extracted from all suspected skin biopsies using commercially available kits following manufacturer's instruction with some modifications (DNeasy Blood & Tissue Kits®, Qiagen, Germany). Polymerase chain reaction (PCR) was performed to confirm LSDV using a previously reported primer set (forward; GTGGAAGCCAATTAAGTAGA and reverse; GTAAGAGGGACATTAGTTCT) targeting the inverted repeat region (ITR) of the genome (Stram et al., 2008). In brief, The PCR reaction was set up in a 50 µL final volumes containing 25 µL master mix, 2.5 µL forward primer, 2.5 µL reverse primer, 5.0 µL DNA template and 15 µL nuclease free water. The PCR conditions had an initial denaturation step of 95°C for 1 min followed by 35 cycles of denaturation at 94°C for 30s, annealing at 49°C for 30s, extension at 72°C for 70s and a final extension step at 72°C for 5 min. Finally, 5 µL of amplified amplicons were taken and stained using 1% ethidium bromide followed visualization of the band (1237bp) after agarose gel (1%) electrophoresis (Figure 5).

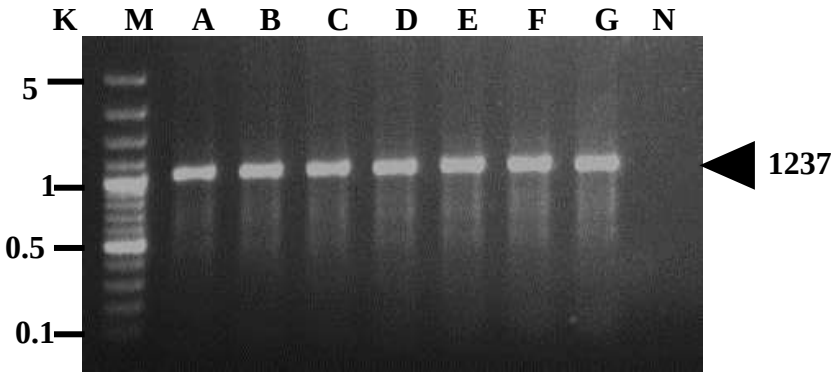

Figure 5: Agarose PCR showed 1237bp band size specific for LSD. (M= 100bp plus ladder; A-G= Positive samples, NC= Negative control)

3.5 Nucleotide sequencing and phylogenetic analysis

Four randomly selected LSDV positive PCR amplicons were gel purified using Wizard® SV Gel and PCR Clean-Up System (Promega, USA) and sequenced by sanger dideoxy sequencing (Macrogen®, Korea). The sequence read data were then manually cleaned up using chromatogram and deposited in GenBank to obtain accession number. NCBI BLAST was performed for each of the sequence reads and the ITR region from a diverse range related LSDV and other poxvirus genome sequences (Nt sequence identity 100%-70%) were retrieved (N=63) and aligned using MAFFT v7.017 using G-INS-i (gap open penalty 1.53; offset value 0.123) alignment algorithm (Katoh et al., 2002). The programme jModelTest 2.1.3 favoured a general-time-reversible model with gamma distribution rate variation and a proportion of invariable sites (GTR+I+G4) for the phylogeny (Darriba et al., 2012). Maximum-likelihood (ML) phylogenetic trees was reconstructed using the program PhyML v3.1 (Guindon and Gascuel, 2003) and FigTree v1.4 was used to generate the consensus tree (Smith et al., 2009). The Genbank accession number, organism name, host, collection date and sampling country were used in parenthesis within the tree taxa. The proportion of bootstrap support (%) was demonstrated in each branch while multiple taxa showing polytomy and closely related isolates were collapsed for better visualization. The Bangladeshi isolates (CVASU) of LSDV were shown in blue taxa (Fig 7)

3.6 Statistical analysis

All data were inserted and coded in Microsoft office Excel 2016 spread sheet and both univariable and multivariable analysis was performed using generalized linear mixed models in STATA-IC 13. Farm was included in the model as random effect. Backward elimination procedure was followed and a p-value ≤ 0.05 was considered significant in both univariable and multivariable model. Prevalence map along with location and size of the farm was created using QGIS 3.12.0 (Fig 6).

CHAPTER- IV: RESULTS

4.1 Prevalence of Lumpy skin disease

Among the 19 farms having 3327 animals, the overall prevalence of LSD disease was 10% (confidence interval: 9.4 to 11%). The farm level highest frequency was 63.33% in one of the farms located in Chadgaon region and the lowest was 4.22% in a farm at Sitakunda region of Chattogram district (Figure 2). The prevalence ranges from 20-42% in farms of Chadgaon, Double mooring, Pahartali, Hathazari regions and it was below 20% in farms at Sitakunda and Chattogram port of the study area (Figure 6).

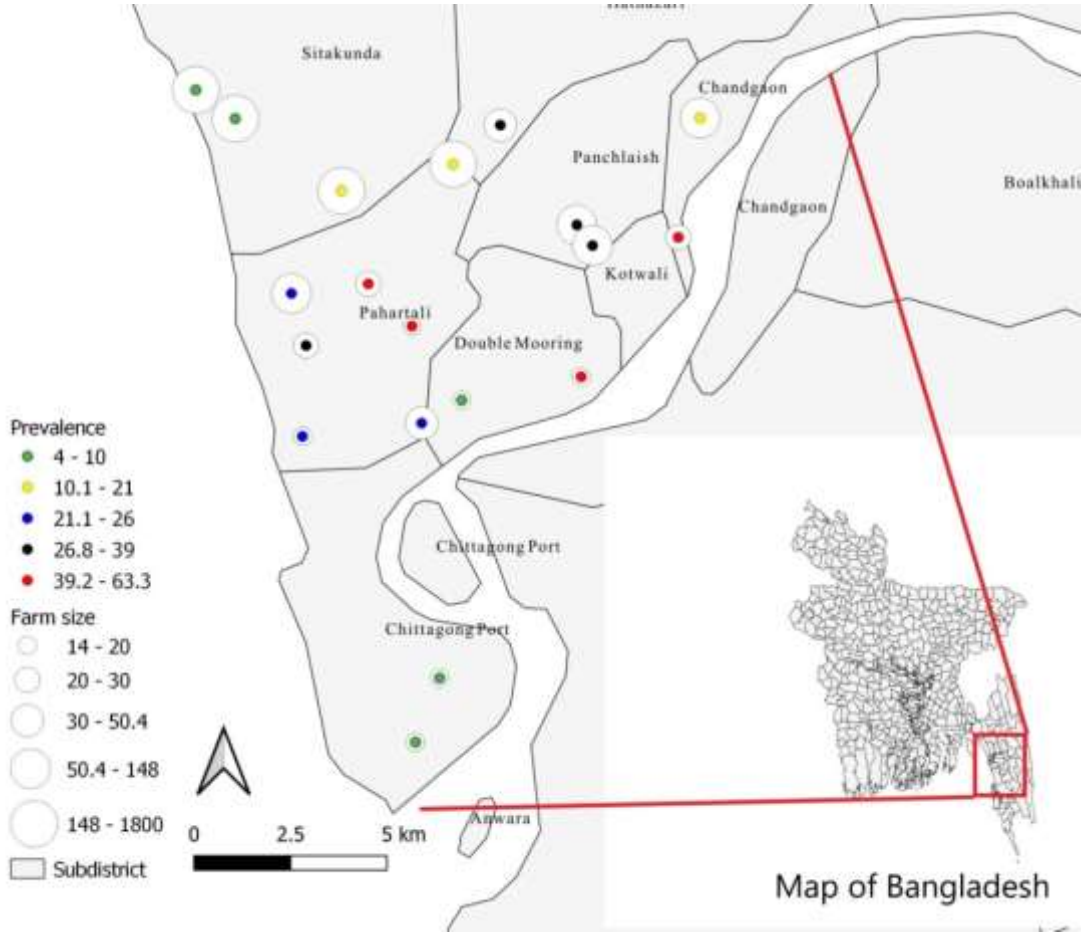

Figure 6: Map showing the location and farm size (circle) along with the number infected animals (farm level frequency, %) in each farm.

4.2 Risk factors associated with the occurrence of Lumpy skin disease

Prevalence was observed the lowest in bulls (5%). Univariable analysis showed that Odds Ratio (OR) of having the disease in calves, cows and heifers were 1.37, 2.52, 3.51 times higher compared to bulls, respectively (Table 1). Females were more susceptible

(OR=2.26) than the male. In terms of lactation, with increasing lactation number decrease in prevalence was observed; odds of having the disease in 1st lactation was 7 times higher compared to 4th lactation. The univariable analysis also showed that local cattle were less susceptible than the crossbred. Besides, introduction of new animals, sources of water supply and floor types in the farm act as potential risk factors the disease (Table 1). In multivariable model, crossbred (p=0.0080, OR=3.58) and female (p= <0.0001, OR=3.96) cattle had significantly higher chance of getting the disease compared to their counterparts (Table 2).

Table 1: Risk factors associated with Lumpy skin disease in cattle farms of Chattogram metropolitan area from the univariable logistic regression analysis

Variables	**Level**	**N (Animals)**	**Positive**	**OR**	**p-value**
			N (%)		
		3327	345 (10)		
Breed	Cross	3220	340 (11)	2.40	0.0500
	Local	107	5 (5)	Ref	
Types of animal	Calf	669	43 (6)	1.37	<0.0001
	Heifer	281	42 (15)	3.51	
	Cow	2272	255 (11)	2.52	
	Bull	105	5 (5)	Ref	
Sex	Female	3071	332 (11)	2.26	0.0040
	Male	256	13 (5)	Ref	
*Lactation	1	107	98 (92)	7.70	<0.0001
	2	267	105 (39)	4.25	
	3	1780	50 (3)	1.10	
	4	118	2 (2)	Ref	
Introduction of new animal	Yes	62	13 (21)	2.34	0.0070
	No	3265	332 (10)	Ref	
Water source	Pond	50	14 (28)	3.46	<0.0001

	Underground (tubewell)	3277	331 (10)	Ref	
Floor	Brick	72	13 (18)	1.93	0.0300
	Cemented	3255	332 (10)	Ref	

*OR calculated only including lactating cow; OR=Odds ratio

Table 2: Risk factors associated with Lumpy skin disease in cattle farms of Chattogram district from the multivariable generalized linear mixed model (logistic regression) analysis.

Variables	**Level**	**Estimates**	**SE****	**OR***	**CI (95%)**	**p-value**
Intercept		-3.71				
Breed	Cross	1.277	0.605	3.58	1.40-9.17	0.0080
	Local	0		Ref		
Sex	Female	1.377	0.479	3.96	2.16-7.27	<0.0001
	Male	0		Ref		
Random effect of farm		1.003	0.186			

*OR = Odds Ratio, **SE = Standard Error of the mean, CI=Confidence Interval

4.3 Molecular identification of Lumpy skin disease virus

All of the collected skin biopsies were PCR positive for LSDV. Among them, a total of 4 (four) samples were sequenced randomly for obtaining GenBank accession (MT070969-72) and subsequent phylogenetic analyses. The ML tree reconstructed from the inverted terminal repeat region (ITRs) of closely related poxviruses revealed that most LSD_CVASU isolates belong to a strongly supported (100% bootstrap value) clade dominated by LSDV strains. LSDV isolated from different parts of the world (mostly Africa and Middle East) over three decades (1997-2019) timeframe. Isolate LSD_CVASU_M1 and M3 (MT070969 and MT070971) together formed a sister branch to the LSDV isolate from Egypt (EU350218) with a moderate bootstrap support (55%) while for isolate M4 (MT070972) the phylogenetic resolution was not clear and demonstrated some relatedness (58% bootstrap support) with Sheep pox reference sequence (CAPIS1ITR) (Figure. 3). Isolate M2 on the other hand showed stronger bootstrap support (80%) towards recent isolates of LSDV from Egypt (KF588352,

KR052866 and KF58835). The phylogenetic reconstruction thus reaffirm that the viral isolates from the nodular skin biopsies were LSDV genotypes most closely related to those from Egypt (Figure. 7).

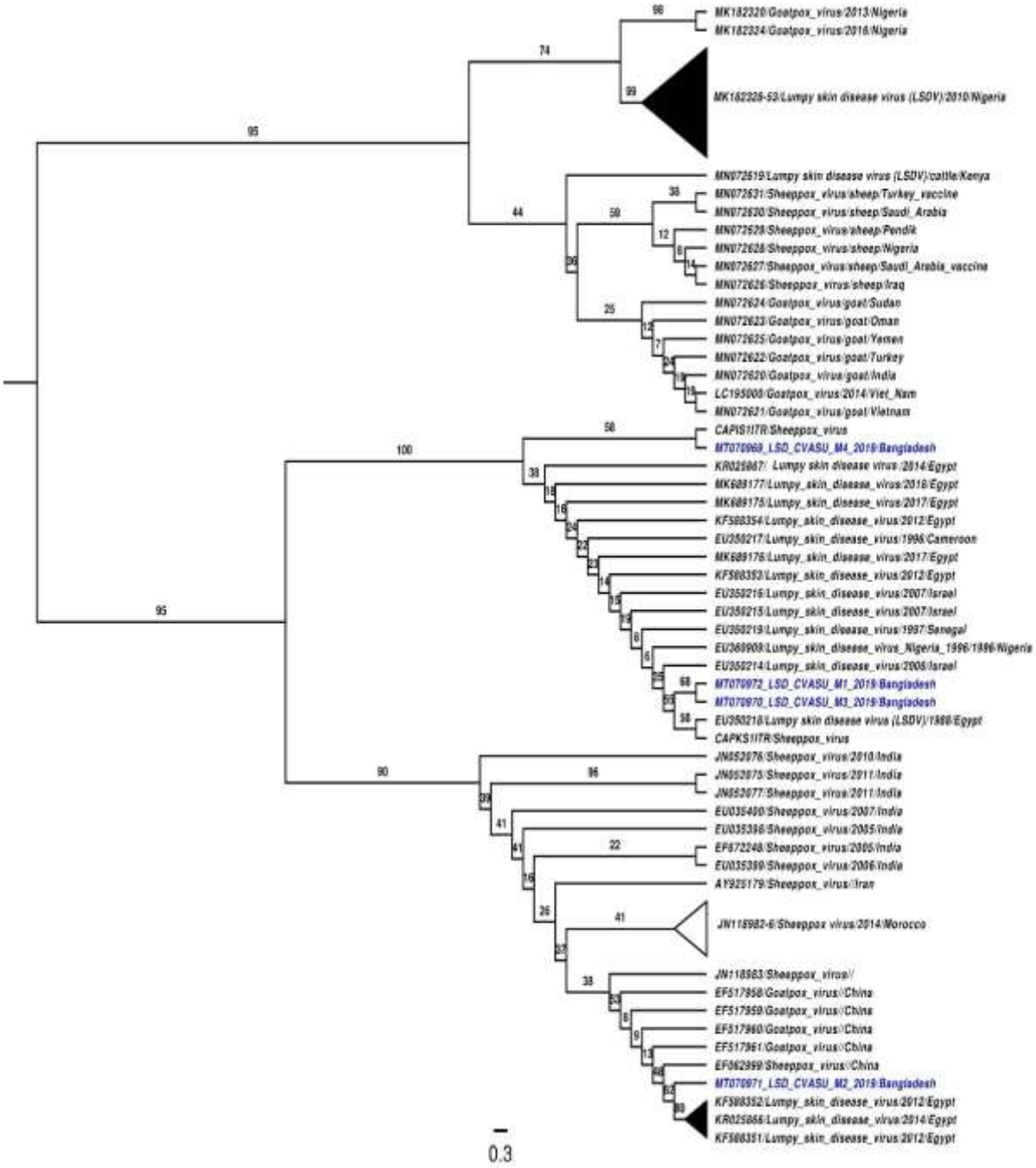

Figure 7**:** Maximum likelihood (ML) tree rooted at midpoint branches based on the ITR region of poxvirus genome demonstrating phylogenetic relatedness of LSDV isolates form Chattogram, Bangladesh (blue taxa). Clades suggesting polytomies were collapsed and shown in cartoon, the bootstrap statistics (percentage) were shown as branch support numbers.

4.4 Histological features of the skin biopsies (nodules)

The histological features of the skin biopsies include granulomatous and pyogranulomatous dermatitis with multifocal to diffuse deep dermal necrosis and panniculitis (Figure 8). In majority of the cases, the superficial and deep dermis had moderate to severe perivascular and periadnexal infiltrates of lymphocytes, plasma cells, macrophages and fewer neutrophils. However, in many cases there were multifocal abscessation formed by a lytic necrotic center, degenerative neutrophils, multiple layers of mononuclear infiltration and encapsulating fibroplasia. The subcutaneous tissue and pannicular fat were spared in most cases, however, in few animals there were pannicular infarction and vasculitis with infiltration of macrophages, lymphocytes and plasma cells. In the overlying epidermis, acanthosis and orthokertatotic hyperkeratosis were common in most cases with few exceptions of ulceration and acute neutrophilic infiltration in the superficial dermis. Hair follicles of the skin biopsies were partially destroyed and replaced with necrotic epithelium, mixed cellular infiltrates, and hair debris (furunculosis).

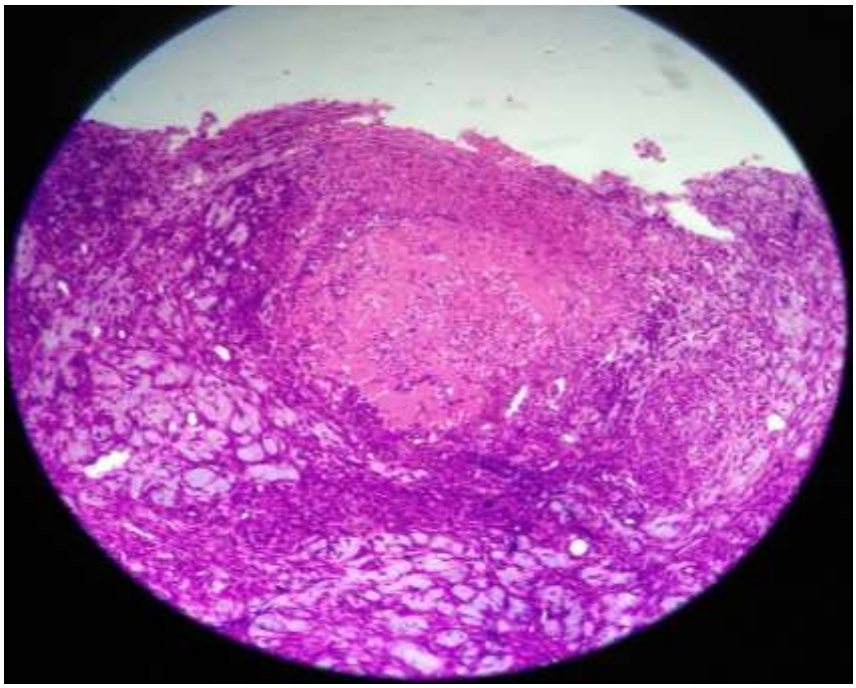

Figure 8: Histological feature of LSD affected nodular lesions. Figure demonstrated deep dermis and sub cutis; focal granulomatous lesion comprised of necrotic debris and encircling mononuclear cell infiltration. [Magnification of image 100X]

CHAPTER-V: DISCUSSION

Bangladesh was free of LSD before mid of 2019 and the very first LSDV infection was reported in Anwara, Karnaphuli and Patiya upazila, Chattogram to OIE in August 2019 (Anonymous, 2019a). This investigation summarises the clinical outbreaks of LSD in the commercial cattle population in Chattogram district unrevealing the disease burden and associated risk factors. The epidemiological data was supported by histopathological features of the clinically characteristic nodular skin lesions as well as PCR based molecular identification and phylogenetic analyses.

The overall prevalence of LSD in Chattogram district was 10% similar to some previous studied in Saudi Arabia, Ethiopia and Turkey who reported around 6-12% prevalence in their cattle population (Kasem et al., 2018; Şevik and Doğan, 2017; Abera et al., 2015; Al-Salihi and Hassan, 2015). Body and colleagues (2012) observed a much higher prevalence (27.9%) in cattle of Oman which was higher than the overall prevalence of the current study. The higher or lower prevalence of disease might have been influenced by many factors such as geography, farm management and biosecurity, seasons, availability of arthropods vectors, trading of animals in and out of the country, disposal of the dead animals. Although we didn't observe any mortality in the study population, some of the previous studies reported 0.99 to 2.12% of mortality (Kasem et al., 2018; Gari et al., 2010). Comparatively shorter duration of the actual study period might be a reason for the paucity of mortality. However, clinical form of LSD is a production disease and generally associated with economic loss in terms of production and treatment expenditure (Babiuk et al., 2008).

Analysis of risk factors in this study indicated that crossbred cattle are more susceptible to LSD than local indigenous cattle which was consistent with the findings of previous research (Klement, 2018; Rammahi and Jassim, 2015). Higher susceptibility of crossbred cattle might be due to lower disease resistance capability in comparison to indigenous breeds (Tageldin et al., 2014). The higher number of crossbred animals (96.79%) was more compared to local (3.21%) cattle that might explain the variation of the results. Heifers were affected largely with LSD in comparison to bulls, calves and cows. In previous studies higher morbidity was recorded in younger cattle (< 2 years) (Kasem et al., 2018) and calves (0.5-1 year) (Molla et al., 2018). This might be due to management system of the farms where heifer was kept in poor hygienic condition in comparison to other animals (calf, cows or bull), moreover, less nutrition were given to the heifer due to non-productivity. Female animals were more prone to LSD compared to males which was

consistent with previous research (Ayelet et al., 2014; Magori-Cohen et al., 2012; Salib and Osman, 2011). Higher frequency of LSD in female cattle could be due to their exposure to many stress conditions e.g., pregnancy, parturition and sometimes less amount of feed supplied compared to their actual requipment (Kasem et al., 2018). However, higher number of the female animals during sampling could be another reason of higher frequency of such disease in female. We observed an inverse relationship with lactation number in the occurrence of LSD in cattle. Farm specific risk factors such as introduction of new animals to the farm demonstrated a significantly higher chance to be infected with the virus or its transmission which was supported by the previous findings (Gari et al., 2010; Munyeme et al., 2008; Macpherson, 1994).

The histopathological features of the suspected nodular skin biopsies demonstrated granulomatous and pyogranulomatous dermatitis with occasional vasculitis and pannicular involvement (Figure 4) which are merely non-specific lesions. However, similar histological features of suspected skin lesions were documented in many prior studies with confirmed LSD cases (Abdallah et al., 2018; El-khabaz, 2014; Body et al., 2012; Stram et al., 2008). Unsurprisingly, we could not identify any intracytoplasmic inclusion bodes or so-called ‘sheep pox’ cells (SPCs) or ‘cellules claveleuses’ of Borrel in any of the skin biopsies (El-Neweshy et al., 2013). Although SPCs or intracytoplasmic inclusions often considered confirmatory histopathological findings for LSD (Abdallah et al., 2018; Body et al., 2012), they are rarely found in natural LSD cases (El-Neweshy et al., 2013; House et al., 1990) and often associated only with acute phage infections. In the present study, all tissue sections were stained with H&E and the histological features leaned towards subacute to chronic stage infections. Future studies should incorporate immunohistochemistry of the tissue section using anti LSDV monoclonal antibodies to reveal replicating virus particles in macrophages and epithelial cells of dermis.

The PCR based molecular test targeting ITR region of the LSDV have successfully confirmed all suspected cases of LSD in this study and the local genotype circulating in Chattogram district was deposited in GenBank as well (Gene bank accession no. MT070969-MT070972). The ML tree reconstructed from the ITR region of all related poxviruses suggested that the LSDV strains circulating in Bangladesh are closely related to that in Middle East and North Africa as 3 out of 4 sequences had closest phylogenetic relationship with isolates from Egypt. However, the ITR region of the genome used for amplification and sequencing of LSDV is a pseudogene, relatively conserved and

homologous to many other poxvirus genomes (Gershoni and Black, 1989). Therefore, the phylogenetic reconstruction had lack of discriminatory resolution, as presented by relatively low bootstrap supports in many branches and positioning LSDV genotypes with sheep pox and goat poxvirus strains in some clades (Figure 3). This limitation might have imposed a negative implication defining the possible source of the outbreak based on evolutionary relatedness of geographically distant strains. Further studies should incorporate sequencing at least three different core gene groups along with concatenation or partitioning approach for alignment and subsequent phylogenetic analyses to reconstruct a comprehensive evolutionary tree with better discriminatory power and resolution.

In Bangladesh, there was no previous outbreak of LSD in any of the susceptible species including cattle. Many factors might have been involved with the current outbreak and transmission across the country. There are both legal and illegal cattle trading occurs every year from neighboring countries; India and Myanmar. Further, throughout the year livestock mobility across the country is quite high which usually reaches its peak during Eid-ul-Adha (a holy festival of Muslim) as thousands of temporary wet markets are established to meet the demand (Anik, 2019). Notable that this outbreak was reported just a month after festival mentioned. It is plausible that unregulated and illegal import of live animal without prior health check or quarantine measures have embarked the clinical outbreak of LSD. Unrestricted in-country movements of livestock even after the first reporting might have significantly aggravated the viral transmission (Tuppurainen, E., Alexandrov, T. & Beltrán-Alcrudo, 2017) However, there was also an outbreak of such disease occurred in China and Odisha of India in August, 2019 (Sudhakar et al., 2020; Anonymus, 2019) and this could be an unexplored link to this outbreak as cattle movements were speculated as a risk factor (Klausner et al., 2017). Within farm LSDV transmission is further related with the biosecurity measures and other management practices in the farms. We found a positive co-relation between the communal water supply as well as the floor made of brick as observed by others (Tuppurainen and Oura, 2012; Babiuk et al., 2008). Although we didn't check for individual animals for presence of ectoparasites but, overall, we have observed those in all most all the farms which may play a role in the transmission of this virus reported by previous research (Ince et al., 2016). Future research should be directed for identification of the specific vectors to overcome the limitation of this study.

CHAPTER-VI: CONCLUSION

The current study investigated the outbreak of LSDV infection in commercial farms of Southern Bangladesh unveiling the overall prevalence and risk factors associated with the disease. This study also suggests a plausible source of the outbreak based on limited genomic data and evolutionary assays. As there is no effective vaccine against this economically important disease, further research should be focused on the molecular characterization of the whole genome of the local strain of LSDV to promote development of a suitable vaccine candidate. The data generated in this study would be beneficial to the veterinary practice in Bangladesh. The findings will guide us to take appropriate measures to prevent further relapse or outbreak of this disease in future.

CHAPTER-VII: REFERENCES

Abdallah, F.M., El Damaty, H.M., Kotb, G.F., 2018. Sporadic cases of lumpy skin disease among cattle in Sharkia province, Egypt: Genetic characterization of lumpy skin disease virus isolates and pathological findings. Vet. World 11, 1150–1158. https://doi.org/10.14202/vetworld.2018.1150-1158

Abdulqa, H.Y., Rahman, H.S., Dyary, H.O., Hasan, H., 2016. iMedPub Journals Reproductive Immunology : Open Access Lumpy Skin Disease 1–6. https://doi.org/10.21767/2476-1974.100025

Abera, Z., Degefu, H., Gari, G., Kidane, M., 2015. Sero-prevalence of lumpy skin disease in selected districts of West Wollega zone, Ethiopia. BMC Vet. Res. 11, 1–9. https://doi.org/10.1186/s12917-015-0432-7

Abutarbush, S.M., 2017. Lumpy skin disease (knopvelsiekte, pseudo-urticaria, neethling virus disease, exanthema nodularis bovis), in: Emerging and Re-Emerging Infectious Diseases of Livestock. Springer International Publishing, pp. 309–326. https://doi.org/10.1007/978-3-319-47426-7_14

Agianniotaki, E.I., Babiuk, S., Katsoulos, P.-D., Chaintoutis, S.C., Praxitelous, A., Quizon, K., Boscos, C., Polizopoulou, Z.S., Chondrokouki, E.D., Dovas, C.I., 2018. Colostrum transfer of neutralizing antibodies against lumpy skin disease virus from vaccinated cows to their calves. Transbound. Emerg. Dis. 65, 2043–2048. https://doi.org/10.1111/tbed.12983

Ahmed, S.S.U., Ersbøll, A.K., Biswas, P.K. and Christensen, J.P., 2010. The space–time clustering of highly pathogenic avian influenza (HPAI) H5N1 outbreaks in Bangladesh. *Epidemiology & Infection*, *138*(6), pp.843-852

Ahmed, W.M., Zaher, K.S., 2008. Observations on lumpy skin disease in local Egyptian cows with emphasis on its impact on ovarian function. African J. Microbiol. Res. 2, 252–257.

Aiel, K., 2009. Lumpy skin disease. Bov. & Ovine 22–67.

Al-Salihi, K.A., Hassan, I.Q., 2015. Lumpy Skin Disease in Iraq: Study of the Disease Emergence. Transbound. Emerg. Dis. 62, 457–462. https://doi.org/10.1111/tbed.12386

Aleksandr, K., Olga, B., David, W.B., Pavel, P., Yana, P., Svetlana, K., Alexander, N., Vladimir, R., Dmitriy, L., Alexander, S., 2020. Non-vector-borne transmission of lumpy skin disease virus. Sci. Rep. 10, 1–12. https://doi.org/10.1038/s41598-020-64029-w

Alemayehu, G., Zewde, G., Admassu, B., 2013. Risk assessments of lumpy skin diseases in Borena bull market chain and its implication for livelihoods and international trade. Trop. Anim. Health Prod. 45, 1153–1159. https://doi.org/10.1007/s11250-012-0340-9

Alexander, R., Plowright, W., Africa, D.H.-B. of epizootic diseases of, 1957, undefined, n.d. Cytopathogenic agents associated with lumpy skin disease of cattle.

Ali, BH, H.O., 1977. Investigation of the first outbreaks of lumpy skin disease in the Sudan. Elsevier.

Alim MA, Das S, Roy K, Masuduzzaman M, Sikder S, Hassan MM, S.A.& H.M., 2012. Prevalence of hemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. Pak. Vet. J. 32, 221–224.

Alkhamis, M.A., VanderWaal, K., 2016. Spatial and temporal epidemiology of lumpy skin disease in the Middle East, 2012-2015. Front. Vet. Sci. 3, 1–12. https://doi.org/10.3389/fvets.2016.00019

Allepuz, A., Casal, J., Beltrán-Alcrudo, D., 2019. Spatial analysis of lumpy skin disease in Eurasia—Predicting areas at risk for further spread within the region. Transbound. Emerg. Dis. 66, 813–822. https://doi.org/10.1111/tbed.13090

Ambilo, A., Melaku, A., 2013. Major Skin Diseases of Cattle: Prevalence and Risk Factors in and around Hawassa, Southern Ethiopia, Journal of Advanced Veterinary Research.

Anik, S.B., 2019. India's ban on cattle export: A blessing for Bangladesh. Dly. Dhaka Trib.

Annandale, C., Irons, P., Bagla, V., Osuagwuh, U., Venter, E., 2010. Sites of Persistence of Lumpy Skin Disease Virus in the Genital Tract of Experimentally Infected Bulls. Reprod. Domest. Anim. 45, 250–255. https://doi.org/10.1111/j.1439-0531.2008.01274.x

Anonymous. (2019a). *Situation Report: Lumpy skin disease in Bangladesh*. https://fscluster.org/sites/default/files/documents/sitrep_lsd_20191210.pdf

Anonymous. (2019b). *Lumpy skin disease worries Meherpur cattle farmers | The Daily Star*. https://www.thedailystar.net/country/news/lumpy-skin-disease-worries-meherpur-cattle-farmers-1834018

Ardestani, E.G., Mokhtari, A., 2020. Modeling the lumpy skin disease risk probability in central Zagros Mountains of Iran. Prev. Vet. Med. 176, 104887. https://doi.org/10.1016/j.prevetmed.2020.104887

Aspden, K., Van Dijk, A.A., Bingham, J., Cox, D., Passmore, J.A., Williamson, A.L., 2002. Immunogenicity of a recombinant lumpy skin disease virus (neethling vaccine strain) expressing the rabies virus glycoprotein in cattle. Vaccine 20, 2693–2701. https://doi.org/10.1016/S0264-410X(02)00203-7

Awad, W.S., Ibrahim, A.K., Mahran, K., Fararh, K.M., Moniem, M.I.A., 2010. Evaluation of different diagnostic methods for diagnosis of Lumpy skin disease in cows. Trop. Anim. Health Prod. 42, 777–783. https://doi.org/10.1007/s11250-009-9486-5

Awadin, W., Hussein, H., Elseady, Y., Babiuk, S., Furuoka, H., 2011. Detection of Lumpy Skin Disease Virus Antigen and Genomic DNA in Formalin-Fixed Paraffin-Embedded Tissues from an Egyptian Outbreak in 2006. Transbound. Emerg. Dis. 58, 451–457. https://doi.org/10.1111/j.1865-1682.2011.01238.x

Ayelet, G., Haftu, R., Jemberie, S., Belay, A., Gelaye, E., Sibhat, B., Skjerve, E. and Asmare, K., 2014. Lumpy skin disease in cattle in central Ethiopia: outbreak investigation and isolation and molecular detection of the virus. *Rev. Sci. Tech*, *33*(3), pp.877-87

Ayelet, G., Haftu, R., Jemberie, S., Belay, A., Gelaye, E., Sibhat, B., Skjerve, E. and Asmare, K., 2014. Lumpy skin disease in cattle in central Ethiopia: outbreak investigation and isolation and molecular detection of the virus. *Rev. Sci. Tech*, *33*(3), pp.877-87

Babiuk, S., 2018. Replication in a host, in: Lumpy Skin Disease. Springer International Publishing, pp. 37–40. https://doi.org/10.1007/978-3-319-92411-3_9

Babiuk, S., Bowden, T.R., Boyle, D.B., Wallace, D.B., Kitching, R.P., 2008a. Capripoxviruses: An Emerging Worldwide Threat to Sheep, Goats and Cattle. Transbound. Emerg. Dis. 55, 263–272. https://doi.org/10.1111/j.1865-

1682.2008.01043.x

Babiuk, S., Bowden, T.R., Parkyn, G., Dalman, B., Manning, L., Neufeld, J., Embury-Hyatt, C., Copps, J., Boyle, D.B., 2008b. Quantification of Lumpy Skin Disease Virus Following Experimental Infection in Cattle. Transbound. Emerg. Dis. 55, 299–307. https://doi.org/10.1111/j.1865-1682.2008.01024.x

Babiuk, S., Bowden, T.R., Parkyn, G., Dalman, B., Manning, L., Neufeld, J., Embury-Hyatt, C., Copps, J., Boyle, D.B., 2008c. Quantification of Lumpy Skin Disease Virus Following Experimental Infection in Cattle. Transbound. Emerg. Dis. 55, 299–307. https://doi.org/10.1111/j.1865-1682.2008.01024.x

Banglapedia, 2019. Banglapedia [WWW Document]. URL http://en.banglapedia.org/index.php?title=Livestock (accessed 9.19.20).

Bedeković, T., Šimić, I., Krešić, N., Lojkić, I., 2018. Detection of lumpy skin disease virus in skin lesions, blood, nasal swabs and milk following preventive vaccination. Transbound. Emerg. Dis. 65, 491–496. https://doi.org/10.1111/tbed.12730

Best, N., Richardson, S., Thomson, A., 2005. A comparison of Bayesian spatial models for disease mapping. Stat. Methods Med. Res. https://doi.org/10.1191/0962280205sm388oa

Body, M., Singh, P.K., Hussain, H.M., Al-rawahi, A., Al-maawali, M., Al-lamki, K., Al-habsy, S., 2012. Clinico-histopathological findings and PCR based diagnosis of lumpy skin disease in the Sultanate of Oman. Pak. Vet. J. 32, 206–210.

Boshra, H., Truong, T., Nfon, C., Bowden, T.R., Gerdts, V., Tikoo, S., Babiuk, L.A., Kara, P., Mather, A., Wallace, D.B., Babiuk, S., 2015. A lumpy skin disease virus deficient of an IL-10 gene homologue provides protective immunity against virulent capripoxvirus challenge in sheep and goats. Antiviral Res. 123, 39–49. https://doi.org/10.1016/j.antiviral.2015.08.016

Bowden, T.R., Coupar, B.E., Babiuk, S.L., White, J.R., Boyd, V., Duch, C.J., Shiell, B.J., Ueda, N., Parkyn, G.R., Copps, J.S., Boyle, D.B., 2009. Detection of antibodies specific for sheeppox and goatpox viruses using recombinant capripoxvirus antigens in an indirect enzyme-linked immunosorbent assay. J. Virol. Methods 161, 19–29. https://doi.org/10.1016/j.jviromet.2009.04.031

Brenner, J., Bellaiche, M., Gross, E., Elad, D., Oved, Z., Haimovitz, M., Wasserman, A.,

Friedgut, O., Stram, Y., Bumbarov, V., Yadin, H., 2009. Appearance of skin lesions in cattle populations vaccinated against lumpy skin disease: Statutory challenge. Vaccine 27, 1500–1503. https://doi.org/10.1016/j.vaccine.2009.01.020

Chihota, C.M., Rennie, L.F., Kitching, R.P., Mellor, P.S., 2001. Mechanical transmission of lumpy skin disease virus by Aedes. Epidemiol. Infect. 126, 317–321. https://doi.org/DOI: https://doi.org/10.1017/S0950268801005179

Chihota, C. M., Rennie, L.F., Kitching, R.P., Mellor, P.S., 2001. Mechanical transmission of lumpy skin disease virus by Aedes aegypti (Diptera: Culicidae). Epidemiol. Infect. 126, 317–321. https://doi.org/10.1017/S0950268801005179

Coetzer, J.A.W. and Tuppurainen, E., 2004. Lumpy skin disease. Infectious diseases of livestock. Oxford Univ. Press 1268–1276.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. JModelTest 2: More models, new heuristics and parallel computing. Nat. Methods. https://doi.org/10.1038/nmeth.2109

Davies, F.G., 1991. Lumpy skin disease, an African capripox virus disease of cattle. Br. Vet. J. https://doi.org/10.1016/0007-1935(91)90019-J

Davies, F.G., Krauss, H., Lund, J., Taylor, M., 1971. The laboratory diagnosis of lumpy skin disease. Res. Vet. Sci. 12, 123–127. https://doi.org/10.1016/s0034-5288(18)34204-8

DLS, 2019a. Livestock Economy [WWW Document]. URL http://www.dls.gov.bd/site/page/22b1143b-9323-44f8-bfd8-647087828c9b/Livestock-Economy (accessed 9.19.20).

Dürr, S., Bonfoh, B., Schelling, E., Kasymbekov, J., Doherr, M.G., Toktobaev, N., Schueth, T. and Zinsstag, J., 2013. Bayesian estimation of the seroprevalence of brucellosis in humans and livestock in Kyrgyzstan. *Revue scientifique et technique (International Office of Epizootics)*, *32*(3), p.801

EFSA, 2015. Scientific Opinion on lumpy skin disease. EFSA J. 13, 3986. https://doi.org/10.2903/j.efsa.2015.3986

El-kenawy, A.A., and E.-T., 2011. Lumpy skin disease virus identification in different tissues of naturally infected cattle and chorioallantoic membrane(CAMs) of

unembryonated chicken eggs using immunofluorescence, immunoperoxidase techniques and polymerase chain reaction. Int. J. Virol. 7, 158–166.

El-khabaz, K.A.S., 2014. Rapid Laboratory Diagnosis of Lumpy Skin Disease By Using Pcr Technique. Assiut Vet. Med. J. 60, 37–41.

El-Mandrawy, S.A.M., Alam, R.T.M., 2018. Hematological, biochemical and oxidative stress studies of lumpy skin disease virus infection in cattle. J. Appl. Anim. Res. 46, 1073–1077. https://doi.org/10.1080/09712119.2018.1461629

El-Neweshy, M.S., El-Shemey, T.M. and Youssef, S.A., 2013. Pathologic and immunohistochemical findings of natural lumpy skin disease in Egyptian cattle. Pak. Vet. J. 33, 60–64.

Elliott, P., Wartenberg, D., 2004. Spatial epidemiology: Current approaches and future challenges. Environ. Health Perspect. https://doi.org/10.1289/ehp.6735

Erster, O., Rubinstein, M.G., Menasherow, S., Ivanova, E., Venter, E., Šekler, M., Kolarevic, M., Stram, Y., 2019. Importance of the lumpy skin disease virus (LSDV) LSDV126 gene in differential diagnosis and epidemiology and its possible involvement in attenuation. Arch. Virol. 164, 2285–2295. https://doi.org/10.1007/s00705-019-04327-5

Fauquet, C., Mayo, M., Maniloff, J., Desselberger, U., 2005. Virus taxonomy: VIIIth report of the International Committee on Taxonomy of Viruses.

Foody, G.M., 2006. GIS: health applications. Prog. Phys. Geogr. Earth Environ. 30, 691–695. https://doi.org/10.1177/0309133306071152

G.F. El-bagoury, M.E.I.R.E.M.E.-N.A.S.E.-H., 2011. Isolation and Identification of Lumpy Skin Disease Virus from Naturally Infected Buffaloes at Kaluobia, Egypt. Glob. Vet. 7, 234–237.

Gangnon, R.E., Clayton, M.K., 2000. Bayesian detection and modeling of spatial disease clustering. Biometrics. https://doi.org/10.1111/j.0006-341X.2000.00922.x

Gari, G., Biteau-Coroller, F., LeGoff, C., Caufour, P., Roger, F., 2008. Evaluation of indirect fluorescent antibody test (IFAT) for the diagnosis and screening of lumpy skin disease using Bayesian method. Vet. Microbiol. 129, 269–280. https://doi.org/10.1016/j.vetmic.2007.12.005

Gari, G., Mekonnen, G., Sibhat, D., Abebe, A., Sahle, M., Abie, G., 2015. Participatory disease surveillance (PDS) of sheep and goats deseases in selected districts of Afar Regional State: Particular focus on Pestes des petit ruminants (PPR) and sheep and goat pox disease (SGP). Ethiop. Vet. J. 19, 83. https://doi.org/10.4314/evj.v19i1.8

Gari, G., Waret-Szkuta, A., Grosbois, V., Jacquiet, P., Roger, F., 2010. Risk factors associated with observed clinical lumpy skin disease in Ethiopia. Epidemiol. Infect. 138, 1657–1666. https://doi.org/10.1017/S0950268810000506

Gari Jimolu, G., 2011. Epidemiological Study of Lumpy Skin Disease and Its Economic Impact in Ethiopia.

Gelaye, E., Lamien, C.E., 2019. Lumpy skin disease and vectors of LSDV, in: Transboundary Animal Diseases in Sahelian Africa and Connected Regions. Springer International Publishing, pp. 267–288. https://doi.org/10.1007/978-3-030-25385-1_13

Gershon, P.D., Ansell, D.M., Black, D.N., 1989. A Comparison of the Genome Organization of Capripoxvirus with That of the Orthopoxviruses, JOURNAL OF VIROLOGY.

Gershoni, P.D., Black, D.N., 1989. A capripoxvirus pseudogene whose only intact homologs are in other poxvirus genomes. Virology 172, 350–354. https://doi.org/10.1016/0042-6822(89)90138-4

Gharban, H.A.J., Al-Shaeli, S.J.J., Al-Fattli, H.H.H., Altaee, M.N.K., 2019. Molecular and histopathological confirmation of clinically diagnosed lumpy skin disease in cattle, Baghdad Province of Iraq. Vet. World 12, 1826–1832. https://doi.org/10.14202/vetworld.2019.1826-1832

Gomo, C., Kanonhuwa, K., Godobo, F., Tada, O., Makuza, S.M., 2017. Temporal and spatial distribution of lumpy skin disease (LSD) outbreaks in Mashonaland West Province of Zimbabwe from 2000 to 2013. Trop. Anim. Health Prod. 49, 509–514. https://doi.org/10.1007/s11250-017-1222-y

Gubbins, S., 2019. Using the basic reproduction number to assess the risk of transmission of lumpy skin disease virus by biting insects. Transbound. Emerg. Dis. 66, 1873–1883. https://doi.org/10.1111/tbed.13216

Gubbins, S., Stegeman, A., Klement, E., Pite, L., Broglia, A., Cortiñas Abrahantes, J.,

2020. Inferences about the transmission of lumpy skin disease virus between herds from outbreaks in Albania in 2016. Prev. Vet. Med. 181, 104602. https://doi.org/10.1016/j.prevetmed.2018.12.008

Guindon, S., Gascuel, O., 2003. A Simple, Fast, and Accurate Algorithm to Estimate Large Phylogenies by Maximum Likelihood. Syst. Biol. 52, 696–704. https://doi.org/10.1080/10635150390235520

Hayder al Rammahi, A.J., 2015. Epidemiological and diagnostic study of first lumpy skin disease outbreak in southern baghdad district. Asian Acad. Res. J. Multidiscip. 1.

Heine, H.G., Stevens, M.P., Foord, A.J., Boyle, D.B., 1999. A capripoxvirus detection PCR and antibody ELISA based on the major antigen P32, the homolog of the vaccinia virus H3L gene. J. Immunol. Methods 227, 187–196. https://doi.org/10.1016/S0022-1759(99)00072-1

House, J.A., Wilson, T.M., Nakashly, S. el, Karim, I.A., Ismail, I., Danaf, N. el, Ayoub, N.N., Moussa, A.M., 1990a. The Isolation of Lumpy Skin Disease Virus and Bovine Herpesvirus- from Cattle in Egypt. J. Vet. Diagnostic Investig. 2, 111–115. https://doi.org/10.1177/104063879000200205

Ince, Ö.B., Çakir, S., Dereli, M.A., 2016. Risk analysis of lumpy skin disease in Turkey. Indian J. Anim. Res. 50, 1013–1017. https://doi.org/10.18805/ijar.9370

Islam, S., Barua, S.R., Moni, S.P., Islam, A., Rahman, A.K.M.A., Chowdhury, S., 2020. Seroprevalence and risk factors for bovine brucellosis in the Chittagong Metropolitan Area of Bangladesh. Vet. Med. Sci. vms3.348. https://doi.org/10.1002/vms3.348

Issimov, A., Kutumbetov, L., Orynbayev, M.B., Khairullin, B., Myrzakhmetova, B., Sultankulova, K., White, P.J., 2020. Mechanical Transmission of Lumpy Skin Disease Virus by Stomoxys spp. (Stomoxys calsitrans, Stomoxys sitiens, Stomoxys indica), Diptera: Muscidae. Animals 10, 477. https://doi.org/10.3390/ani10030477

Jia-yu, Y., Qian, Z., Fei-fei, D., Chuan-jie, T., Hui, P., Yuan-yuan, S., Yong-feng, Z., Jian-li, W., Jiang, S., Zhi-jing, X., 2018. Emergence of novel canine parvovirus type 2 and its pathogenesis in raccoon dogs. Vet. Microbiol. 216, 7–12. https://doi.org/10.1016/j.vetmic.2018.01.016

Kahana-sutin, Klement, E., Lensky, I., Gottlieb, Y., 2017. High relative abundance of the stable fly Stomoxys calcitrans is associated with lumpy skin disease outbreaks in

Israeli dairy farms. Med. Vet. Entomol. 31, 150–160. https://doi.org/10.1111/mve.12217

Kahrs, R.F., 2001. *Viral diseases of cattle*. Iowa State University Press.

Kara, P.D., Afonso, C.L., Wallace, D.B., Kutish, G.F., Abolnik, C., Lu, Z., Vreede, F.T., Taljaard, L.C.F., Zsak, A., Viljoen, G.J., Rock, D.L., 2003. Comparative sequence analysis of the South African vaccine strain and two virulent field isolates of Lumpy skin disease virus. Arch Virol 148, 1335–1356. https://doi.org/10.1007/s00705-003-0102-0

Kasem, S., Saleh, M., Qasim, I., Hashim, O., Alkarar, A., Abu-Obeida, A., Gaafer, A., Hussien, R., AL-Sahaf, A., Al-Doweriej, A., Bayoumi, F., Hodhood, A., Abdelatif, M., 2018. Outbreak investigation and molecular diagnosis of Lumpy skin disease among livestock in Saudi Arabia 2016. Transbound. Emerg. Dis. 65, e494–e500. https://doi.org/10.1111/tbed.12769

Katsoulos, P.-D., Chaintoutis, S.C., Dovas, C.I., Polizopoulou, Z.S., Brellou, G.D., Agianniotaki, E.I., Tasioudi, K.E., Chondrokouki, E., Papadopoulos, O., Karatzias, H., Boscos, C., 2018. Investigation on the incidence of adverse reactions, viraemia and haematological changes following field immunization of cattle using a live attenuated vaccine against lumpy skin disease. Transbound. Emerg. Dis. 65, 174–185. https://doi.org/10.1111/tbed.12646

Kazutaka Katoh, Kazuharu Misawa, Kei-ichi Kuma, T.M., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066.

Kiplagat, S.K., Kitala, P.M., Onono, J.O., Beard, P.M., Lyons, N.A., 2020. Risk Factors for Outbreaks of Lumpy Skin Disease and the Economic Impact in Cattle Farms of Nakuru County, Kenya. Front. Vet. Sci. 7, 259. https://doi.org/10.3389/fvets.2020.00259

Klausner, Z., Fattal, E., Klement, E., 2017. Using Synoptic Systems' Typical Wind Trajectories for the Analysis of Potential Atmospheric Long-Distance Dispersal of Lumpy Skin Disease Virus. Transbound. Emerg. Dis. 64, 398–410. https://doi.org/10.1111/tbed.12378

Klement, E., 2018. Economic impact of lumpy skin disease, in: Lumpy Skin Disease.

Springer International Publishing, pp. 7–9. https://doi.org/10.1007/978-3-319-92411-3_3

Klement, E., Broglia, A., Antoniou, S.E., Tsiamadis, V., Plevraki, E., Petrović, T., Polaček, V., Debeljak, Z., Miteva, A., Alexandrov, T., Marojevic, D., Pite, L., Kondratenko, V., Atanasov, Z., Gubbins, S., Stegeman, A., Abrahantes, J.C., 2020. Neethling vaccine proved highly effective in controlling lumpy skin disease epidemics in the Balkans. Prev. Vet. Med. 181, 104595. https://doi.org/10.1016/j.prevetmed.2018.12.001

Lamien, C.E., Lelenta, M., Goger, W., Silber, R., Tuppurainen, E., Matijevic, M., Luckins, A.G., Diallo, A., 2011. Real time PCR method for simultaneous detection, quantitation and differentiation of capripoxviruses. J. Virol. Methods 171, 134–140. https://doi.org/10.1016/j.jviromet.2010.10.014

Lawson, A., Browne, W., Rodeiro, C., 2003. Disease mapping with WinBUGS and MLwiN.

Lawson, A., Williams, F., 2001. An introductory guide to disease mapping.

Lawson, A.B., Williams, F.L.R., 2001. An introductory guide to disease mapping. An Introd. Guid. to Dis. mapping.

Le Goff, C., Lamien, C.E., Fakhfakh, E., Chadeyras, A., Aba-Adulugba, E., Libeau, G., Tuppurainen, E., Wallace, D.B., Adam, T., Silber, R., Gulyaz, V., Madani, H., Caufour, P., Hammami, S., Diallo, A., Albina, E., 2009. Capripoxvirus G-protein-coupled chemokine receptor: A host-range gene suitable for virus animal origin discrimination. J. Gen. Virol. 90, 1967–1977. https://doi.org/10.1099/vir.0.010686-0

Loth, L., Gilbert, M., Osmani, M., … A.K.-P. veterinary, 2010, undefined, n.d. Risk factors and clusters of highly pathogenic avian influenza H5N1 outbreaks in Bangladesh. Elsevier.

Lubinga, J.C., Tuppurainen, E.S.M., Mahlare, R., Coetzer, J.A.W., Stoltsz, W.H., Venter, E.H., 2015. Evidence of Transstadial and Mechanical Transmission of Lumpy Skin Disease Virus by *Amblyomma hebraeum* Ticks. Transbound. Emerg. Dis. 62, 174–182. https://doi.org/10.1111/tbed.12102

Lumpy skin disease | EURL-capripox.be [WWW Document], n.d. URL https://www.eurl-capripox.be/lumpy-skin-disease (accessed 2.17.21).

Luna, L., 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology.

Macauley, J.M., Summers, J.K., Engle, V.D., 1999. Estimating the ecological condition of the estuaries of the Gulf of Mexico. Environ. Monit. Assess. 57, 59–83. https://doi.org/10.1023/A:1005944829407

Machado, G., Korennoy, F., Alvarez, J., Picasso-Risso, C., Perez, A., VanderWaal, K., 2019. Mapping changes in the spatiotemporal distribution of lumpy skin disease virus. Transbound. Emerg. Dis. 66, 2045–2057. https://doi.org/10.1111/tbed.13253

Macpherson, C.N.L., 1994. The effect of transhumance of the epidemiology of animal diseases," 1994, The Kenya Veterinarian.

Mafirakureva, P., Saidi, B., Mbanga, J., 2017a. Incidence and molecular characterisation of lumpy skin disease virus in Zimbabwe using the P32 gene. Trop. Anim. Health Prod. 49, 47–54. https://doi.org/10.1007/s11250-016-1156-9

Magori-Cohen, R., Louzoun, Y., Herziger, Y., Oron, E., Arazi, A., Tuppurainen, E., Shpigel, N.Y., Klement, E., 2012. Mathematical modelling and evaluation of the different routes of transmission of lumpy skin disease virus. Vet. Res. 43. https://doi.org/10.1186/1297-9716-43-1

Manić, M., Stojiljković, M., Petrović, M., Nišavić, J., Bacić, D., Petrović, T., Vidanović, D., Obrenović, S., 2019. Epizootic features and control measures for lumpy skin disease in south-east Serbia in 2016. Transbound. Emerg. Dis. 66, 2087–2099. https://doi.org/10.1111/tbed.13261

Mathijs, E., Vandenbussche, F., Haegeman, A., King, A., Nthangeni, B., Potgieter, C., Maartens, L., Van Borm, S., De Clercq, K., 2016. Complete genome sequences of the Neethling-like lumpy skin disease virus strains obtained directly from three commercial live attenuated vaccines. Genome Announc. 4. https://doi.org/10.1128/genomeA.01255-16

Menasherow, S., Rubinstein-Giuni, M., Kovtunenko, A., Eyngor, Y., Fridgut, O., Rotenberg, D., Khinich, Y., Stram, Y., 2014. Development of an assay to differentiate between virulent and vaccine strains of lumpy skin disease virus (LSDV). J. Virol. Methods 199, 95–101. https://doi.org/10.1016/j.jviromet.2013.12.013

Mercier, A., Arsevska, E., Bournez, L., Bronner, A., Calavas, D., Cauchard, J., Falala, S., Caufour, P., Tisseuil, C., Lefrançois, T., Lancelot, R., 2018a. Spread rate of lumpy skin disease in the Balkans, 2015-2016. Transbound. Emerg. Dis. 65, 240–243. https://doi.org/10.1111/tbed.12624

Milovanović, M., Dietze, K., Milicévić, V., Radojičić, S., Valčić, M., Moritz, T., Hoffmann, B., 2019. Humoral immune response to repeated lumpy skin disease virus vaccination and performance of serological tests. BMC Vet. Res. 15, 1–9. https://doi.org/10.1186/s12917-019-1831-y

Molla, W., de Jong, M.C.M., Gari, G., Frankena, K., 2017. Economic impact of lumpy skin disease and cost effectiveness of vaccination for the control of outbreaks in Ethiopia. Prev. Vet. Med. 147, 100–107. https://doi.org/10.1016/j.prevetmed.2017.09.003

Molla, W., Frankena, K., Gari, G., Kidane, M., Shegu, D., de Jong, M.C.M., 2018. Seroprevalence and risk factors of lumpy skin disease in Ethiopia. Prev. Vet. Med. 160, 99–104. https://doi.org/10.1016/j.prevetmed.2018.09.029

Möller, J., Moritz, T., Schlottau, K., Krstevski, K., Hoffmann, D., Beer, M., Hoffmann, B., 2019. Experimental lumpy skin disease virus infection of cattle: comparison of a field strain and a vaccine strain. Arch. Virol. 164, 2931–2941. https://doi.org/10.1007/s00705-019-04411-w

Munyeme, M., Muma, J.B., Skjerve, E., Nambota, A.M., Phiri, I.G.K., Samui, K.L., Dorny, P., Tryland, M., 2008. Risk factors associated with bovine tuberculosis in traditional cattle of the livestock/wildlife interface areas in the Kafue basin of Zambia. Prev. Vet. Med. 85, 317–328. https://doi.org/10.1016/j.prevetmed.2008.03.006

Mwanandota, J.J., Macharia, M., Ngeleja, C.M., Sallu, R.S., Yongolo, M.G., Mayenga, C., Holton, T.A., 2018. Validation of a diagnostic tool for the diagnosis of lumpy skin disease. Vet. Dermatol. 29, 532-e178. https://doi.org/10.1111/vde.12690

Ochwo, S., VanderWaal, K., Munsey, A., Nkamwesiga, J., Ndekezi, C., Auma, E., Mwiine, F.N., 2019. Seroprevalence and risk factors for lumpy skin disease virus seropositivity in cattle in Uganda. BMC Vet. Res. 15, 236. https://doi.org/10.1186/s12917-019-1983-9

OIE, 2019. (No Title) [WWW Document]. URL https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=31742 (accessed 3.16.20).

OIE, 2017. LUMPY SKIN DISEASE Fact Sheet 1–5.

Orlova, E.S., Shcherbakov, A. V., Diev, V.I., Zakharov, V.M., 2006. Differentiation of capripoxvirus species and strains by polymerase chain reaction. Mol. Biol. 40, 139–145. https://doi.org/10.1134/S0026893306010183

Osman, F.A.S. and A.H., 2011. Incidence of lumpy skin disease among Egyptian cattle in Giza Governorate, Egypt. Vet. World 4, 162–167.

Otter, A., Mackintosh, A., Hateley, G., 2016. Investigation of suspect lumpy skin disease cases in England and Wales. Vet. Rec. https://doi.org/10.1136/vr.i6127

Peck, D.B., 2017. The economic efficiency and equity of government policies on brucellosis: comparative insights from Albania and the United States of America. ncbi.nlm.nih.gov.

Pfeiffer, D., Epizooties, M.H.-J.-O.I. des, 2002, undefined, n.d. and management (Part One)-Diagnostics-Geographical information systems as a tool in epidemiological assessment and wildlife disease management. Paris: L'Office, 1982-.

Prozesky, L., Barnard, B.J., 1982. A study of the pathology of lumpy skin disease in cattle. Onderstepoort J. Vet. Res. 49, 167–175.

Robson, D.S., Chapman, D.G., 1961. Catch Curves and Mortality Rates. Trans. Am. Fish. Soc. 90, 181–189. https://doi.org/10.1577/1548-8659(1961)90[181:ccamr]2.0.co;2

Rouby, S., Aboulsoud, E., 2016. Evidence of intrauterine transmission of lumpy skin disease virus. Vet. J. 209, 193–195. https://doi.org/10.1016/J.TVJL.2015.11.010

Saegerman, C., Bertagnoli, S., Meyer, G., Ganière, J.-P., Caufour, P., De Clercq, K., Jacquiet, P., Fournié, G., Hautefeuille, C., Etore, F., Casal, J., 2018. Risk of introduction of lumpy skin disease in France by the import of vectors in animal trucks. PLoS One 13, e0198506. https://doi.org/10.1371/journal.pone.0198506

Saez, M., Petició, C.S.-G.E. a, 2007, undefined, n.d. Estadística y epidemiología espacial. torrossa.com.

Salib, F.A., Osman, A.H., 2011. Incidence of lumpy skin disease among Egyptian cattle in Giza Governorate, Egypt. Vet. World 4, 162–167. https://doi.org/10.5455/vetworld.2011.162-167

Sameea Yousefi, P., Mardani, K., Dalir-Naghadeh, B., Jalilzadeh-Amin, G., 2017. Epidemiological Study of Lumpy Skin Disease Outbreaks in North-western Iran. Transbound. Emerg. Dis. 64, 1782–1789. https://doi.org/10.1111/tbed.12565

Sanz-Bernardo, B., Haga, I.R., Wijesiriwardana, N., Hawes, P.C., Simpson, J., Morrison, L.R., MacIntyre, N., Brocchi, E., Atkinson, J., Haegeman, A., De Clercq, K., Darpel, K.E., Beard, P.M., 2020. Lumpy Skin Disease Is Characterized by Severe Multifocal Dermatitis With Necrotizing Fibrinoid Vasculitis Following Experimental Infection. Vet. Pathol. 57, 388–396. https://doi.org/10.1177/0300985820913268

Seet, B.T., Johnston, J.B., Brunetti, C.R., Barrett, J.W., Everett, H., Cameron, C., Sypula, J., Nazarian, S.H., Lucas, A., McFadden, G., 2003. P <scp>OXVIRUSES AND</scp> I <scp>MMUNE</scp> E <scp>VASION</scp>. Annu. Rev. Immunol. 21, 377–423. https://doi.org/10.1146/annurev.immunol.21.120601.141049

Şevik, M., Doğan, M., 2017a. Epidemiological and Molecular Studies on Lumpy Skin Disease Outbreaks in Turkey during 2014-2015. Transbound. Emerg. Dis. 64, 1268–1279. https://doi.org/10.1111/tbed.12501

Şevik, M., Doğan, M., 2017b. Epidemiological and Molecular Studies on Lumpy Skin Disease Outbreaks in Turkey during 2014–2015. Transbound. Emerg. Dis. 64, 1268–1279. https://doi.org/10.1111/tbed.12501

Shalaby, M.A., El-Deeb, A., El-Tholoth, M., Hoffmann, D., Czerny, C.P., Hufert, F.T., Weidmann, M., Abd El Wahed, A., 2016. Recombinase polymerase amplification assay for rapid detection of lumpy skin disease virus. BMC Vet. Res. 12, 1–6. https://doi.org/10.1186/s12917-016-0875-5

Smith, G.J.D., Vijaykrishna, D., Bahl, J., Lycett, S.J., Worobey, M., Pybus, O.G., Ma, S.K., Cheung, C.L., Raghwani, J., Bhatt, S., Peiris, J.S.M., Guan, Y., Rambaut, A., 2009. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza a epidemic. Nature 459, 1122–1125. https://doi.org/10.1038/nature08182

Sprygin, A., Pestova, Y., Wallace, D.B., Tuppurainen, E., Kononov, A. V., 2019. Transmission of lumpy skin disease virus: A short review. Virus Res.

https://doi.org/10.1016/j.virusres.2019.05.015

Stram, Y., Kuznetzova, L., Friedgut, O., Gelman, B., Yadin, H., Rubinstein-Guini, M., 2008. The use of lumpy skin disease virus genome termini for detection and phylogenetic analysis. J. Virol. Methods 151, 225–229. https://doi.org/10.1016/j.jviromet.2008.05.003

Stubbs, S., Oura, C.A.L., Henstock, M., Bowden, T.R., King, D.P., Tuppurainen, E.S.M., 2012. Validation of a high-throughput real-time polymerase chain reaction assay for the detection of capripoxviral DNA. J. Virol. Methods 179, 419–422. https://doi.org/10.1016/j.jviromet.2011.11.015

Sudhakar, S.B., Mishra, N., Kalaiyarasu, S., Jhade, S.K., Hemadri, D., Sood, R., Bal, G.C., Nayak, M.K., Pradhan, S.K., Singh, V.P., 2020. Lumpy skin disease (LSD) outbreaks in cattle in Odisha state, India in August 2019: Epidemiological features and molecular studies. Transbound. Emerg. Dis. tbed.13579. https://doi.org/10.1111/tbed.13579

Swiswa, S., Masocha, M., Pfukenyi, D.M., Dhliwayo, S., Chikerema, S.M., 2017. Long-term changes in the spatial distribution of lumpy skin disease hotspots in Zimbabwe. Trop. Anim. Health Prod. 49, 195–199. https://doi.org/10.1007/s11250-016-1180-9

Tageldin, M.H., Wallace, D.B., Gerdes, G.H., Putterill, J.F., Greyling, R.R., Phosiwa, M.N., Al Busaidy, R.M., Al Ismaaily, S.I., 2014. Lumpy skin disease of cattle: An emerging problem in the Sultanate of Oman. Trop. Anim. Health Prod. 46, 241–246. https://doi.org/10.1007/s11250-013-0483-3

Taylor, R.A., Berriman, A.D.C., Gale, P., Kelly, L.A., Snary, E.L., 2019. A generic framework for spatial quantitative risk assessments of infectious diseases: Lumpy skin disease case study. Transbound. Emerg. Dis. 66, 131–143. https://doi.org/10.1111/tbed.12993

Tian, H., Chen, Y., Wu, J., Shang, Y., Liu, X., 2010. Serodiagnosis of sheeppox and goatpox using an indirect ELISA based on synthetic peptide targeting for the major antigen P32. Virol. J. 7, 245. https://doi.org/10.1186/1743-422X-7-245

Tulman, E.R., Afonso, C.L., Lu, Z., Zsak, L., Sur, J.-H., Sandybaev, N.T., Kerembekova, U.Z., Zaitsev, V.L., Kutish, G.F., Rock, D.L., 2002. The Genomes of Sheeppox and Goatpox Viruses. J. Virol. 76, 6054–6061. https://doi.org/10.1128/JVI.76.12.6054-

6061.2002

Tuppurainen, E., Alexandrov, T. & Beltrán-Alcrudo, D., 2017. Lumpy skin disease – A manual for veterinarians [WWW Document]. Food Agric. Organ.

Tuppurainen, E.S.M., 2018. Decontamination and disinfection, in: Lumpy Skin Disease. Springer International Publishing, pp. 103–105. https://doi.org/10.1007/978-3-319-92411-3_22

Tuppurainen, E.S.M., Oura, C.A.L., 2012. Review: Lumpy Skin Disease: An Emerging Threat to Europe, the Middle East and Asia. Transbound. Emerg. Dis. 59, 40–48. https://doi.org/10.1111/j.1865-1682.2011.01242.x

Tuppurainen, E.S.M., Pearson, C.R., Bachanek-Bankowska, K., Knowles, N.J., Amareen, S., Frost, L., Henstock, M.R., Lamien, C.E., Diallo, A., Mertens, P.P.C., 2014. Characterization of sheep pox virus vaccine for cattle against lumpy skin disease virus. Antiviral Res. 109, 1–6. https://doi.org/10.1016/j.antiviral.2014.06.009

Ugarte, M., Ibáñez, B., and, A.M.-S.E.R., 2005, undefined, 2005. Detection of spatial variation in risk when using CAR models for smoothing relative risks. Springer 19, 33–40. https://doi.org/10.1007/s00477-004-0202-8

Vidanović, D., Šekler, M., Petrović, T., Debeljak, Z., Vasković, N., Matović, K., Hoffmann, B., 2016. Real-time PCR assays for the specific detection of field Balkan strains of Lumpy skin disease virus. Acta Vet. Brno. 66, 444–454. https://doi.org/10.1515/acve-2016-0038

Weiss, K.E., 1968. Lumpy Skin Disease Virus. Springer, Berlin, Heidelberg, pp. 111–131. https://doi.org/10.1007/978-3-662-39771-8_3

Young, E., Basson, P.A., Weiss, K.E., Weiss, P.A.&, 1970. EXPERIMENTAL INFECTION OF GAME ANIMALS WITH LUMPY SKIN DISEASE VIRUS (PROTOTYPE STRAIN NEETHLING), vet. Res. Pretoria : Government Printer.

Zeedan, G.S.G., Mahmoud, A.H., Abdalhamed, A.M., El-Razik, K.A.E.H.A., Khafagi, M.H., Zeina, H.A.A.A., 2019. Detection of lumpy skin disease virus in cattle using real-time polymerase chain reaction and serological diagnostic assays in different governorates in Egypt in 2017. Vet. World 12, 1093–1100. https://doi.org/10.14202/vetworld.2019.1093-1100

RECOMMENDATIONS

The findings from the study should contemplate a number of points that can be adopted for LSD control. Firstly movement control of the animals as well as impenetrable quarantine measures. Secondly vector should be eliminated by adopting farm hygienic improvement also has significant impact on LSD control.

LIMITATION AND FUTURE STUDY

This study cannot point out specific sources of the disease in the study area along with the possible vectors. Subsequent research should take those into consideration. Besides that the single primer used for disease detection and phylogenetic analysis may affect the findings as well. Future study may emphasis the identification of vectors responsible for disease spread in the area. Using varieties of control measure without proper investigation such as goat pox vaccine used in the field to halt the outbreak in the study area required strict investigation.

Annex-I

Composition of preservatives used in sample preservation and processing:

- **Bouin's solution**

  Prepare 75ml saturated aqueous solution of picric acid

  Add 25ml formalin (40% aqueous solution of formaldehyde) to give 100ml total volume

  Add 5ml glacial acetic acid

  Fix tissue by submersion in Bouin's fluid for 6 hours

  Transfer fixed tissue to 70% alcohol

- **Buffered formalin**

  To produce 10L pour a base 1L distilled water into a suitable container.

  Add 40g sodium dihydrogen orthophosphate (monohydrate)

  Add 65g disodium hydrogen orthophosphate (anhydrous)

  Add 1L formalin (40% aqueous solution of formaldehyde)

  Add a further 8L water for use

  Immerse samples and fix for 12-24 hours

  Samples may be stored in this fixative if required

Biography

F. M. Yasir Hasib passed the Secondary School Certificate Examination in 2009 followed by Higher Secondary Certificate Examination in 2011. He obtained his Doctor of Veterinary Medicine Degree in 2017 (held in 2018) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh. Now, he is a candidate for the degree of MS in Veterinary Pathology, under the Department of Pathology and Parasitology, Faculty of Veterinary Medicine, CVASU. He has immense interest to work in immunopathology and vaccine research.