RISK FACTORS ASSOCIATED WITH THE OCCURRENCE OF LUMPY SKIN DISEASE IN CATTLE

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This Master's thesis is found to be satisfactory in all aspects for submission

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Abstracts

Lumpy skin disease (LSD), a viral disease transmitted by vectors, poses a significant threat to the cattle industry. Its emergence as a trans-boundary disease in the Indian subcontinent has raised concerns, including in Bangladesh since 2019. LSD adversely affects milk production, causes weight loss, fragile skin, and decreased market value of affected animals. An unmatched case-control study was conducted, involving 303 households (case-control ratio of 1:2). With a non-response rate of 10%, 98 cases and 196 controls were targeted, providing 80% study power to detect an odds ratio (OR) of 2.5 with 95% confidence. Face-to-face interviews were conducted with household owners in two Upazilas. Multivariate regression models were developed using retained variables ($P \leq 0.05$), testing for interaction through collinearity. The study revealed that dairy cattle are seven times more likely to experience LSD compared to beef-type animals ($P=0.004$). Additionally, calves have a 12-fold higher susceptibility to LSD infection than bulls. Crossbred animals were found to be 1.35 times more susceptible to LSD than local breeds. Vaccination emerged as a protective factor, with non-vaccinated animals having a 3.65 times higher probability of developing LSD. Vaccination is a crucial and cost-effective prevention and control strategy to mitigate the impact of LSD. Considering the higher susceptibility of dairy and cross-bred cattle and calves, a targeted intervention with a comprehensive approach integrating vaccination and risk mitigation measures can help minimize the effects of LSD on the cattle industry. In a developing country like Bangladesh, 20% people are directly and 50% people are indirectly depending on livestock for their livelihood. So, it is high time to intervene the spread of LSD by proactively addressing the risk factors and improve the socio-economic condition of our people.

Keywords: Lumpy Skin Disease (LSD), Odds ratio, Multivariate regression model.

CHAPTER 1: INTRODUCTION

Lumpy skin disease (LSD) is a viral disease that is considered as wreaking havoc on the cattle industry. It has moved beyond boundaries in recent years and is now considered a transboundary emerging disease in the Indian subcontinent (Kumar et al. 2021). Lumpy skin disease is caused by the Lumpy Skin Disease Virus (LSDV), which belongs to the family *Poxviridae*. According to OIE, the disease was initially identified in Zambia in 1929 and was endemic throughout Africa (Gupta et al. 2020). Bangladesh has experienced the threatening consequences of LSD since 2019 (Talukdar et al. 2020).

Emaciation, lasting skin damage, decreased milk production, miscarriage, infertility, high expenditures of supportive care, and mortality are all effects of LSD that are well-known to have an adverse effect on cattle (Mat et al. 2021). The disease is also enlisted in the OIE list of notifiable diseases, that causes around $886.34 and $1.066 losses per animal for dairy and beef cattle, respectively (Mat et al. 2021). A herd-level LSD outbreak resulted in a median total economic loss of USD 1176 in Ethiopia (Molla et al. 2017). Besides these economic losses, the treatment cost of LSD is also high. As a consequence of this disease, farmers are struggling to manage their livelihoods (Enamul et al. 2020). A recent study of (Chouhan et al. 2022b) revealed that for a total of 403 LSD cases, accounted losses of 3781916.00 BDT (44493.13 US dollars). The overall average loss per case was 110.41 US dollars, or 9384.41 BDT.

There are number of risk factors associated with the occurrence of LSD. Among that agricultural methods, grazing and watering management, influx of new animals, herd size, and encounters with sheep and goats are some of the factors to consider as the risk for the distribution of LSD in Ethiopia (Gari et al. 2010). These factors may be varied from area to area and country to country. So, it is important to figure out the risk factors associated with the occurrence of LSD in different areas of our country.

Recently, the Department of Livestock Services (DLS) reported that LSD was identified in Bangladesh for the first time on 22/07/2019 in the Chattogram district, where the initial attack rate was 18% with no mortality (Hasib et al. 2021a)(Talukdar et al. 2020). In Bangladesh, after its outbreak, limited number of study has been conducted (Hasib et al. 2021b)(Chouhan et al. 2022a)(Talukdar et al. 2020). (Talukdar et al. 2020)(Parvin et al. 2022)Vaccines are recently produced at government level at Goat Pox Vaccine Section of Livestock Research Institute, Mohakhali under the DLS. Besides, Bangladesh Livestock Research Institute is trying to develop an active seed from circulating strain. Some vaccines are also imported privately by Bengal Overseas Ltd (Lumpy vax), Nasco Agro Product (Lumpy Shield), ACI Ltd (Bovivax LSD), and Incepta Pharmaceuticals Ltd (LSD-NDOLL) ("Lumpy Skin Disease: Economic Importance and Its Control Measures - ACI Limited" n.d.). FAO also supports government by supplying and introducing the vaccine in some selected areas(Talukdar et al. 2020). FAO vaccinated 8467 cattle at three upazilas in 2021, and during follow up, they observed the occurrence of LSD in vaccinated vs unvaccinated cattle in selected areas. So, it is high time to take necessary action to determine the risk factors of LSD in cattle of Bangladesh to reduce the disease burden and prepare an effective control plan.

Though there are some reports of epidemiological studies there is still opportunity for thorough epidemiological study with a sizable sample size and other criteria that have not yet been studied in our country Therefore, the study was carried out with the following objectives.

i. To identify the risk factors associated with LSD occurrence in cattle in Bangladesh.

CHAPTER 2: REVIEW OF LITERATURE

2.1 History of lumpy skin disease (LSD)

In 1929, northern Zambia experienced the discovery of a novel cattle skin ailment. It was believed that the ailment was brought on by either a plant poisoning or an allergic reaction to insect bites because the etiology of the illness was unknown (MacDonald, 1931; Morris, 1931 quoted by Weiss, 1968). The condition was first known as pseudo-urticaria or "lumpy sickness." The disease, which had not previously been documented and was provisionally known as "Ngamiland cow disease," experienced another epidemic in October 1943 in Ngamiland, Bechuanaland Protectorate (Botswana) (Von Backstrom, 1945). In 1945, the illness spread to South Africa from where it had originated in the Transvaal, as well as to southern Rhodesia (Zimbabwe) (Houston, 1945). The first demonstration of the infectious agent being transmitted by inoculating cattle with a suspension of skin nodules was made by Thomas and colleagues in 1945. The diagnosis of lumpy skin disease in East Africa dates back to 1957 in Kenya (MacOwan, 1959), 1971 in Sudan (Ali and Obeid, 1977), 1973 in Chad and Niger, 1974 in Nigeria (Nawathe et al. 1978), and 1983 in Somalia. Despite all control and eradication efforts, the illness has grown endemic in Egypt since the initial outbreak in Ismailia in May 1988. (Ali et al. 1990). In Israel, an LSD epidemic broke out in 1989. It was hypothesized that the illness was disseminated from the Egyptian epidemic by insect vectors carried by the wind or inside the vehicles (Yeruham et al. 1995). The illness was completely eradicated from Israel thanks to a ring vaccination program within 50 km of the epidemic, the slaughter of all diseased and in touch cattle, sheep, and goats, as well as restrictions on cattle transportation. In Bahrain in 1993, 1994, and 2002; Iran in 1996 and 2001; the United Arab Emirates in 2000; Kuwait in 1991; and Oman in 1984, outbreaks or isolated cases of LSD have been reported, according to the OIE's annual report on the state of animal health around the world (OIE). In the majority of South Africa, lumpy skin condition recurs frequently. A more recent outbreak in Egypt in Asian water buffaloes were reported by Sharawi & Abd El-Rahim (2011). A laboratory unconfirmed outbreak of LSD on a dairy farm in Oman affecting up to 35% of the total herd with 12% fatality was reported by Kumar (2011).

2.2 Etiology

Alexander et al. (1957) was the first to isolate the Neethling type virus in tissue culture, despite the fact that Thomas and Maré (1945) and Von Bacstrom (1945) also showed that LSD is infectious. Prydie and Coackley validated these findings two years later (1959). The virus that causes lumpy skin disease is a member of the Poxviridae family, which is made up of multiple genera and the subfamilies Entomopoxvirinae and Chordopoxvirinae (Table 1). It is a large (300 nm) pleomorphic, double-stranded, unsegmented DNA virus that is classified in the genus *Capripoxvirus* of the family *Poxviridae*, subfamily *Chordopoxvirinae* (vertebrate poxviruses). It has only one serotype and is closely related to goat pox (GTPV) and sheep pox viruses (SPPV), the only other members of the genus *Capripoxvirus*.

Table 1. Genera within the Poxviridae family

Genus	Viruses
Capripoxvirus	Sheep pox, goat pox, lumpy skin disease viruses
Orthopoxvirus	Buffalo pox, camel pox, cow pox, vaccinia, ectromelia, monkey pox, rabbit pox, raccoon pox, tatera pox, variola and vole pox viruses
Parapoxvirus	Pseudocow pox, bovine opular stomatitis, contagious pustular dermatitis (orf), squirrel para pox viruses and parapoxvirus of red deer
Suipoxvirus	Swine pox virus
Avipoxvirus	Fowl pox, canary pox, junco pox, pigeon pox, quail pox, sparrow pox, starling pox, turkey pox, mynah pox and pcittacine pox viruses
Leporipoxvirus	Hare fibroma, myxoma, rabbit (Shope) fibroma, and squirrel fibroma viruses
Molluscipoxvirus	Molluscum contagiosum virus
Yatapoxvirus	Yaba and tana pox viruses

The largest animal virus is the poliovirus. Capripoxviruses range in size from 320 to 260 nm. With the exception of the parapoxviruses, the viruses in the several genera of chordopoxviruses share a similar shape. Poxvirions have an oval or brick form. Over 100 polypeptides are organized in a core, two lateral bodies, a membrane, and an envelope within the virion. Important structural components for the interaction with the host cell are the membrane and envelope. Without causing cell rupture, mature virions that are discharged from the cell are encased. Two layers of cellular lipids and various virus-specific polypeptides can be found in the envelope. Therefore, the majority of the virions released by the host cell's rupture are not enclosed. Viruses with and without envelopes can spread disease (Fenner et al. 1987). The lipoprotein bilayer that makes up the outer membrane shields the lateral and core bodies. It has asymmetrical arrays of "filaments" of tubular protein. There are two lateral bodies of unknown nature, and the core is fashioned like a dumbbell. Transcriptase and other enzymes are among the proteins that make up the viruses' core (Fenner et al. 1987). Even while each species has its own unique polypeptides, the virion has a large number of antigens, the majority of which are shared by all the individuals of the same genus (Fenner et al. 1987). Capripoxvirus strains were shown to share 80% of their DNA by restriction endonuclease analysis, which was performed on both field samples and vaccine strains (Black et al. 1986).

2.3 The characteristics of the LSD virus

The development of cytopathic changes and intracytoplasmic inclusion bodies in cell cultures (Alexander et al. 1957; Prydie and Coackley, 1959; Munz and Owen, 1966). The development of macroscopic lesions (pocks) in the chorioallantoic membranes of embryonated chicken eggs (Alexander et al. 1957; Van Rooyen et al. 1969). Production of generalized skin lesions in rabbits (Alexander et al. 1957). Morphological and antigenic similarities with sheep and goat pox viruses is also found (Kitching and Smale, 1986).

Between pH 6.6 and 8.6 the lumpy skin disease virus is stable. It is sensitive to ether and chloroform, and the detergent sodium dodecyl sulphate easily inactivates it (Weiss, 1968; Plowright and Ferris, 1959). LSD virus replication is accompanied by the development of intracytoplasmic inclusion bodies (Alexander et al. 1957; Prydie and Coackley, 1959; Prozesky and Barnard, 1982). Virion entry into the host cell can occur either through endocytosis or plasma membrane fusion. Within minutes of infection, the transcriptase released from the virion core initiates the synthesis of the mRNA. After infection, 1.5 to 6 hours later, the first polypeptides finish the core's uncoating before the viral DNA synthesis process actually starts (Fenner et al. 1987). Virion development takes place in the cytoplasm in microscopic inclusion bodies. Eosinophilic intracytoplasmic inclusion bodies can be seen when examining tissue culture monolayer cells stained with haematoxylin and eosin and infected with the LSD virus.

The inclusion bodies have a spherical or ad hoc shape. Within a single cell, there could be one or many inclusion bodies (Weiss, 1968).

The LSDV exhibit a remarkable degree of resistance to a range of environment conditions. Weiss (1968) reported that the virus can survive for at least 33 days in skin lesions. More recently, Tuppurainen et al. (2005), using nucleic acid detection and virus isolation techniques, reported a longer period of survival of the virus in blood and skin of experimentally infected cattle. They succeeded in isolating LSDV from skin lesions as long as 39 days post-infection and detected viral DNA in skin biopsies for up to 92 days post-infection. The virus is inactivated in 2 hours at 56°C degrees (OIE 2010a). It is phenol-labile (2% for 15 min) and is susceptible to highly alkaline or acid pH solutions, ether (20%), chloroform and formalin (1%) and can be inactivated by sodium dodecyl sulphate (10%) (Weiss 1968).

2.4 Epidemiology

In endemic locations, lumpy skin disease typically recurs periodically, or it may trigger epidemics that spread very quickly over a territory or nation (Davies, 1991). Experimental and field data have shown that LSD is not a very infectious substance. In natural epidemics, the morbidity rates range from 3 to 85%. Only 40 to 50 percent of infected animals with artificially induced illnesses had clinical symptoms. Typically, the death rate is under 10% (Thomas and Maré, 1945). Despite the fact that differences between the capripoxvirus strains collected over a 20-year period were not discovered (Kitching et al. 1989), it was suggested that the variation in mortality and morbidity rates may be caused by the involvement of strains with varying levels of pathogenicity, the effectiveness of the disease’s transmission by the mosquito, or both vector, and route of infection (Carn and Kitching, 1995b). In experiments, it has been shown that intradermal injection of the LSD virus primarily causes localized lesions at the site of inoculation, as opposed to intravenous inoculation, which causes more severe sickness and extensive lesions (Carn and Kitching, 1995a).

The results of transmission studies are not clear; while one study showed that *Aedes aegypti* is capable of mechanical transmission of LSDV (Chihota et al. 2001), another failed to achieve LSDV transmission from infected to susceptible cattle using mosquitoes (*Anopheles stephensi*), the stable fly (*Stomoxys calcitrans*) and the biting midge (*Culicoides nubeculosus*) (Chihota *et al.* 2003). These inconclusive results may be due to low levels of viraemia in the blood of infected animals that contribute to the inefficient transmission of LSDV by biting flies feeding on blood alone (Carn & Kitching, 1995). Recently, Tuppurainen et al. (2010) reported the potential role of ixodid ticks in the transmission of LSDV.

Stomoxys calcitrans and *Biomyia fasciata* were caught after being fed on affected cattle, and the virus that causes lumpy skin disease was identified from these species (Weiss, 1968). Utilizing *Stomoxys calcitrans*, the capripoxvirus transmission between sheep was proven (Kitching and Mellor, 1986) and between cattle using the mosquito, Aedes aegypti (Chihota et al. 2001) as a vector. Field reports confirm that outbreaks in the absence of a significant population of biting flies decrease (Yeruham et al. 1995) and that the disease diminishes with the onset of the dry season and the reduction in the number of biting flies (Nawathe et al. 1978). Carn and Kitching (1995b) concluded that the low titre of LSD virus present in blood of animals during the viraemic stage is not sufficient for mechanical transmission to occur by biting flies feeding on blood alone and that they must feed on skin lesions to obtain sufficient amount of virus for transmission to take place. Acute skin lesions contain high titres of virus that are sufficient to contaminate the mouthparts of biting insects (Carn and Kitching, 1995b). The virus has also been shown to be present in semen: however, the role of semen in the transmission of the virus is not clear (Tuppurainen et al. 2005; Bagla et al. 2006; Annandale et al. 2010).

All types of cattle, including both sexes and all ages, are vulnerable. Frequently, young calves are more badly impacted. LSD virus can experimentally infect sheep and goats (Weiss, 1986).Following experimental infection, capripoxvirus causes comparable lesions in sheep and cattle (Burdin, 1959). Cattle that were intradermally infected with the sheep pox virus (Isiolo strain) developed skin lesions that were clinically identical to those caused by the LSD (Neethling) virus (Capstick, 1959). With the exception of two reports of Arabian Oryx (Oryx leucoryx) in Saudi Arabia and five instances of Asian water buffalo (Bubalus bubalis) in Egypt (Ali et al. 1990), natural infection has not been reported in any other ruminant species (Greth et al. 1992).

In other sections of this book, the protection provided by herd vaccination is covered in more detail. Several studies, mostly carried out in Africa, looked at the impact of various factors at the herd level. The main limitation of the majority of this research is the ineffective adjustment for confounding variables such region, meteorological conditions, and immunization. As a result, the outcomes are somewhat variable. Herd size was found to be positively correlated with the risk for LSD in a study conducted in Ethiopia (Hailu et al. 2014). In Turkey, the similar relationship was discovered (Sevik and Dogan, 2016). However, it should be kept in mind that larger herds have a higher likelihood of having at least one case of LSD, by coincidence. Therefore, it is not necessarily true that huge herds are more vulnerable to the virus or more exposed to it. Agro-pastoral farming practices, the introduction of new livestock, and communal watering and grazing are other risk factors identified in this study. In a different Ethiopian study, it was discovered that feedlot cattle had a greater risk of contracting LSD than herds that were under comprehensive management (Ayelet et al. 2014). Although this difference was not statistically significant, the incidence in Turkey's beef herds was higher than that in its dairy herds (Sevik and Dogan 2016). In Zimbabwe, resettlement farms had the highest rates of LSD morbidity; however, the authors explain this finding by pointing out that veterinary care is more readily available in these areas. (Gomo et al. 2017).

Risk factors for Animals African-born cattle of the zebu type tend to be less prone to LSD infection and may experience widespread skin lesions, but they also experience less severe clinical illness and higher survival rates than cattle imported from other continents (Davies, 1991). Studies carried out in Ethiopia, Turkey, and Oman showed similar results (Gari et al. 2010; Tageldin et al. 2014; Sevik and Dogan, 2016). While the vaccination had no protective effect on cross breeds, the morbidity rate among vaccinated zebu cattle was more than four times greater than that of unvaccinated cattle. These results could be the result of an unreliable definition of morbidity and a lack of confounding effect control (Ayelet et al. 2013). Results on age-related LSD susceptibility are inconclusive. While some studies (Ayelet et al. 2013, 2014) found increased morbidity in young animals, others (Sevik and Dogan, 2016) demonstrated no association between morbidity and age or even decreased morbidity in calf studies (Magori-Cohen et al. 2012). The influence of gender on LSD susceptibility is likewise inconsistently reported. (MagoriCohen et al. 2012; Ayelet et al. 2013, 2014). In case of Bangladesh, Seasonal variation and associated breeding of high number of mosquito and fly (vector) are mentioned as an important risk factor for LSD transmission. (Khalil et al. 2021) Breed is also considered as an important host factor for the cattle population of Bangladesh as cross breed animals are more prone to LSD than that of indigenous cattle (Chouhan et al. 2022a). Animal of different age also have shown susceptibility to LSD infection in different level. LSD is more fatal for the young animal. The female milking cows are more commonly affected by LSDV, where as the bulls are almost resistant(Molla et al. 2017).

2.5 Clinical signs

The disease's progression could be acute, subacute, or chronic. Generalized skin lesions only appear in 40 to 50% of experimentally infected animals; many instances are asymptomatic (Weiss, 1968). According to Haig (1957), LSD takes between four and fourteen days to develop in natural conditions while it takes between two and four weeks to develop experimentally (Prozesky and Barnard, 1982; Carn and Kitching, 1995b). There is a biphasic febrile reaction that can reach temperatures higher than 41 °C in animals who develop clinical illness. For 4 to 14 days, they are febrile. Depression, a lack of desire to move, inappetence, salivation, lachrymation, and a nasal discharge that may be mucoid or mucopurulent accompany this. Lachrymation may be followed by conjunctivitis, corneal opacity, and, in rare circumstances, blindness. Prescapular, precrural, and subparotid lymph nodes in particular are typically noticeably swollen in the superficial lymph nodes. Clinical disease is characterized by a biphasic febrile reaction that can reach 41 °C. This may persist for 7 days (OIE, 2009). Clinical signs observed during this stage include salivation, lachrymation and mucopurulent nasal discharge. Ocular lesions in some cases may become advanced including conjunctivitis followed by lachrymation and may eventually lead to blindness (Coetzer, 2004). Skin nodules are classical manifestations of LSD and have been well described (Coetzer, 2004; Babiuk et al. 2008b). These nodules are usually widespread and may include the genitalia, udder, perineum, vulva, ears, limbs and skin around the head. These nodules can be 2-5 cm in diameter and necrotic skin lesions may extend from the dermis and hypodermis into the surrounding tissues (Prozesky & Barnard 1982).

In most cases, nodular skin lesions erupt 48 hours after the febrile reaction starts. They could be few and sparse, or they could be incredibly numerous and cover the entire body. The skin of the head, neck, perineum, genitalia, udder, and limbs are preference sites. Nodules affect the skin, subcutaneous tissue, and occasionally even the underlying muscles. They range in size from 5 to 50 mm, are confined, hard, spherical, and elevated. On the conjunctiva, snout, nostrils, mucous membrane of the mouth, larynx, trachea, oesophagus, and abomasum, ulcerative lesions might develop. Small nodules may become ulcerated and sequestered or they may resolve spontaneously. It's possible to develop secondary bacterial infections or fly larvae infestations. Large nodules may become fibrotic and persist for several months; also known as “sit fasts”. Severe subcutaneous oedema of the ventral body parts, such as the dewlap, brisket, limbs, udder, scrotum, and vulva, may develop in certain severely affected animals. The skin on the oedematous limbs may turn necrotic and flake off, leaving behind deep sores that could subsequently become bacterially infected. Mastitis may be brought on by edematous and necrotic lesions in the udder. Pneumonia can develop in some animals with necrotic lesions in the lungs and trachea. A localized collapse of the trachea and eventual asphyxia may occur as a result of connective tissue contracting in healed tracheal wounds. Bulls often become infertile for a short period of time, but occasionally severe orchitis might cause them to become permanently sterile. Abortion may occure and be in pregnant cows. In case of Bangladesh, the most prominent clinical signs include the development of firm raised skin nodules to 50 mm in diameter at any part of the body especially, around the head, neck, genitals, and limbs. Scabs developed in the center of the nodules after which the scabs fall off, leaving large holes that may become infected. Swelling of limbs and brisket were also found. Enlarged superficial lymph nodes were most common. Swollen and painful joints in lower limbs are also common. (Open et al. 2020)

2.6 Diagnosis

A presumptive diagnosis of the disease can be made based on clinical signs. It has a variable morbidity rate, ranging between 5-85%; mortality rates are variable but usually less than 10% (OIE, 2009). Based on the clinical indications, the condition can be presumed to exist. Rapid laboratory techniques are required to confirm the diagnosis in cases of mild and inapparent disease, albeit this can be challenging to do. Direct fluorescent antibody test (FAT), cell culture isolation, transmission electron microscopy (TEM), or conventional serological tests such as the serum virus neutralization test (SNT), indirect fluorescent antibody test (IFAT), and agar-gel immunodiffusion test can all be used to detect antibodies in the laboratory to make the diagnosis of LSD (AGID)

Tuppurainen et al. 2005; Orlova et al. 2006; Stram et al. 2008), real-time PCR (Babiuk et al. 2008b) and dot blot hybridization (Awad et al. 2010). Regular histopathology and immunohistological staining offer a comparatively cheap tool for disease diagnosis. For the detection of LSD antibodies, indirect and antigen trapping ELISA as well as polymerase chain reaction (PCR) have recently been found successful. Immunohistochemistry, using immunoperoxidase staining, can be used to visualize LSDV antigens in infected tissues (Babiuk et al. 2008b; Annandale et al. 2010). This method is laborious, time-consuming, and not a high throughput assay and therefore not easily used to screen large animal populations.

Transmission electron microscopy is the most rapid diagnostic technique and permits reliable detection of LSDV particles in fresh or formalin-preserved samples (Woods, 1988). It has been used in outbreaks (Nawathe et al. 1978; Khalafalla et al. 1993) as well as experimental infections (Aspden et al. 2003; Tuppurainen et al. 2005). It has the advantage of not requiring specific reagents, which is not the case with serological and molecular tests (Goldsmith & Miller, 2009). However, access to a transmission electron microscopy as well as a competent microscopist may not be available in most LSD endemic countries (Zheng et al. 2007). Unlike serological and molecular tests, it is not suitable for primary screening of large number of samples. Furthermore, it cannot differentiate between SPPV, GTPV and LSDV (Kitching & Smale, 1986). Lastly, where orthopoxviruses are endemic in cattle (Yeruham et al. 1996; Singh et al. 2008), transmission electron microscopy can only differentiate between these viruses and LSDV when specific immunological staining techniques are used (Babiuk et al. 2008a).

The use of virus isolation (VI) to detect LSDV and the cell lines used has been summarised in the literature (Binepal et al. 2001). The LSDV is commonly isolated using primary lamb kidney (LK) or primary lamb testis cells. Foetal lung, skin, muscle and endothelial cells can also be used (Davies, 1991a; Binepal et al. 2001). Growth is indicated by the development of cytopathic effect (CPE) which may become evident after 4 to 10 days in most cell cultures (Davies, 1991a). Primary cell culture of bovine dermis cells (BDC) prepared from a foetal calf's ear can be used to isolate LSDV (Tuppurainen et al. 2005; Bagla et al. 2006). An ovine testis cell line (OA3.Ts) for LSDV isolation was recently evaluated and the observed CPE were similar to those obtained with the commonly used primary LK cells (Babiuk et al. 2007). Distinct viral plaques indicative of LSDV growth could be detected in this cell line by immunostaining with capripoxvirus-specific antiserum. Lumpy skin disease virus can be isolated from nodular skin lesions, ocular, nasal and saliva swabs and buffy coat (Carn & Kitching, 1995). Although the use of VI techniques to isolate LSDV from semen is not very sensitive (Irons et al. 2005), VI tends to be in general more sensitive than rapid antigen assays and less expensive than molecular tests (Leland & Ginocchio, 2007).

2.6.1 Differential diagnosis

It is possible to mistake LSD with the skin lesions caused by pseudo lumpy skin disease (caused by the bovine herpesvirus 2), insect bites, Demodex infection, onchocerciosis, besnoitiosis, and dermatophilosis (Barnard et al. 1994). Rinderpest, cow viral diarrhea/mucosal illness, and bovine malignant catarrhal fever are diseases that cause mucosal sores that can be mistaken for LSD (Barnard et al. 1994).

2.7 Economic importance

Even though the morbidity and mortality rates of LSD are typically low, the prolonged loss of productivity in dairy and beef cattle, the decline in body weight, the mastitis, and the severe orchitis, which can cause temporary infertility and occasionally permanent sterility, make it an economically significant disease of cattle in Africa. Cows that are pregnant may miscarry, and their sterility may persist for several months (Weiss, 1968). Animals with extensive damage to their hides have permanently diminished value for the leather industry (Green, 1959). In case of Bangladesh, the average economic loss per case was 9384.41 BDT (110.40 US $). The higher economic loss per case was accounted in Mymensingh district (10248.91 BDT ≈ 120.58 US $) than Gaibandha district (8211.52 BDT ≈ 96.61 US $). The loss was higher in crossbred cattle (9709.58 BDT≈114.23 US $) compared to indigenous cattle (7595.94 BDT ≈ 89.36 US $). The total estimated annual loss due to LSD in Mymensingh and Gaibandha districts was 7763.25 million BDT (91.33 million US $). In Gaibandha district, the estimated annual loss was 2666.20 million BDT (31.37 million US $) while in Mymensingh district it was 5097.05 million BDT (59.97 million US $)(Chouhan et al. 2022a).

2.8 Control

The only practical means of controlling LSD in endemic areas is immunization. The primary means of disease control in nations where LSD is absent is the restriction or outright prohibition of the importation of animals and animal products from endemic nations that might carry live virus (Carn, 1993). LSD-free nations may impose import or transit bans on live domestic and wild bovine animals as well as on bovine sperm in an effort to prevent the disease's spread from endemic regions. When importing from an area where the disease is endemic, veterinary administrations should demand the presentation of an international veterinary health certificate attesting that the animals being imported do not exhibit clinical symptoms of the illness and that they have received their LSD vaccination more than 30 days but less than three months before shipment. They should stipulate that the cattle must spend at least 28 days in a quarantine facility in the exporting nation before being sent. The submission of an international veterinary certificate attesting that the donor animal does not exhibit any clinical signs of LSD on the day of semen collection or for the subsequent 28 days, as well as the donor having spent the previous 28 days in a quarantine station in the exporting nation, should be requirements for the importation of bovine sperm. The importation of bovine products that might include live virus should either be prohibited, or the importing nation should demand that those items be handled in a way that guarantees the LSD virus will be eliminated before the importation (OIE's web page, recommendations for the importation of live bovine species and bovine semen from endemic countries to LSD free countries).

The killing of diseased and in-contact animals, ring vaccination within a 25–50 km radius, mobility restrictions for animals, and destruction of contaminated hides should typically be enough to eliminate the illness in non-endemic areas in the event of an LSD outbreak (Carn, 1993).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study design, areas and population

An unmatched case-control study was conducted on 303 households to identify the potential risk factors associated with the occurrence of LSD from August to November 2022. The sampling units were households that had at least two cattle (Alkhamis et al. 2020). Mirsharai upazila from Chattogram district and Nawabgonj upazila from Dinajpur district were selected for data collection (Fig. 1). The selection of these two districts was based on the outbreak history of LSD. On the other hand, FAO had selected these two districts as their vaccination sites.

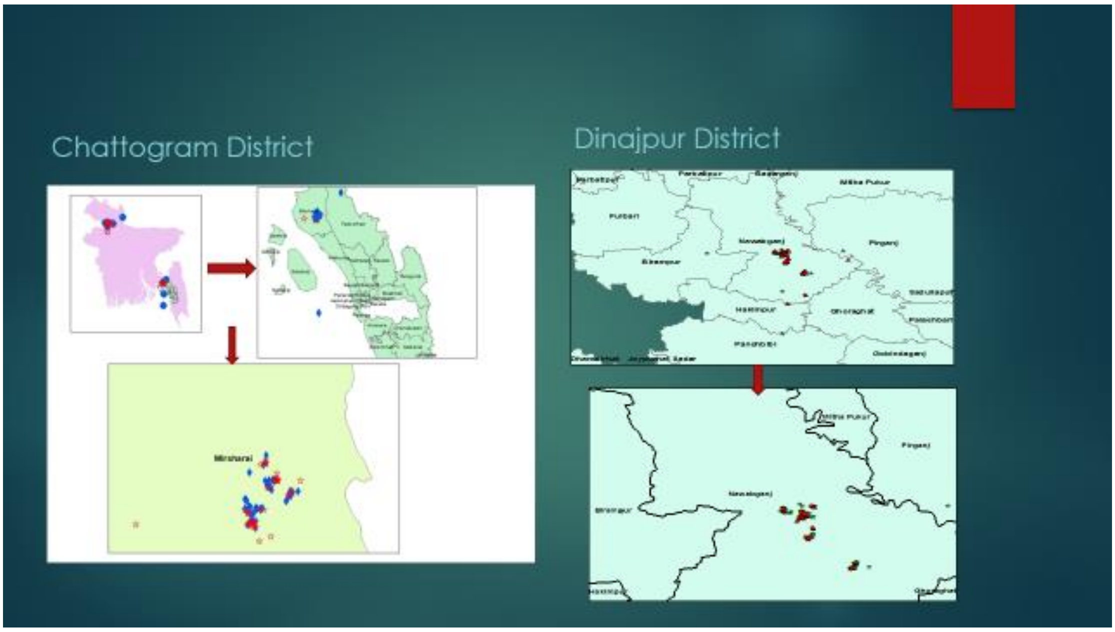

Fig. 1. Study areas.

3.2 Sample size

To maximize the effectiveness of the study two controls were matched with each case. In order to have 80% power of the study, to detect an odds ratio (OR) of 3 with 95% confidence, 98 cases, and 196 controls were needed considering the non-response rate of 10%. This computation was based on an assumption of a moderate correlation between case and control exposures (Alkhamis et al. 2020). A total of 303 households was selected for data collection. Among 303 samples, 152 animals (half of the samples) were selected from Mirsarai and the rest (151) from Nawabganj upazila.

3.3 Case definition

The animal having the following sign and symptoms was considered as the case of LSD-

- Firm raised skin nodules to 50 mm in diameter at any part of the body especially, around the head, neck, genitals, and limbs.
- Scabs developed in the center of the nodules after which the scabs fall off, leaving large holes that may become infected.
- Swelling of limbs and brisket.
- Enlarged superficial lymph nodes (Abutarbush 2014).

3.4 Selection of cases

Selected households were identified by collecting hospital data of the concerned area and data was also be collected from discussion with Upazilla Livestock Officers, Veterinary Surgeons, private practitioners, vaccinators, and other hospital staff. Discussion with the dairy association and other farmers was also provided information about cases and controls. A household containing at least one affected cattle within the last six months was considered a case household (Alkhamis et al. 2020).

3.5 Selection of controls

All households which had not reported a single case for the last six months was considered for control household. In the same village were given a distinct identification number, and a random selection process was used to choose unaffected households (controls) until the required number was achieved (Alkhamis et al. 2020)**.**It was 1:2. Control was taken from the nearest area of the occurring case depending on a neighborhood basis.

3.6 Data collection

Data were collected through face-to face interview of the household owners. It contains demographic data of farm and farm owners, information about age, sex, farming system, vaccination status, disease history, movement history, vaccination, etc (Alkhamis et al. 2020).

3.7 Data analysis

Collected data were entered in an excel spreadsheet and then sort the data and code if necessary. The sorted and coded data were then analyzed using STATA-17. Statistical tests and logistic regression were used to generate odds ratios and estimate the strength of evidence at the household level between putative risk factors and occurring at least one case of LSD. Variables associated with univariable logistic regression analysis was retained if the P value was ≤0.2 for the further multivariable model. The final multivariable model was prepared by using the variables that were retained if the P-value was ≤0.05. Interaction between variables was tested by the collinearity test (Ali Farah Gumbe, 2018).

CHAPTER 4: RESULTS

The present study showed that the association between the different exposure factors like demographic information, risk related factors, hygienic condition related data and protective factor like vaccination with the dependent variable (Occurrence of Lumpy Skin Disease).

In case of analysis of demographic information of respondents (owner of animals), it has been found that, by occupation most of the animal and farm-owners were farmers (67.55%), some were businessman (22.19%) and here it was noticeable that the participation of women was really negligible in farming of large animals (3.97%) (Fig. 2). In case of educational status, the major part (79.73%) of farmers had achieved SSC or more. However, these variables related to demographic information found insignificant.

In case of farm related variables, we took a number of variables for regression analysis, variables are included for multiple logistic regression, which have p value <=0.2. We got three types of case-farms consisting of Dairy, Beef and both dairy and beef type of animals, which had significant association (P=0.004) with the occurrence of Lumpy Skin Disease. Where in milk producing animals the frequency of disease occurrence (68,62.96%) was higher than that of the beef producing breed (5,4.63%) (Table 3).

In case of breed, Deshi (Indigenous) and Cross bred animals were observed and the breed was likely to be associated with the occurrence of Lumpy Skin Disease (P=0.094), where the analysis showed that the percentage of disease was higher in indigenous animals (54.63%) than the Cross breed (45.37%).

In case of different type of animals in terms of gender and age it has been found that calves were more susceptible to LSD with the frequency of 72 (66.67%) in diseased animal whereas in case of control animal the frequency is 04(2.06%). The frequency of disease LSD in case of infected cow was 27 (25.00%) and in non-infected animal it's 15 (7.73%). On the other hand, the bull was usually found resistant LSD infection with the frequency of 93(47.94%) in control group. The Univariate analysis has shown that the occurrence of LSD was highly associated with type of animals.

Table 2. Factors related to Farm management and husbandry practices**.**

Variable	**Total**	**Case**	**Control**	**P-Value**
Type of animal by purpose	**Frequency (%)**	**Frequency (%)**	**Frequency (%)**	
Dairy	209 (69.21)	68 (62.96)	141 (72.68)	0.004
Beef	25 (8.28)	5 (4.63)	20 (10.31)	
Dairy & Beef	68 (22.52)	35 (32.41)	33 (17.01)	
Breed				
Deshi	184 (60.93)	59 (54.63)	125 (64.43)	0.094
Cross	118 (39.07)	49 (45.37)	69 (35.57)	
Type of Animal by gender				
Cow	42 (13.91)	27 (25.00)	15 (7.73)	
Bull		93 (30.79)	93(47.94)	0.000
Calf	76 (25.17)	72 (66.67)	04(2.06)	
Heifer	63 (20.86)	4 (3.70)	59(30.41)	
Lactating and pregnant cow	28 (9.27)	5 (4.63)	23(11.86)	

Considering different age group of animal we found that, the young animals with the age group of 01to 03 months had the highest susceptibility to LSD infection (58.32%), whereas the lowest infection rate was observed in animals with more than six months of age (9.72%).

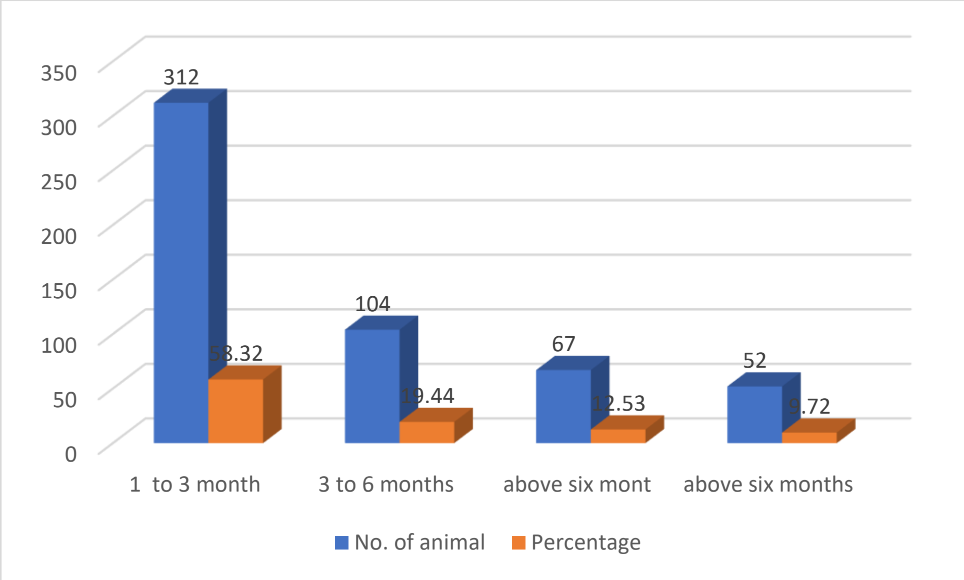

Fig. 3. Frequency and percentage of animal affected by LSD in terms of age group.

For measurement of husbandry practice the variables we took in account was floor type of animal habitat, grazing habit of animal, type and condition of pasture land, location of ponds and bushy area near animal houses, distance of animal house from owner's house and vaccination practice.

The association was measured @ 20% level of significance during univariate analysis and we found some variable significantly associated with the disease occurrence. The overall hygienic condition of cattle house was categorized in three groups and we found the highest frequency (262 ,86.75%) for poor category of hygienic management which was associated with LSD infection (P=0.276). We also found that most of the owner (95.03%) didn't use sanitizer for disinfecting the cattle house, which is co-related with disease occurrence (p= 0.145). Cleaning frequency of cattle house was associated with LSD infection too (p= 0.067), where the analysis revealed that some owners clean their farm daily (117 ,38.74%), some did it weekly (120,39.74%) or after a regular interval (65, 21.52%).

In case of management practices, we found a significant level of association (p=0.025) between the type of pasture, where the animal graze and the disease occurrence @ 20% level of significance. The analysis showed the highest frequency (82, 75.93%) of disease occurrence, who graze in dry low lands, where water remains logged during rainy season and usually act as a breeding place of mosquitoes and flies. From this study it has been documented that, vaccination was likely to be the most protective factor to control Lumpy Skin Disease (LSD) and showed a high level of significance (P=0.00).

We analysed the factors related to hygienic measures and management practices of and around the farm or animal house we had observed the overall hygienic condition of animal habitat, use of sanitizers, mosquito and fly repellents in and around the animal house, cleaning frequencies of animal house and proper manure disposal as hygienic management (Table 3).

Table 3. Factors related to hygienic condition.

Variable	**Case Frequency (%)**	**Control Frequency (%)**	**P-Value**
Hygienic condition			0.276
Good	11 (10.19)	12 (6.19)	
Moderate	4 (3.70)	13 (6.70)	
Poor	93 (86.11)	169 (87.11)	
Grazing			0.881
Yes	102 (94.44)	184 (94.85)	
No	6 (5,56)	10 (5.15)	
Use of sanitizer			0.145
Yes	8 (7.41)	7 (3.61)	
No	100 (92.69)	187 (96.39)	
Use of fly repellant			0.828
Yes	30 (27.78)	35 (18.04)	
No	78 (72.22)	159 (81.96)	
Use of mosquito repellent			0.829
Yes	96 (88.89)	174 (89.69)	
No	12 (11.11)	20 (10.31)	
Grazing			0.881
Yes		184 (94.85)	
No		10 (5.15)	
Cleaning procedure			0.067
Daily	55 (50.93)	62 (31.96)	
Weekly	34 (31.48)	86 (44.33)	

Some times	19 (17.59)	46 (23.71)	
Pond near animal house			0.423
Yes	68 (62.96)	81 (41.75)	
No	40 (37.04)	113 (58.25)	
Bushy areas near animal house			0.458
Yes	107 (99.07)	190 (97.94)	
No	1 (0.93)55	4 (2.06)	
Distance from house			0.379
<=50	55 (50.93)	109 (56.19)	
<=100	53 (49.07)	85 (43.81)	
Floor Type			0.782
Concrete	14 (12.96)	21 (10.82)	
Mud	58 (53.70)	114 (58.76)	
Herring Bond	00	1(0.52)	
Brick & Straw	15 (13.89)	25 (12.89)	
Cement	21 (19.44)	33(17.01)	
Pasture type			0.025
Dry High Land	18 (16.67)	40 (20.62)	
Dry Low Land	82 (75.93)	134 (69.07)	
Marshy Low Land	2 (1.85)	12 (6.19)	
Vaccination			0.000
Yes	00(00)	52 (48.15)	
No	194(99.00)	56(51.0)	
Manure disposal			0.573
Yes	99 (91.67)	174 (89.69)	
No	9 (8.33)	20 (10.31)	

For further analysis, the multivariable logistic regression analysis took into account the explanatory variables with $p \leq 0.2$ in the bivariable analysis. Additionally, the Crammer's V factor was used to evaluate the multicollinearity among the explanatory variables. During observation of multicollinearity among the explanatory variable we found collinearity among use of sanitizer and hygienic condition of animal habitat. So, for further multivariate regression analysis we exclude these two exposures variable and found farm type, breed, type of animal and vaccination are significantly associated with dependent variable (disease).

In case of multivariate analysis, we found that the chance of LSD infection was seven times higher in dairy cattle than that of beef type animals. Similarly, the calves were 12 times more prone to LSD infection than the bull. We also found that the cross-bred animals were 1.35 times more susceptible to the occurrence of LSD than that of deshi breed. We found vaccine as a protective factor, which is useful to prevent LSD. The probability of developing LSD in non-vaccinated animal was 3.65 times higher than the vaccinated one (Table 4)

Table 4. Findings of multivariate regression.

Variable	**Total Frequency (%)**	**Odds Ratio**	**P-Value**	**CI (95%)**
Farm Type				
Dairy	209 (69.21)	0.207871	0.042	0.077582-1.21296
Beef	25 (8.28)	ref	-	-
Both	68 (22.52)	0.761968	0.216	.4617647- .64881
Type of Animal				
Cow	42 (13.91)	0.2394	0.000	.056224 - 1.46268
Bull		93(30.79)		
Calf	76 (25.17)	0.390	0.218	0.873073-1.744852
Breed				
Deshi	184 (60.93)	ref	-	-
Cross	118 (39.07)	0.3492	0.038	0.8085352-1.51455
Vaccination				
Yes	8(2.65)	0.646	0.003	.1822626 – 1.95492
No	294(75.35)	ref	-	-

CHAPTER 5: DISCUSSION

LSD is a disease which causes serious economic loss due to declined milk production, low market price of cachectic animals and deteriorated skin quality. In case of such kind of disease, Prevention is the most effective way to mitigate the huge economic loss rather than treatment. Vaccination is the most effective measure of prevention and control of LSD. This study will help to figure out the role of vaccine to reduce the prevalence of LSD throughout the country and it will also play a crucial role in preparing further control program.

According to the findings of Gari et al. (1987), LSD infection is more common in dairy cattle than the beef and draught perpose cattle. In this study it was also observed that the dairy type cattle were more prone to LSD infection than the beef type cattle. The main cause behind this species susceptibility might be associated with breed and transmission through common milker in a same area. In our country, the beef breed is usually reared for a short period of time aimed to a festival. So, during this short time the beef type animals are commonly don't get chance to be infected.

In the study of Rahman et al. (2022) the findings present that the female animals are more susceptible to LSD occurrence. Similarly inthis study, another potential risk factor came to light in final regression analysis was the gender and age of animals. The female cattle specially the cows were very much prone to the LSD infection. Another important finding of this study is that bulls were very resistant to LSD infection whereas the calf was found most susceptible to LSD and in most cases, it is fatal also.

In the study of Chouhan et al. (2022) it is observed that the rate of infection of LSD is higher in cross bred animal than that of local breed. In this study similar findings were observed after regression analysis, it has been found that the cross-bred animals were more susceptible than the dashi breed. The vaccine has been proved as a protective factor to control the disease effectively. It has been found that more than 90% animal, which has been vaccinated have developed immunity (Don't developed disease).

CHAPTER 6: CONCLUSIONS

The study sheds light on the risk factors of LSD in Mirsarai upazilla of Chattogram district and Nawabgonj upazilla of Dinajpur district in Bangladesh. This case-control study addresses the risk factors like cross breed, dairy and female animals are highly associated with the occurrence of LSD. These risk factors highlight the significance of proper vaccination. This study provides evidence for the policy makers that they should collaborate with relevant stakeholders for the control of LSD and to minimize the enormous economic loss caused by LSD. The study also encourages the intervention measures by addressing the risk factors and resulting the containment of outbreaks.

CHAPTER 7: RECOMMENDATIONS

a) Farmers should be made aware of the use of LSD vaccine.

b) Training of farm owners for improvement of biosafety measures on farms.

c) Vaccination activities must be conducted before the outbreak of the disease. The Upazilla Livestock Office can take initiative to collect the required number of vaccines and apply it in cows. In particular, necessary steps should be taken to bring the calves under vaccination.

D) If the required number of vaccines are not available, farmers should be made aware about the collection of vaccines from commercial sources at local level and their application to animals.

E) Individuals engaged in the treatment of cattle at the village level should be aware of the means of transmission of the disease and measures should be taken to increase the awareness of the villagers.

F) Infected cows should be treated and advised following "Lampy Skin Disease Management Guidelines for Bangladesh" and other necessary measures should be taken.

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Annex 1

Department of Livestock Services

Epidemiology Unit

Questionnaire for the case-control study of lumpy skin disease outbreak investigation in Dinajpur and Chattogram districts

ID No: Date of interview:

Farmers Demography

1. Name of the farmer:

2. Address:

3. Gender: 4.Mobile No:

5. GPS location:

6. Occupation:

7. Income Source: □ Business □ Job □ Labour □ Any other (Please specify…)

8. Educational Study:

□ No institutional study □ Primary □ Secondary

□ Higher Secondary □ Tertiary □ Any other (Please describe….)

9. Type of farm: □ Intensive □ Semi-intensive □ Open □ Other (Please specify…)

10.Spatial location: Length: Width:

11. Number of animals □ Calf: □ Heifer: □ Cow: □ Bull: □ Any other: □ Total:

12. The number of sick animals due to LSD for last six months:

13. No. of the sold animal during last six months:

14. Number of dead animals last six months due to LSD:

15. What did you do with the dead animals ?

□ Buried □ thrown in nearby pond or field □ Other (Please specify…)

16. Price value of dead animal: Decrease price of sold animal due to LSD:

Management/Risk-related information

17. How do you dispose the manure?.....................................

18. How do you clean your cattle house?

 By using hose pipe By swiping By removing manure only Other (Specify please…)

19. How frequently do you clean your cattle house?

 Twice a day Daily Every alternative day Twice a week _____/day;

20. Do you use sanitizer to clean your cattle house?

 Yes No

21. If yes, Please mention their name…………

22. Do you use fly repellent?

 Yes No

23. If yes, Please mention their name…………

24. Do you use the mosquito net in your cattle house?

 Yes No

25. Do you use mosquito repellent?

 Yes No

26. If yes, Please mention their name…………

27. Do you have any stagnant water bodies near your house?

 Yes No

28. Please specify the distance between the water bodies and the shed........................

29. Do you have a bushy area around your house?

 Yes No

30. Please specify the distance between the bushy areas and the shed.............................

31. How is the overall hygienic condition of your cattle house? (Hygienic Guideline)

 Poor Moderate Good

32. What is the feeding system of your cattle?

 Stall feeding Grazing Both

33. In the case of grazing where do you take your cattle for grazing?

 In dry high land In dry low land In marshy low land In paddy field Other (Specify please…)

34. What is the floor type of your animal house?

□ Concrete □ mud □ herring bond □ brick with straw □ cement □ Others (Specify please…)

35. For breeding purpose what measure do you use?

□ Natural service □ AI (Artificial Insemination)

36. If AI is used, then when did you last make AI of your cattle? Date…………

37. What is the source of the AI?

□ Govt. Hospital □ Private company (specify name please………….)

38. Age:

39. Sex:

40. Breed

41. What kind of problems did you see when your cow got sick?

□ Fever □ Swollen joint □ Swollen lymph nodes □ Nodules throughout the body □ Ruptured nodules □ pas and maggot formation □ Any others (Please specify…)

42. Do you have any sick animal at present? □ Yes □ No

43. If yes, when does it show the clinical sign? Date…

44. Did you provide any treatment? □ Yes □ No

45. What are the medicines given for treatment? Specify, please…

46. Who facilitates the treatment?

□ Govt. Vet □ Private practitioner □ Quack □ Self-arranged

47. Please calculate the cost of treatment for all LSD affected animal in your house……………

48. Did you observe any abnormal symptoms after recovery? Specify please………..

49. Did you vaccinate your animal against LSD? □ Yes □ No

50. If yes, when did you vaccinate? Date………

51. Who facilitates the vaccination program?

□ Govt. Vaccinator □ Quack □ Private practitioner □ Self-arranged (Please Specify)…………

52. What is the source of vaccine?

□ Govt. Hospital □ Private company □ FAO

53. Please show me the vial of vaccine (for Identification of Seed) or in case of vaccination by FAO or Govt. office the data about vaccine seed will be collected…………………………..

54. What is the stage of parity of your animal?

□ 1st □ 2nd □ 3rd □ 4th □ more

55. If AI is used, then when did you last make AI of your cattle? Date…………

56. Have you recently introduce new animals in the farm'?

□ Yes □ No

57. If yes, Where did you buy your cattle from?

□ cattle market □ neighbour house □ same village □ middle man □ other (specify……)

58. When did you buy your cattle?

□ Date 1……….. □ Date 2……….. □ Date 3………..□ more ………..

59. After introduction of new animals did you keep them isolated ?

□ Yes □ No

60. If yes, For how many days you maintained this isolation?..................

61. if any cattle become sick or affected with LSD did you isolate them?

□ Yes □ No

62. If yes, For how many days you kept them isolated?........................

63. Please mention three most important factors related to occurrence of LSD.

1………………..

2. ……………….

3. ………………..

64. Please give your opinion about the prevention and control of LSD?..................

NB: Hygienic Guideline :

1.□ Poor - Cleaning of floor with only water regularly

- Un-authorized people and vehicle access is not prohibited,

- Water does not run out perfectly within 15 minutes during cleaning or rain and the floor remains wet most the time.

-no separate feed store room, feed are stored within the burn.

-No washing of milkers' hand with antiseptics before milking.

2. □ Moderate - Cleaning of floor with disinfectant once or twice per week.

- Water runs out perfectly from the pen within 15 minutes during cleaning but during heavy rain water stays for more than 15 minutes.
- locked separate feed store room without proper ventilation.

3. Good - Cleaning of floor with disinfectants every day, un-authorized people and vehicle access in farm premises is prohibited.
    - Water runs out perfectly from the pen within 15 minutes during cleaning and rainy season and leaves the pen dry.
    - Locked separate feed store room with proper ventilation, locked separate feed store room with proper ventilation.(Chowdhury et al. 2017)

Parameters for Grazing land criteria:

1. dry high land- No stagnant water through the year round
2. dry low land - No stagnant water except rainy season
3. marshy low land- Stagnant water remain almost throughout the year
4. paddy field- land used for cultivation

Reference:

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Annex 2:

Photo Gallery:

Biography

This is Dr. Jobaida Khanam, a Government employee I have worked as a veterinarian for the Department of Livestock Services (DLS) for the past seven years. Now I am doing my second MSc in applied veterinary epidemiology through One Health Institute in collaboration with GHD (Global Health Development) and CVASU (Chattogram Veterinary and Animal Sciences University) and the first cohort of the FETPV program. I received first place in her master's degree in veterinary microbiology in 2011 (CGPA: 3.96 out of 4.00) and second place in my bachelor's degree in 2009 (DVM, CGPA 3.78 out of 4.00) from Hajee Mohammod Danesh Science and Technology University.