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ORIGINAL RESEARCH

Seroprevalence and Associated Risk Factors of Lumpy Skin Disease of Cattle in Selected Districts of Afar Region, Ethiopia

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Background: Lumpy skin disease (LSD) is one of Ethiopia's most economically significant transboundary livestock illnesses. The disease has a significant economic impact on pastoral household livestock owners, who rely significantly on their cattle as a source of income.

Methods: A cross-sectional study was undertaken in selected districts of Afar region from November 2018 to May 2019 primarily intended to estimate the prevalence of lumpy skin disease serologically in local Afar cattle as well as identify potential associated factors. A multistage sampling method was employed to select study districts, peasant association, herd size and study units. A total of 384 sera were processed using serum neutralization test (SNT) method to detect antibodies against lumpy skin disease virus. Relevant data were refined and further analyzed using stata version 14.

Results: In the study districts, the overall animal level seroprevalence was found to be 7.6% (N = 29/384; 95% confidence interval: 4.90–10.20) and the overall herd level prevalence was found to be 20.8% (n = 15/72; 95% confidence interval: 11.42–30.18). Only district was shown to be statistically significant (P = 0.004) in terms of LSD occurrence among the relevant factors studied. Cattle in Chifra district were 20.18 times more likely to contract LSD infection than cattle in Dubti district, when Asayita district was used as the reference group.

Conclusion: The present study finding confirmed the presence of the disease in the study districts of afar region and coordinated intervention set to be in place.

Keywords: afar, cattle, LSD, associated factors, seroprevalence, serum neutralization test

Introduction

Ethiopia is the first in Africa with huge livestock population, with an estimated 59.5 million cattle, 31.3 million sheep, 32.74 million goats, and 54.5 million chickens.[1] In Ethiopia, the livestock subsector is a significant contributor to the majority of the population's livelihood as a source of meat, milk, drought power, and income, particularly in pastoral and agro-pastoral areas.[2] Despite having the highest livestock figure in Ethiopia, production and productivity has remained low, owing to livestock diseases and other constraints.[1] In Ethiopia in general and the study areas in particular, livestock diseases are the leading cause of significant economic losses to pastoral livestock owners. Various fragmented study reports show that livestock diseases are widespread across the country, with yearly mortality rates of 8–10% for cattle herds, 15% for sheep and 12% for goat flocks respectively. Furthermore, diseases were estimated to reduce livestock productivity by 50–60% per year.[3,4] Transboundary livestock diseases have continued to cause significant devastating losses in Ethiopia's livestock sector, possibly by imposing trade restrictions, aggravating poverty. Lumpy skin disease (LSD), foot and mouth disease (FMD), and contagious bovine pleuropneumonia (CBPP) are three of the most economically important livestock diseases in developing countries including Ethiopia.[5,6]

Lumpy skin disease (LSD) is one of the most economically important transboundary viral infections affecting particularly of cattle. Lumpy skin disease (LSD) is a highly infectious disease caused by lumpy skin disease virus

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(LSDV) that belongs to the *Capripoxvirus* genus of Poxviridae family.[7,8] The genus Capripoxvirus is comprised of three genetically closely related important virus species, such as sheep pox virus (SPV), goat pox virus (GPV) and LSDV, affecting sheep, goats and cattle respectively.[9] Arthropod vectors, mechanical means and tick species of the family Ixodidae mainly play an important role in the transmission of LSDV. More importantly, Stomoxys calcitrans (Stable fly) has been reported as the most probable vector for LSDV due to its abundance and being associated with outbreaks.[10,11] As a result, LSD episodes mainly occurred during the rainy season, when bug activity is at its peak[12] and the severity of the disease is also depends on virulence nature of the viral strain, susceptibility of the host, immune status of the cattle, animal breed and other related factors.[13] Clinically, the disease is characterized by elevated fever, enlarged lymph nodes and bordered nodules on the skin, emaciation, edema of the skin, nasal discharge, and death.[8,14] Since the disease was first discovered in Zambia in 1929, LSD has gradually and widely expanded over Africa, the Middle East, Southeastern Europe, Central Asia, and more recently South Asia and China. Several countries in Africa, states of the Middle East, and Turkey currently have endemic to cases of the disease. Recently, LSD was introduced into China, Bangladesh and India, beginning from July 2019. In 2020, the disease then spread to other parts of China and India as well as Nepal and Bhutan, indicating the continued and widespread presence of the disease.[15] In case of Ethiopia, the disease was first discovered in 1981 in northwestern regions,[16] and it has since spread to practically all of the country's territory. According to epidemiological studies conducted in various regions of Ethiopia, LSD seroprevalence ranges from 6.4% to 31% at the animal level and up to 64% at the herd level.[17,18] Due to its rapid spread and significant economic losses, the World Organization for Animal Health (OIE) categorized LSD as a notifiable disease under "Cattle diseases and infections".[19–21] Lumpy skin disease virus (LSDV) mostly affects ruminants, imposing financial pressure on pastoral livestock owners whose main source of income is livestock production and rearing.[22] Its substantial economic losses of the disease in livestock industry results from a sharp decline in milk yield and meat production, permanent damage to skin and hide, body weight loss, abortion, retarded growth and infertility. Trade limitations and national and international livestock movement are to blame for the LSD's indirect economic losses.[23–25] Morbidity rates may vary significantly during LSD outbreaks and reach up to 100%, whereas mortality due to LSD varies between 1 and 3%, but up to 40% have been reported in severe outbreak case.[26] Control and prevention of LSDV rely on application of vaccination, restricted animal movement and vector control.[8] Vaccination is reported to be the most effective among the methods used for controlling LSD in both disease endemic and non-endemic areas.[27,28] Currently, the strain KSGP-O180 is being used for vaccine to control the LSD outbreak in Ethiopia.[29] Ethiopia has been striving to control LSD using mass vaccination at a specified season as well as following a report of cases. In the study areas, the annual vaccination coverage seems extremely below the immunity threshold of the population that is necessary to control LSD outbreak occurrence.[30] Moreover, unpublished reports have been coming out from animals' owner and experts about a suspected vaccine failure.[31]

Despite the fact that LSD has a significant economic impact, especially in pastoral areas of afar region, there is lack of studies on LSD disease and associated factors that contribute for occurrence the disease. Therefore, this study was initiated to estimate the seroprevalence of LSD at the animal and herd level as well as its associated factors in the study areas.

Materials and Methods

Description of the Study Areas

The study was conducted from November, 2018 to May, 2019 in three districts namely (Asayita, Dubti and Chifra), which are located in the administrative zone one of Afar Region, Ethiopia. The Afar Pastoral Region is located in northeast of Ethiopia between 39°34′ to 42°28′E longitude and 8°49′ to 14°30′N latitude (Figure 1). The region shares common international boundaries with Eritrea in the northeast and Djibouti in the east and it is characterized by an arid and semi-arid climate with low and erratic rainfall. Rainfall is bi-modal throughout the region, with a mean annual rainfall below 500 mm in the semi-arid western escarpments and decreasing to 150 mm in the arid zones to the east. The region's elevation varies from 120 meters below sea level in the Danakil depression to 1500 meters above sea level elsewhere. Temperatures range from 20°C. at higher elevations to 48°C. at lower levels. Pastoralists, who rely heavily on livestock production for their livelihood, make up the majority of the human population in the afar region. In the afar

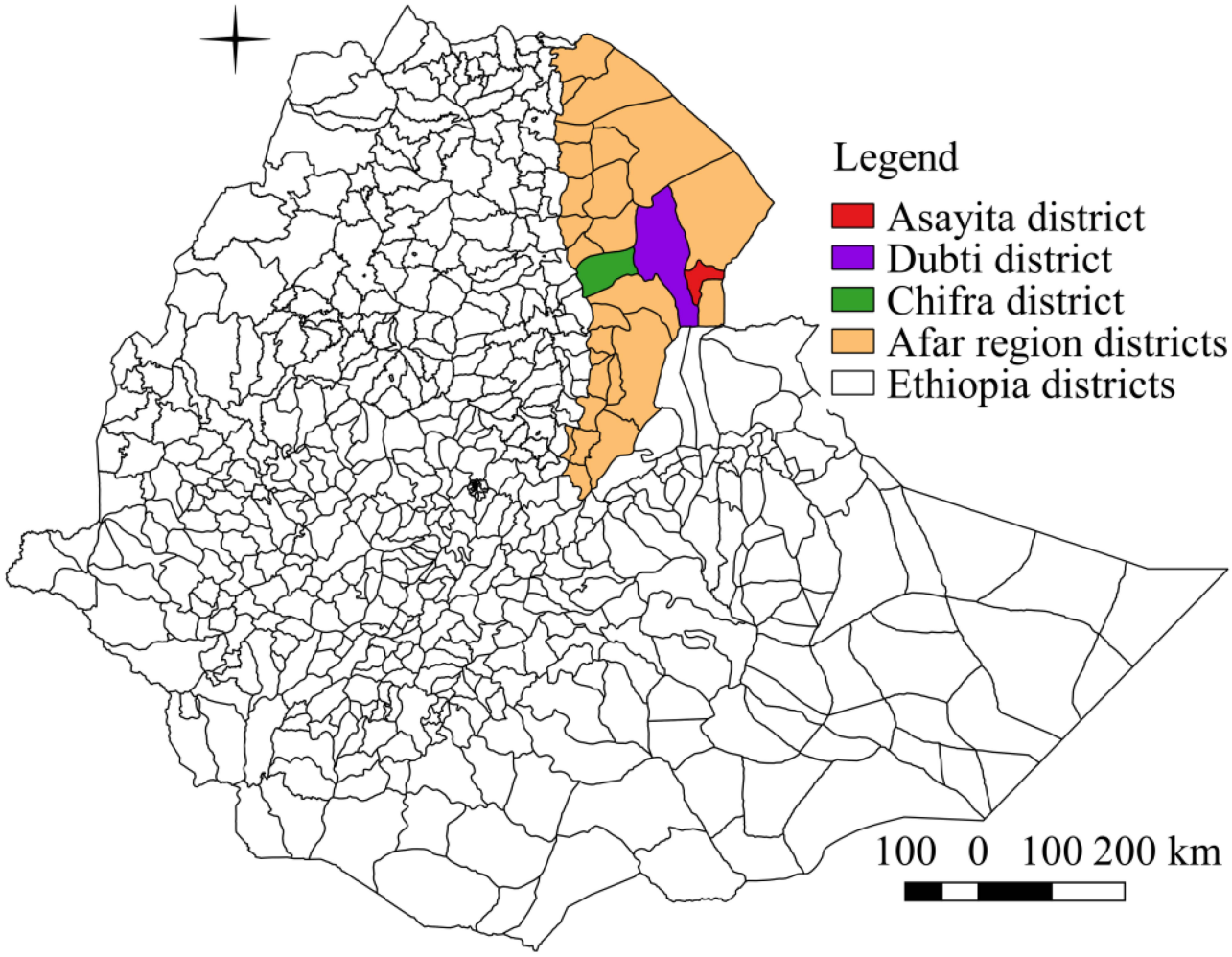

Figure 1 Map of the study areas.

region, there are approximately 1.9 million Afar breed cattle, 90% of which are handled under a pastoral production system and the other 10% in an agro-pastoral production system.[1]

Study Population

The study populations were non-vaccinated cattle for the last six months and aged above six months. Basically, the neutralizing antibodies developed against LSD vaccine are supposed to be short lived and get cleared before six months. However, cattle vaccinated before six months and young animals aged six months and above months were included in the study for assurance of antibody absence. The age groups of the study animals were categorized as Young (≤3.5 years), Adult (3.5 years<x ≤5.5 years) and Old (>5.5 years).[32] In addition, herd size was grouped as small (<40 cattle), medium (40–75 cattle) and large (>75 cattle) according to.[33] Some studies revealed that LSD infection level varied among the study population. Hence, the aim of categorizing the study population in this study was to check whether LSDV infection is affected by herd size or not from highly infectious nature of this virus.

Study Design and Period

A cross sectional study design was carried out from November 2018 to May 2019 aimed to estimate the seroprevalence of LSD and to assess associated factors selected districts of Afar region.

Sampling Technique and Sample Size Determination

The sampling method employed in this study was simple random sampling to select the study population since the study districts were purposively selected based on higher study population, access to transportation, history of no vaccination for the last six months, absence of outbreak cases and willingness of pastoralists to participate in this research work. Moreover, the study kebeles within each district were selected purposively. Lastly, the study units were randomly selected in each kebele. As no previous study was found on LSD in cattle in the study areas, 50% expected prevalence, 95% confidence level and 5% absolute precision or marginal error were used respectively. Taking these variables into equation, the total number of animals to be included in the study was determined applying the formula.[34]

$$n = \frac{Z^2 x\ Pexp(1 - Pexp)}{d^2}$$

Where n = required sample size; d = desired absolute precision (0.05); Z = Multiplier from normal distribution at 95% Confidence interval (1.96); P_{exp}= expected prevalence (50%); (1-P_{exp}) = Probability of having no disease 50% (0.5). Based on the equation, a total of 384 study units were determined to be sampled of all the study districts. A total of 147, 97, and 140 sera were collected from Asayita, Dubti and Chifra, respectively using proportional sample allocation strategy.

Sample Collection and Transportation

With precaution and gloved hands, approximately 8–10mL blood was drawn from jugular vein of cattle using disposable needles and 10mL non-heparinized vacutainer tube and 21 Gauge needles of 384 animal units. Following sample collection, vacutainer tubes were labeled, packed and transported to Samara University, college of veterinary medicine (SU-CVM) laboratory and kept overnight at room temperature to allow the blood to clot at a slant position. Then, the serum samples were transferred into identically labeled sterile cryovials and stored at −20°C refrigerator prior to laboratory analysis. Finally, the sera were transported to National Veterinary Institute (NVI), Bishoftu and the samples were processed using serum neutralization test (SNT) for detection of LSD antibody presence in the blood of the sampled cattle.

Administration of Questionnaire Survey

Open and closed ended questionnaires were prepared and administered to herd owners to assess potential risk factors of the disease alongside with sample collection. Respondents from each district were randomly selected and interviewed to assess associated risk factors of the disease. The questionnaires were interpreted into Afaraf language. Herd owner having cattle were the sampling units for questionnaire survey. Using herd owner as study unit, a total of 72 herd owners were selected proportionally allocating 27 to Asayita, 20 to Dubti and 25 to Chifra. All needed epidemiological data were collected, tabulated, coded and analyzed using stata software version 14 (Stata Corp, College Station, TX, USA).

Serological Analysis

Serum Neutralization Test

The serum neutralization test (SNT) protocol was employed by OIE.[8] The SNT was performed using a constant-virus /varying-serum method as described by.[35] Briefly, each serum was tested in duplicate wells at serial dilutions of 1/5, 1/25, 1/125, 1/625 and 1/ 3125. In 96 well flat-bottomed tissue culture microtiter plates, Kenyan sheep pox virus (KS1) was employed at 100 TCID50 per well. To acquire consistent results, the Vero cell was utilized as the culture host for the assay.[36] The plates were incubated at 37 °C in an atmosphere containing 5% carbon dioxide for 9 days. The presence of cytopathic effect (CPE) was examined using an inverted phase-contrast microscope at day 4 and final reading was made at day 9. In this study, both positive and negative control samples were used during this laboratory work. Kenyan sheep pox (KS1 O-180) Capripox virus strain was used as positive control. The serum samples were considered LSD positive, when CPE was inhibited either in both or in one of the duplicate wells at 1/25 or higher dilutions.

Data Management and Analysis

Both the laboratory analytical output and the necessary questionnaire response data were recorded, coded, and filtered in Microsoft Excel before being uploaded to Stata 14.0 (Stata Corp, College Station, TX, USA) for data analysis. To determine frequencies, diagrams, and tables, descriptive statistics were used. Association between risk factors and the disease positivity was assessed using Chi-square (χ^2). Bivariate logistic regression was computed to estimate the magnitude association between risk factors and the disease. In all the analyses, confidence level at 95% was calculated and the $P<0.05$ was used for statistical significance level between the potential confounders and the outcome variable.

Limitation of the Study

Limitation of the present study supposed to be sampling bias in selecting study populations and false positive or negative result by SNT during laboratory work.

Results

Animal Level Seroprevalence

Out of 384 serum samples tested using SNT, the overall seroprevalence at animal level was found to be 7.6% (95% CI= 4.90–10.20; n=29/384) and herd level seroprevalence was found to be 20.8% (95% CI= 11.42–30.18; n=15/72) seropositive for the presence of antibodies against LSDV infection. Descriptive computations are summarized (Table 1). The majority of the animals studied, n = 312 (81.25%), were female animals, while the rest, n = 72 (18.75%), were male animals. In comparison to Dubti and Asayita districts, Chifra had more seropositive animals (N = 140, or 12.14%) than the other two districts.

Herd Level Seroprevalence

In an attempt to assess the attributes of herd over the disease occurrence, a total of 72 herds were examined over the three study districts of which only 15 herds (20.8%; n=15/72; 95% CI= 11.42–30.18) have been confirmed at least one positive cattle by SNT test for antibodies against LSDV.

Associated Factors of Lumpy Skin Disease

In the current study, four factors were considered presumably supposed to have influence the infection and establishment of LSD disease among the study animals applying Chi-square (χ^2) statistics. As a result, only one study district (P = 0.004) was

Table 1 Descriptive Statistics Result Showing Variables with Animal Level Seroprevalence

Variable	Category	Frequency (No)	Positive	Prevalence in % (95% CI)
Sex	Female	312	25	8.00 (5.25–11.60)
	Male	72	4	5.556 (1.53–13.62)
Age	Young	77	4	5.19 (0.29–10.90)
	Adult	210	21	10 (5.90–14.10)
	Old	97	4	4.12 (0.16–8.10)
Herd Size	Small	124	8	6.45 (2.15–10.77)
	Medium	142	10	7.04 (2.84–11.25)
	Large	118	11	9.32 (4.08–14.56)
District	Asayita	147	1	0.68 (−0.65–2.00)
	Dubti	97	11	11.34 (5.15–17.56)
	Chifra	140	17	12.14 (6.74–17.69)

Table 2 Logistic Regression Analysis of Risk Factors for LSD Infection

Variable	Category	Animals Sampled	Positive	X^2	P-value
Age	Young	77	4	4.048	0.132
	Adult	210	21		
	Old	97	4		
Sex	Female	312	25	0.506	0.477
	Male	72	4		
Herd size	Small	124	8	0.797	0.671
	Medium	142	10		
	Large	118	11		
District	Asayita	147	1	16.162	0.000
	Dubti	97	11		
	Chifra	140	17		

Table 3 Bivariate Logistic Regression Output by Districts

District	No of Animals Sampled	Positive	COR	P-value	95% CI
Asayita	147	1	-	-	-
Dubti	97	11	18.67	0.005	2.369–147.162
Chifra	140	17	20.18	0.004	2.648–153.798
Total	384	29			

found to be correlated to LSD occurrence. Seropositivity variations were observed among age groups; however, they were statistically insignificant. Adult cattle showed higher seropositivity than young and old animals, however, the difference was statistically insignificant (Table 2).

Bivariate logistic regression analysis was computed using 95% CI and P<0.05 to assess the strength of association between the disease and risk factors (Crude Odds Ratio=COR) with the occurrence of disease as depicted (Table 3). In comparison to study animals identified in Asayita district, study animals found in Chifra district were 20.18 times more likely to be at risk of acquiring LSD.

Discussion

Lumpy skin disease seroprevalence reports of have been reported with various serological results in different agro-ecological parts. There was no even a single scientific study finding on the seroprevalence of LSD and its associated factors in the study districts of afar region except unpublished reports of woreda animal health bureau. That is why the current study is the first of its kind to estimate the seroprevalence of LSD in cattle using serum neutralization test (SNT) and assesses associated risk factors in afar region, Ethiopia. In the present study animal level seroprevalence of LSD infection was found to be 7.6% which is comparable with previous reports of 6.43% at West Wollega Zone of Oromia region,[37] 7.4% in north-eastern Ethiopia[38] and 7% at around Nekemt.[39] In contrast to this study, recent studies have found that the Southern region has a higher LSD seroprevalence of 11.6%,[40] 27.9% at Woliso town[41] and 28% in three districts in Amhara's eastern region.[42] This seropositivity variation could be due to differences in individual animal breed, immune status, interaction of cattle with other animals, production system, variation in geographical area,

sampling period and testing methods employed for the studies, sample size, seasons, agro-ecological conditions and introduction of new animals without screening which could affect the incidence of LSD.[42,43]

In this study the herd level seroprevalence was compared with other previous studies and showed lower finding than previous herd level prevalence in lowland (50%), midland (26%) and highland agro-climate zones (64%) in Ethiopia.[17] Seroprevalence at the herd level was reported to be 52.6% in central and northwestern Ethiopia, and 5.95% in western Ethiopia[44] and 44% in eastern part of Ethiopia.[45] This herd level LSD seroprevalence variation could be due to vectors population availability in the study areas, density of livestock at the grazing and watering points, husbandry methods, rainy seasons, geographical situations and introducing new animals from outside without screening that would be affect the occurrence of LSD.[46] Among the associated factors assessed, only the study area was found to be statistically significant with regard to LSD serostatus. LSD occurrence and age groups were found to be statistically insignificant. Adult and old cattle, on the other hand, have a higher risk of developing the disease than young animals. The results of this seroprevalence study are consistent with previous findings.[44] This difference could be due to fatigue from lactation and intense effort for milk and draft animals as well as stress.[42,44,46] The lower prevalence of LSD in young animals, on the other hand, could be associated to traditional young animal care practices that separate young animals from the herd, possibly reducing vector exposure.[38,47]

Furthermore, this study found no significant association between sex and LSD serostatus, which is consistent with the previous study results, despite females having a relatively high seroprevalence.[44] In contrast, statistically significant association between sex and serostatus of LSD was reported by previous seroprevalence studies.[39,46] These seroprevalence results variations might be due to female animals are usually kept longer by farmers while males are sold off at a younger age and thus the effect of sex may be an artifact of duration of exposure.[18] This study found a significantly variation in LSD seroprevalence across the study districts, which is consistent with previous seroprevalence reports.[37,44,45] This seroprevalence discrepancy could be because of differences in herd management system, the presence of flooding and irrigation in the study areas that may facilitate multiplication of potential mechanical vectors to enhance disease transmission, herd sizes, sharing common boundary with neighboring states, and introduction of new animals without screening could contribute for occurrence of LSDV.[17,40,45] In addition, in the present study, even though no statistical association between herd size and LSD serostatus was observed, it has been found a relative high seroprevalence of LSD in medium and large herd size as compared to small herd size. Previous LSD serostatus studies back up this assertion.[40] Furthermore, cattle with larger herd sizes were found to be more affected than cattle with smaller herd sizes. Stressful conditions, frequency of transmission, intensity of LSDV exposure and other related factors could all influence LSD serostatus variation in herd size.[41]

Conclusions

The current study finding indicated that the overall LSD seroprevalence of cattle was 7.6% at individual animal and 20.8% at herd level. Hence, the current study revealed that LSD was prevalent in the study areas. Unlike other risk factors, the study areas were found to be associated factors for occurrence of the disease. Therefore, further studies should be conducted to estimate its region wise seroprevalence and assess the economic impact of the disease. From transboundary nature and economic impact point of view, the disease requires technically sound and coordinated efforts for its prevention and control. Therefore, the study findings strongly urge a prompted and coordinated interventional to be set in place.

Data Sharing Statement

The data sets used and/or analyzed during the current study could be available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Written ethical approval and written informed consent for this study was obtained from Samara University, College of Veterinary Medicine of Animal Research Ethics and Review committee (reference AREC019/2019). Written informed consent was also obtained from the herd owners to take samples from their cattle and for further research use of the

samples. The reason for this written informed consent is that, participants were required for interview and the individual participants were not subjected to any harm as much as their privacy is kept confidential. Confidentiality of collected data and the scientific honesty during write up was considered. These written informed consents were documented.

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Disclosure

The authors declare that they have no competing interests in the publication of this paper.

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