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Epidemiological studies on lumpy skin disease in cattle and buffaloes

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Abstract
Lumpy skin disease is an emerging vector borne viral disease in India and was reported in many states including Karnataka. In the present study, index case was recorded in Bidar district in July 2020 and subsequently cases were reported from different talukas of Bidar district. The morbidity of LSD in Bidar district was 6.00 per cent and 0.17 per cent for cattle and buffaloes respectively and the overall morbidity rate was 3.55 per cent. Common clinical signs recorded were high fever, round circumscribed skin nodules, lymph node enlargement and brisket to limb edema. The age-wise and sex-wise prevalence study revealed, adult and male cattle were more susceptible to LSD. In case of breeds, all breeds were equally prone to infection. LSD infection peaked during the month of August through October and gradually decreased to lowest level during the month of December, which positively correlates significantly with temperature, relative humidity and rainfall (r=1.00). The climate with hot humid environment might have favored the up surging of vectors and occurrence of LSD in Bidar district. Vector study revealed, *Haematobia* (horn) flies that are suspected to be vector of LSDV were found to be predominant in the LSD affected farms.

Keywords: Lumpy Skin disease, prevalence, temperature, relative humidity, rainfall, *Haematobia* (horn) flies

Introduction
Lumpy skin disease is an important viral disease of cattle and buffaloes of all breeds and age groups, characterized by high fever, nodular lesions on skin and enlargement of superficial lymph nodes. This is an evolving infectious disease having important economic significance. The causative agent is lumpy skin disease virus (Neethling), an important animal pox DNA virus of the family *Poxviridae*, subfamily *Chordopoxvirinae* and the genus *Capripoxvirus*. This virus has an antigenic relationship and resemblance with goat pox and sheep pox viruses. All three capripox viruses have a common antigen on their membrane and this resemblance is the reason to use sheep pox vaccine against LSD (Radostits *et al*., 2007) [29].
Lumpy skin disease (LSD) virus causes infection in cattle (*Bos indicus* and *Bostaurus*) and water buffaloes (*Bubalus bubalis*). However, cattle are more susceptible (30.80%) compared to buffaloes (1.60%) (El-Nahas *et al*., 2011) [16]. The major way of transmission of LSD virus is by blood feeding arthropods which act as mechanical vector. Rarely transmission occurs through contaminated feed and water or through contact (Ali *et al*., 2012) [4]. Haematophagous arthropods that are likely to serve as LSDV vectors include the following taxa: biting flies like horn flies, stable flies (Diptera: Muscidae) and equine flies (Diptera: Tabanidae), hard ticks (Acari: Ixodidae), mosquitoes (Diptera: Culicidae), *Culicoides* biting midges (Diptera: Ceratopogonidae) etc. (EFSA, 2015) [14].
Major risk factors of outbreak of LSD are warm and humid weather conditions and high abundance of vector populations. Wide range of LSD outbreaks are generally observed during the wet season and are related with heavy rain fall and high level of blood feeding insect's activity(Gari *et al*., 2010) [19].
Lumpy skin disease was reported for the first time in Zambia (North Rhodesia) in the 1929, which was at first assumed as an allergic reaction because of biting insects in cattle (Woods, 1988). In India, it was first reported from Odisha (Khairbani, Betnoti and Mayurbhanj) on 12 August 2019. Subsequently, the outbreaks were reported from other parts of Mayurbhanj and Bhadrak of Odisha (Sudhakar *et al*., 2020) [33]. Since then, there were reports of LSD recorded in different part of the country like Chhattisgarh, Madhya Pradesh, Jharkhand, West Bengal, Assam, Andhra Pradesh, Telangana, Tamil Nadu, Maharashtra, Karnataka and Kerala (Srinivas *et al*., 2020) [32].

Lumpy skin disease is classified as a notifiable disease by the World Organization for Animal Health (OIE) because of its considerable economic impact (Tuppurainen and Oura, 2012) [34]. Due to devastating economic losses occurring to the farmers, it has become necessary to study about risk factors associated with the occurrence of disease and planning control strategies. Control of vectors remains one of the major strategies for prevention and control of disease in the absence of effective vaccine in India.

Materials and Methods

Place of study

In the present study, five talukas of Bidar district and animals presented to Veterinary Clinical Complex, Veterinary College, Nandi Nagar, Bidar (KVAFSU) were included. Five talukas of Bidar district are Bhalki, Basavakalyan, Humnabad, Aurad and Bidar. Bidar is located on northern side of Karnataka state, bordering Maharashtra and Telangana. The livestock population was estimated to be 1,73,634 cattle and 1,25,510 buffaloes (20th livestock census, 2019).

Outbreak data

Lumpy skin disease is a notifiable disease and it is required to be reported. Lumpy skin disease outbreak data of complete Bidar district were obtained from department of Animal Husbandry and Veterinary Science, Bidar and veterinary dispensaries of different villages in Bidar district. The records contain general information about number of infected animals, places of outbreak and time of outbreak. A point prevalence study of LSD outbreak was undertaken in Veterinary Clinical Complex (VCC), Veterinary College, Nandi Nagar, Bidar (KVAFSU) for the period of January 2020 to December 2020. The study includes data on number of LSD infected animals among total cases presented to VCC during outbreak period. Other possible variables including animal details like species (Cattle/Buffalo), breed, sex (Male/Female) and age (<1 years, 1-2 years and >2 years) were also recorded. Numbers of deaths due to disease complications were recorded for further epidemiological studies. Collected data on LSD outbreak were used to obtain descriptive statistics like morbidity, mortality and case fatality rate.

Animals were suspected for lumpy skin disease based on typical clinical signs shown. Further confirmation of LSD infection was done by capripoxvirus generic polymerase chain reaction using scab samples.

Affected animals were examined for all the typical signs which conclude it as LSD infection, which includes, Mild (102-103ºF) or High (>103ºF) rectal temperature, localized or generalized Skin nodules, lymph node enlargement, brisket edema or limb edema, corneal opacity and respiratory involvement. Information about common complications occurred due to lumpy skin disease were recorded. These include reduction in milk yield, reduction in working efficiency, anestrous and abortion.

Meteorological data

Data on meteorological parameters of Bidar district were collected from Agriculture Research Station, Halladkeri, UAS, Raichur, Bidar for a study period. This data includes mean temperature (in ºC), average rain fall (in mm) and relative humidity (in percentage). The average values of each parameter were calculated per month from January 2020 to December 2020. Analysis was performed to investigate the degree of relationship between disease outbreak and other climate variables in and around Bidar using spearman correlation coefficients (*r*) (Alkhamis and Vanderwaal, 2016) [5]. Temperature-humidity index (THI) is a combined single value, which represents both effects of 'air temperature' and 'humidity associated with the level of heat stress'.

$$THI = (1.8 \times AT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26)]$$

Where
AT = Air temperature (°C)
RH = Relative humidity (%)

Vector study

Finding endemic vectors which are abundant in Bidar district was important, as it might be responsible for spread of lumpy skin disease in and around Bidar. Different outbreak sites or affected farms were selected randomly in and around Bidar for the study and prevailing status of vector population were recorded. Flies present on animal body were collected using modified electric insect suction trap (T TOPLINE blowing and sucking dry handheld vacuum cleaner, 1000 watts) and were identified as per the standard protocol based on wings and mouth part (Bhatia *et al*., 2007) [10].

Results

Prevalence study

The incursion of LSD outbreak for the first time in Bidar, Karnataka during 2020 in naive animals showing vigorous clinical picture with a high morbidity were recorded. Where in a total of 630/699 villages (90.12%) were infected, indicating a wide spread of LSD in naive population of cattle and buffaloes in Bidar district.

Morbidity rate of lumpy skin disease in Aurad taluka was 10.91 per cent (4462/40867), which was followed by Bhalki (2916/35246, 8.27%), Humnabad (1764/29247, 6.03%), Basavakalyan (874/42875, 2.03%) and Bidar (411/25399, 1.61%) in case of cattle. Whereas the morbidity rate in buffaloes, in Aurad was 0.45% (134/29649), which was followed by Bhalki (59/22524, 0.26%), Bidar (10/32721, 0.03%), Humnabad (08/20217, 0.03%) and Basavakalyan (03/20399, 0.01%). The morbidity rate of Bidar district were 6.00 per cent and 0.17 per cent for cattle and buffaloes respectively. The overall morbidity rate of LSD in Bidar was 3.55 per cent.

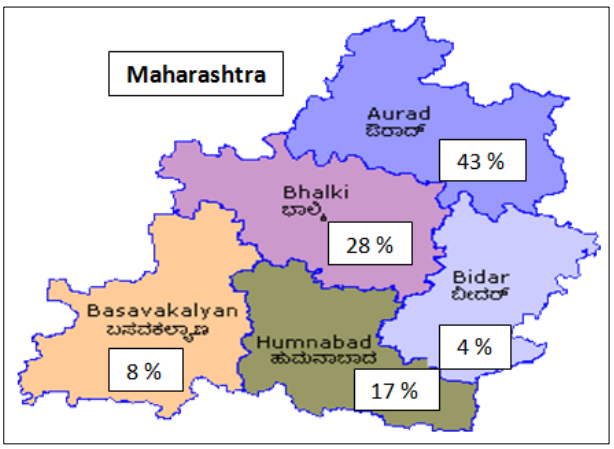

Fig 1: Taluka-wise occurrence of Lumpy skin Disease in Bidar district

Point Prevalence study

A total of 2017animalswere examined for LSD during the period, of which 1199 were cattle and 818 were buffaloes. Among them, 226 cattle and 08 buffaloes were showing LSD infection (Plate 05 and 06). This concludes the prevalence of lumpy skin disease among the study population was 11.60 per cent, whereas higher percentile of prevalence was recorded in cattle (18.84%) than in buffaloes (0.97%). Of which, 14 cattle (Calf-08, Bullock-03 and Cow-03) were succumbed to death due to lumpy skin disease. The overall mortality rate recorded of lumpy skin disease in cattle was 1.16 per cent. However, no mortality was recorded in buffaloes due to LSD during this study period. The case fatality rate was recorded as 6.19 per cent in cattle, whereas no case fatality was noticed in buffaloes.

Polymerase chain reaction

Scab samples were collected from Lumpy skin disease suspected animals and were tested by conventional Capri pox virus generic PCR. In test, amplified products of expected size (192 bp) for the partial fragment of P32 gene were obtained.

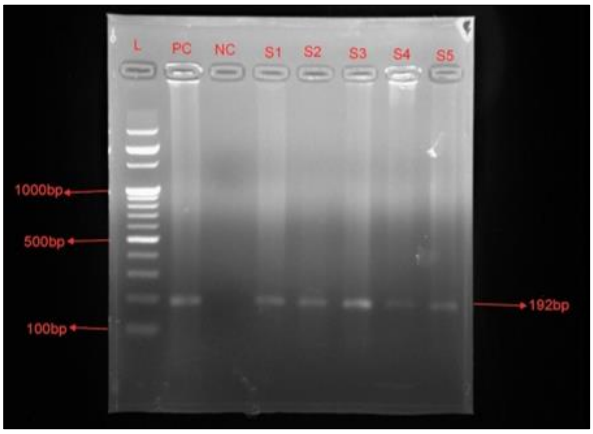

Plate 1: Gel photograph of P32 gene PCR (L-Ladder, PC-Positive control, NC-Negative control and S-Scab sample)

Physical examination

Among studied population, animals have shown clinical signs like enlarged superficial lymph nodes (92.73%), localized (50.85%) and generalized (49.14%) flat-topped nodules, limb oedema (38.88%), high fever (34.61%), mild fever (29.48%), brisket oedema (22.22%) and rarely respiratory involvement (2.56%) and corneal opacity (1.70%).

A: Photograph showing lumpy skin disease infected cattle
B: Photograph showing brisket and limb oedema in lumpy skin disease infected cattle
C: Photograph showing ocular discharge and corneal opacity in lumpy skin disease infected calf
D: Photograph showing nasal discharge and respiratory involvement in lumpy skin disease infected calf

Age

The animals with age more than 2 years showed a higher prevalence of LSD (74.78%) than young animals of age less than one year (16.23%) and were followed by age of one to two years (8.97%). In this study mean age of cattle affected with lumpy skin disease was 5.80±0.29 years whereas mean age of buffaloes infected with lumpy skin disease was 3.16 ± 0.79 years.

Sex

In atotal of 234 animals, 147 (62.82%) were male and 87 (37.17%) were female. This indicates that males were found to be more susceptible when compared to females.

Breed

Out of 226 infected cattles, non-descript were 87 (38.49%), which was followed by Deoni (60 / 26.54%), Red kandhari (52 / 23.00%), Holstein Friesian cross (13 / 5.75%), Jersey cross (09 / 3.98%) and Gir (05 / 2.21%). In case of buffaloes out of 08 cases presented, 07 (87.5%) were non-descript and 01 (12.5%) was Murrah. The results revealed that both descript (59.82%) and non-descript (40.17%) breeds were affected.

Economic significance of disease

Lumpy skin disease has post infection complications in some of infected cattle and buffaloes *viz*., reduced milk yield, anestrous for long period in cows and reduced working

efficiency in bullocks.

Reduction in milk yield
Out of 234 animals infected with lumpy skin disease, 87 were females and among them 09 animals (10.34%) have shown reduction in milk yield as post infection complication due to LSD.

Reduction in working efficiency
Among 234 cases interviewed for post infection complications, 147 were male and among them, 08 bullocks (4.76%) have shown reduced working efficiency after LSD infection.

Anestrous
A total of 87 females were presented out of 234 infected cases of lumpy skin disease and among them, 05 animals (5.74%) were showing anestrous condition for more than 5 months after LSD infection.

Abortion
Out of 234 animals presented with lumpy skin disease, 19 animals were pregnant and none of them were recorded with abortion at any stage of gestation.

Untoward incident
Majority of the cases recovered from the infection after proper treatment within few days to two weeks. However, few animals didn't respond to treatment and succumbed to death (14) or took longer time to recover (7 animals sold out).

Meteorological study
Temperature humidity index (THI)
THI for the months of August, September and October were 81.53, 84.01 and 82.59 respectively. THI was positively correlated with outbreak of LSD in and around Bidar. In accordance with the temperature-humidity index (THI), the prevalence of LSD was higher in September (7885), followed by October (2079) and August (677).Upon analysis, Temperature-humidity index (THI) and number of LSD infected animals during outbreak were positively correlated (r = 1.00 and p<0.01). So, it was concluded, the prevailing Temperature-humidity index (THI) during epidemic period might have favoured the occurrence of LSD infection in cattle and buffaloes.

Mean rainfall (mm)
Rainfall was recorded from March to October in Bidar district in 2020. Initially in summer months it was minimum from 12.60 to 47.90 mm. The rain fall got increased from June and reached the maximum level in September (415.40 mm) and October (221.40 mm). Subsequently no rainfall recorded in November and December months. Rainfall and occurrence of LSD during outbreak were strongly positively correlated (r = 1.00 and p<0.01). Hence, rainfall seems to favour LSD outbreaks in cattle and buffaloes.
Overall, highest numbers of cases were reported in the month of September, where about 7885 positive cases reported among total of 10,641 LSD affected cases in whole Bidar district. This show, the climate with rainy, warmer and humid environment in September might have favoured the occurrence of LSD in cattle and buffaloes in Bidar district.

Table 1: Correlation of climatological factors with occurrence of LSD in cattle and buffaloes (Spearson correlation)

Month	No. of infected animals	THI	'r' value	Mean Rainfall (mm)	'r' value
August	677	81.53	1.00	164.6	1.00
September	7885	84.01		415.4	
October	2079	82.59		221.4	

Note: r= +1: Perfect positive association, 0: No association, -1: Perfect negative association

Vector study
Around 30 farms were visited for finding most abundant and endemic vectors present in the farms. In all the visited cattle and buffalo farms most common vectors present were horn fly (*Haematobia irritans*) and *Culicoides* midges. Among these vectors, *Haematobia* flies were in abundance in all the farms. So, *Haematobia* fly was suspected to be the major vector for transmission of LSD in and around Bidar. *Haematobia* fly's abundance study (July-2020 to June 2021) by visual count method revealed, *Haematobia* fly *abundance* increases from July to November, and decreases in subsequent months. This was coinciding with the outbreaks of LSD in animals. This is another reason of suspicion on *Haematobia* fly as vector for transmitting LSD between affected and healthy animal.

Discussion
Outbreak study
In the present study, a total of 630 (90.12 per cent) villages were recorded with LSD infection in Bidar district. This unprecedented outbreak might be due to naive population getting exposed to new disease for the first time. Hence, most of the villages experienced the outbreak of LSD in cattle and buffaloes. The first case reported in Bidar was from Amlapur, which borders Telangana state and highest prevalence of disease was seen in Aurad and Bhalki talukas, which border Maharashtra state. Both the neighboring states had high occurrence rate of LSD.
The overall prevalence of LSD in Bidar district was 3.55 per cent, similar findings were recorded by Gari *et al*. (2010) [19] and Abera *et al*. (2015) [1].
In point prevalence study, the prevalence of lumpy skin disease among the studied population during July to December 2020 was 11.60 per cent. The study also reported that LSD prevalence was higher in cattle (18.84%) than in buffaloes (0.97%). The findings are in agreement with Ahmed *et al.* (2020) [3] and Abutarbush *et al*. (2015) [2]. However, the low morbidity rate of LSD in buffaloes could be associated with its thicker skin, which can prevent the insect bites and reduces transmission of LSDV (Chihota *et al*., 2003) [11]. The overall mortality rate recorded of lumpy skin disease in cattle was 1.16 per cent. The results were in accordance with Gari *et al*. (2008) [18] and Abutarbush *et al*. (2015) [2]. On contrary no mortalities were recorded by Ayelet *et al*. (2013) [6] and Sudhakar *et al*. (2020) [33]. In addition, higher mortality rate was recorded in calves (57.14%) than adult animals, which might be associated with impaired cellular immunity in younger calves (Hunter and Wallace, 2001) [24]. The case fatality rate recorded was 6.19 per cent during an outbreak period. Similar finding was observed by Abutarbush *et al*. (2015) [2]. In contrary no case fatality rate were recorded by Ayelet *et al*. (2013) [6].
Prevalence is higher in adults than young animals in our study. These results were in correlation with Elmohsen *et al*. (2019) [15] and Faris *et al*. (2021) [17]. Adult animals undergo

stress due to pregnancy and lactation which might be the cause for higher prevalence in adult animals (Faris *et al*.,2021) [17] and this also might be due to the fact that calves are kept indoor, away from biting insects (Ochwo *et al*., 2019) [27]. On the contrary, Ayelet *et al*. (2014) [7] reported that young ones are more susceptible than adult to LSD infection.

The higher prevalence was observed in male than female animals. Present study findings resemble with Gari *et al.* (2011) [20] and it may be possibly linked with male undergoing exhaustion due to draft works and the risk of exposure to stress and fatigue which is assumed to increase susceptibility. On contrary, Ayelet *et al*. (2014) [7] and Elmohsen *et al*. (2019) [15] reported that females are more susceptible to LSD infection than male animals.

In case of both cattle and buffaloes, all breeds are susceptible to LSD infection, and this is in agreement with Ahmed *et al.* (2020) [3] and Sudhakar *et al*. (2020) [33].

Economic significance of LSD

Reduction in milk yield

In present study, 10.34 per cent of animals (10) have shown drastic reduction in milk yield on post infection. This complication of the disease was also recorded by Gari *et al*. (2012) [21], Hailu *et al*. (2015) [23] and Sevik and Dogan (2017) [30]. A sharp decline in the milk production is associated with high fever by viral infection and secondary pathogenic mastitis (Hailu *et al*., 2015) [23].

Reduction in working efficiency

Among 234 cases interviewed for post infection complications, 147 were male in which 5.44 per cent of bullocks (08) have shown reduction in working efficiency in post infection period. Reduction in working efficiency in LSD affected bullocks was also seen by Hailu *et al*. (2015) [23]. Reduced working efficiency is due to anorexia and debility (Gari *et al*., 2012) [21]. Lesions in skin, subcutaneous tissue, and muscles of limbs, together with severe skin inflammation and secondary infection greatly reduce the mobility and rapid deterioration in body condition results in late recovery and reduced working efficiency (Birhanu, 2015) [9].

Anestrous

Total of 87 females were examined out of 234 cases of lumpy skin disease and among them 5.74 per cent of animals were anestrus for more than 5 months after infection. Prolonged anestrous after infection was recorded by Gari *et al*. (2012) [21], Hailu *et al*. (2015) [23], and Manic *et al.* (2019). Anestrous or delayed estrous is due to debility and loss of condition (Gari *et al*., 2012) [21].

Abortion

Out of 234 animals presented with lumpy skin disease, 19 animals were pregnant and none of the animal has shown abortion at any stage of gestation. On contrary Baibuk *et al*. (2008), Gari *et al*. (2012) [21] and Abutarbush *et al*. (2015) [2]. Abortions occur as animals suffer prolonged fever at early gestational stage (Gari *et al*., 2012) [21].

Untoward incident

Majority of the cases recovered from the infection after proper treatment within few days to two weeks. However, few animals didn't respond to treatment and succumbed to death (5.98%) or took longer time to recover (2.99% animals sold out). Delayed recovery due to disease complications was observed by Davies (1991) [12] and Gupta *et al*. (2020) [22]. Extensive nodular lesions produce a hide bound condition, respiratory lesions and limb oedema which may cause a prolonged anorexia and reluctance to move. Rapid deterioration of condition due to dehydration leads animal to be in poor condition for several months and may cause higher rate of mortality (Davies, 1991) [12].

Meteorological study

The pattern of LSD outbreak in Bidar district was in positive correlation with THI, as the numbers of infected animals were more in months with highest THI value. So, it was concluded that as the Temperature-humidity index (THI) increases, number of LSD infection also increases. The finding agreed with Faris *et al*. (2021) [17]. This might be due to, with increased THI, weather conditions will be warm and humid which enhances the growth and spread of the blood feeding insects. These vectors can transmit LSD virus between susceptible and affected animals (Faris *et al*., 2021) [17].

Highest rainfall was seen in correlation with LSD outbreak, which was also peaked in the month of September 2020. Similar findings were observed by Quinn *et al*. (2011) [28] and Molla *et al*. (2017) [26]. Lumpy skin disease outbreaks generally occur during the rainy season when blood feeding insect activity is high. Therefore, epidemics are often connected with heavy rain fall (Quinn *et al*., 2011) [28].

Vector study

Haematobia fly was incriminated as major vector for transmission of LSD in and around Bidar. The results were in accordance with EFSA (2015) [14], EFSA (2017) [13].

Conclusion

The overall prevalence of LSD in Bidar district was 3.55 per cent, affecting 90.12 per cent of villages in Bidar district. Prevalence of LSD was more in cattle (18.84%) when compared to buffaloes (0.97%). Adult animals (>2 years) and male animals were more susceptible to disease. Climate with rainy, warmer and humid environment along with high abundance of horn fly during outbreak period might have favoured the occurrence of LSD in Bidar district.

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