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Field study on the use of vaccination to control the occurrence of lumpy skin disease in Ethiopian cattle

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Abstract

The current study was carried out in central and North-western parts of Ethiopia to assess the efficacy of Kenyan sheep pox virus strain vaccine (KS1 O-180) against natural lumpy skin disease (LSD) infection under field conditions by estimating its effect on the transmission and severity of the disease. For this study, an LSD outbreak was defined as the occurrence of at least one LSD case in a specified geographical area. An observational study was conducted on a total of 2053 (1304 vaccinated and 749 unvaccinated) cattle in 339 infected herds located in 10 sub-kebeles and a questionnaire survey was administered to 224 herd owners. Over 60% of the herd owners reported that the vaccine has a low to very low effect in protecting animals against clinical LSD; almost all of them indicated that the vaccine did not induce any adverse reactions. In the unvaccinated group of animals 31.1% were diagnosed with LSD while this was 22.5% in the vaccinated group ($P<0.001$). Severity of the disease was significantly reduced in vaccinated compared to unvaccinated animals (OR = 0.68, 95% CI: 0.49; 0.96). Unvaccinated infected animals were more likely (predicted fraction=0.89) to develop moderate and severe disease than vaccinated infected animals (predicted fraction=0.84).

LSD vaccine efficacy for susceptibility was estimated to be 0.46 (i.e. a susceptibility effect of 0.54) while the infectiousness effect of the vaccine was 1.83. In other words, the vaccine reduces the susceptibility by a factor of two and increases infectiousness by approximately the same amount. LSD transmission occurred in both vaccinated and unvaccinated animals, the estimated reproduction ratio (R) was 1.21 in unvaccinated animals compared to 1.19 in vaccinated ones, and not significantly different. In conclusion, KS1 O-180 vaccination, as applied currently in Ethiopia, has poor efficacy in protecting cattle populations against LSD, neither by direct clinical protection nor by reducing transmission, and this signifies the urgent need to either improve the quality of the vaccine or to develop potent alternative vaccines that will confer good protection against LSD.

Key words: *Capripoxvirus*, Kenyan sheep pox (KS1 O-180) vaccine, Lumpy skin disease, Reproduction ratio, Severity, Vaccine efficacy

1. Introduction

Lumpy skin disease (LSD) is a disease of cattle caused by LSD virus, a DNA virus, which belongs to the family *Poxviridea*, subfamily *Chordopoxvirinae* and it is of the genus *Capripoxvirus*. The disease is characterized by fever, nodular lesions on the skin and mucous membranes, inflammatory and oedematous swellings of the limbs and brisket, lymphadenopathy, deterioration of body condition and drop in milk production (Davies, 1991; Quinn et al., 2002; Radostits et al., 2007). It has spread to most African countries, Middle East countries and recently to Europe (Davies, 1991; Tuppurainen and Oura, 2012; Tasioudi et al., 2016; WAHID, 2016; Tuppurainen et al., 2017). LSD is endemic in Ethiopia and is a constant threat to the livestock sector since its first occurrence in 1981 (Mebratu et al., 1984; Gari et al., 2010). LSD outbreaks occur frequently in various regional states of the country, despite intensive vaccination campaigns (APHRD, 2012). It is an economically devastating and therefore a notifiable disease as per OIE disease categorization (Gari et al., 2011; Tuppurainen and Oura, 2012; OIE, 2016).

Vaccination, movement control and slaughter of infected and in-contact animals are considered as options for the control of LSD. However, it is widely agreed that vaccination is the most manageable and realistic approach to control the disease in endemic and resource poor countries (Carn, 1993; Tuppurainen et al., 2014). Live attenuated vaccines based on sheep pox virus (for example, Kenyan sheep pox (KS1 O-180), Romanian sheep pox and Yugoslavian RM 65 sheep pox vaccines), goat pox virus (Gorgan goat pox vaccine), and LSDV (Neethling strain vaccine) have been used for the control of LSD (OIE, 2010; Tuppurainen et al., 2014; Gari et al., 2015).

In general vaccination can exert important effects, both at the individual and at the population level. It may help to directly protect vaccinated animals, reduce severity of the disease by reducing all or some of its symptoms or it may reduce transmission of pathogens by lowering susceptibility and/or infectiousness, and thus also indirectly reduce the risk for other vaccinated and unvaccinated individuals to become infected (De Jong, 1994; Halloran et al., 1997; Van der Goot et al., 2007; Aznar et al., 2011; OIE, 2015). The effect of vaccine intervention on the dynamics of infectious diseases, i.e. in the population, can be estimated by the reproduction ratio (R) which is the average number of secondary cases arising from one typically infected animal during its entire infectious period (Diekmann et al., 1990; Heffernan et al., 2005).

LSD vaccine failure has been reported in several countries including Ethiopia. During the 2006 outbreak in Israel, 11% (4.2% in dairy and 33.7% in feedlot cattle) of RM65 (Ramayer strain) vaccinated cattle became infected (Brenner et al., 2009). In Jordan, Abutarbush (2014) reported an overall LSD morbidity of 4.7% in cattle populations vaccinated with RM65 (Jovivac®) and LSD vaccine of unknown origin. Kumar (2011) reported a continued LSD outbreak in Oman for more than three months after vaccination of cattle herds with Kenyan sheep and goat pox vaccine. In Ethiopia, LSD vaccine failure has been reported since 1993 (Carn, 1993). Ayelet et al. (2013) estimated morbidity to be 23.8% in the cattle population of central Ethiopia after vaccination with KS1 O-180 virus strain vaccine. However, a better protection was claimed with Neethling vaccine (1.11% morbidity) and with a 10 times higher dose of the RM65 vaccine (1.85% morbidity) (Ben-Gera et al., 2015). Vaccines in general may give only partial protection (leaky vaccines) or protect only some of the individuals (all-or-nothing) (Smith et al., 1984). In addition, further immunization failure may arise due to insufficient vaccine coverage or factors related to the host, vaccine, or vaccination quality due to handling, reconstitution or administration of the vaccine (Quinn et al., 1999).

Ayelet et al. (2013) and Gari et al. (2015) reported that KS1 O-180 vaccine provides incomplete protection in immunized animals. However, the level of protection and its effect on the severity of the disease have not been documented well under field conditions. KS1 O-180 vaccine is still applied as the sole means of LSD control in Ethiopia. Hence, the aims of this study was to assess the efficacy of KS1 O-180 virus strain vaccine against natural LSD infections under field conditions by estimating its effect on the transmission and severity of the disease.

2. Materials and Methods

2.1. Study and study area

The study consisted of two parts:

(1) A questionnaire survey focusing on herd owners' information regarding several aspects of vaccination which was undertaken in central and North-western parts of Ethiopia (Figure 1). In central Ethiopia, it was undertaken in Ada'a, Sebeta Hawas, Ambo, Dendi, Debrelibanos, Kuyu and Hidabu Abote districts in Oromia National Regional State. In North-western part, the data were collected from Dejen, Gozamen, Hulet Ejju Enessie and Jabitenan districts in Amhara National Regional State. The dominant agricultural production system in the study areas was mixed crop-livestock system. The grazing practice in almost all study areas was open grazing on communal pasture land where animals from a village were herded together.

(2) A vaccine efficacy follow-up study under field conditions was undertaken in the North-western part of Ethiopia in Mota town and the surrounding four rural kebeles (the lowest administrative structure in Ethiopia, in which at least 500 households (3,500 to 4,000 persons) live and cover on average about 53 km^2 and 3 km^2 land area in rural and urban places, respectively) of Hulet Ejju Enessie district, East Gojjam Administrative Zone, Amhara National Regional State (Figure 2). The rural kebeles were Hibre Selam, Debre Gubae, Beza Bizuhan, and Ayen Berhan. Cattle populations of ten sub-kebeles were

enrolled in the study namely Mota (from Mota town), Akobe, Semo, and Shewaber from Hibre Selam, Atetanat and Yerez from Beza Bizuhan, Webmariam from Ayen Berhan, and Kesmender, Komma and Zenabach from Debre Gubae kebele.

2.2. Questionnaire survey

The study population for the questionnaire survey was about 13,200 cattle herd owners living in 33 selected kebeles of 11 districts. These owners were smallholder farmers with the main purpose of subsistence farming, that is: draft power for crop production, milk for consumption, manure for soil fertility and fuel, and cash income. Animals were kept in an extensive management system and most of the herds were composed of local Zebu breed cattle. Animals in this system share communal grazing and watering resources. The term “herd” in this study designates an aggregate of animals kept together day and night and owned by a household.

2.2.1. Study design and data collection

Eleven districts located in the central and north-western parts of Ethiopia were identified for a cross-sectional questionnaire survey. The districts were selected based on the recent LSD outbreak occurrence, location and accessibility. For this study, an LSD outbreak was considered, if at least one case of LSD occurred in a specified geographical area (usually kebele). Three kebeles were randomly selected from each district. From each kebele, five to eight herd owners willing to participate were interviewed. The survey data were collected from a total of 224 herd owners from January 2015 to May 2015.

The data were collected by face to face interview using the local language. After getting an informed consent from the herd owners, the interviewer asked questions about the vaccination status, vaccination frequency, the vaccination service provider, fee of the vaccination, the vaccination date and when the animals become infected if there was any infected animal in his herd. Furthermore, the herd owners were requested to express their opinion on the effectiveness of the vaccine in protecting cattle against LSD and the adverse reactions to the vaccine. The vaccine is considered to be protective from day 21 to one year post vaccination. All responses were recorded in a predesigned response sheet.

2.2.2. Data management and analysis

Descriptive statistics were used to summarise the data on vaccination coverage at herd level, frequency of vaccination, and owner's opinion about the effectiveness and adverse reactions of the LSD vaccine.

2.3. Follow-up study

A follow-up study was carried out after the index case of LSD appeared in Beza Bizuhan kebele at the specific village called Chech on 29 April 2014. The disease stayed restricted in the village for a reasonable period of time but after that it spread to other villages and surrounding kebeles. The selected area for follow-up was Mota town and its surrounding area, representing 10 sub-kebeles. In the area, animals were owned by smallholder farmers with the main purpose of subsistence farming except for six dairy farms which kept cattle for commercial purposes. Most of the herds were composed of local Zebu breed cattle and managed under extensive management. The six dairy herds consisted mainly of Holstein-Zebu cross and were managed under semi-intensive or intensive conditions.

The study population included 7464 heads of cattle grouped in 1203 herds. The cattle population in each sub-kebele (considered as ten separate populations as they were herded on common pasture land within a sub-kebele) was vaccinated partially. This partial coverage was not purposive but due to the failure of the owner to get their animals vaccinated. The vaccination campaign was undertaken at least one month before the entrance of the disease into a specific sub-kebele. The vaccination was provided by the public veterinary service of the Hulet Ejju Enessie district following the index case appearance in the area.

2.3.1. Study design and herd selection

This study was designed as a prospective cohort study. At the beginning of the study, ten cattle populations (i.e. all cattle in a sub-kebele excluding calves less than 6 month old) with partial vaccination coverage and LSD free status were selected. All herds in the selected populations were inspected on a weekly basis for clinical signs of LSD. The herd owners were also asked to report any suspicion of the disease. The sub-kebeles were selected based on their partial vaccination status. We selected populations with different vaccination coverage because that is a pre-requisite to estimate both vaccine efficacy for susceptibility and infectiousness (Longini et al., 1998; Aznar et al., 2011). The vaccination coverage level in the selected 10 sub-kebele cattle populations ranged from 3-95%. Since the vaccine coverage was strictly inferior to 100%, a number of infections within the vaccinated group was expected to occur. The animals, whether vaccinated or not, were followed starting from August 1, 2014 to November 31, 2014, i.e. from the day the first case was detected in the sub-kebele until no more new cases were recorded. If an animal in a herd was diagnosed with LSD and the owner volunteered to participate, the herd was enrolled in the study. Therefore, the main inclusion criteria for a herd were the infection status of the herd and the willingness of the owner to participate. A herd was considered positive if at least one animal showed LSD-characteristic nodular lesions. In total, 448 herds were recorded as being affected and of these, 339 farmers (75.7%) were willing to participate and all their bovines (n=2053) enrolled in the study.

2.3.2. Data collection

In the ten sub-kebeles, infected herds were visited twice a week by animal health professionals and by the first author, and clinical signs were recorded. The severity of LSD was assessed at three levels: mild, moderate and severe. Mild LSD was defined as only few nodular lesions (<5) in some part of the body, mild fever (39-39.5^{0}C) and quick recovery (within a week); the moderate level was assigned if fever, inappetence, many nodular lesions/swelling on the limb or brisket, and weakness was present; severe LSD was scored if high fever (>40^{0}C), extensive nodular lesions/swellings, anorexia, weakness, emaciation or death was observed.

Animal data including breed, sex, age and records like vaccination status, vaccination date and type of vaccine used were compiled for all animals at the first herd visit. Type of herd and sub-kebele were also recorded. The first visit was made by the district veterinary team and the first author. The animal health professionals who collected the data from infected animals were blind for the vaccination status of the affected animals.

Biopsy samples of skin nodules were collected from a sample of the affected animals in each sub-kebele and analysed by conventional and Snapback Real-time PCR (polymerase chain reaction) techniques following the method described by Tuppurainen et al. (2005) and Gelaye et al. (2013) to confirm that the clinically observed disease truly was LSD. A total of 34 skin samples were collected for LSD confirmation.

2.3.3. Vaccine used for control and prevention of LSD

The live attenuated vaccine of KS1-O180 produced by National Veterinary Institute (NVI), Ethiopia was the only vaccine used for prevention and control of LSD in Ethiopia. It recently has been reported that the virus used for the production of KS1-O180 is not a sheep pox virus but was found to be LSDV (Gelaye et al., 2015). The vaccine was prepared in 20 ml vials containing 100 doses and reconstituted by 100 ml of cool and sterile saline water; $10^{3.5}$ $TCID_{50}$

was administered per animal as recommended by the manufacturer. A suspension of 1 ml vaccine was injected subcutaneously at the neck side (NVI, 2010).

2.3.4. Data management and analysis

Descriptive statistics were used to describe the morbidity and mortality in cattle populations with different vaccination coverage.

To analyse the association between the occurrence of LSD infections in animals (i.e. the cases, which are assumed to be binomial distributed) and independent variables (vaccination status, breed, age, sex, herdtype, and location), multivariable logistic regression was performed (STATA version 14). Vaccination status was the main effect of interest while location, breed, age, sex and herdtype were added as additional explanatory variables. All factors were fitted in a multivariable regression model and the final model was obtained by a backward stepwise elimination procedure while checking for confounding. For that purpose confounding was defined as a change of at least 25% in any of the regression coefficients after removing a non-significant ($p>0.05$) variable from the model. Interactions were tested for all combinations of the significant main effects. Generalised estimating equations (GEE, population averaged model) was run using herd as random effect. An exchangeable correlation structure was specified for the random effect and results were expressed as Odds ratio (OR) and its 95% confidence interval (CI).

To estimate the effect of vaccination on the severity (mild, moderate or severe) of LSD, first a univariable and then multivariable (backwards elimination process) ordered logistic regression analysis was run by incorporating breed, age, sex, herdtype, and kebele as potential factor and retaining it in the model as confounder when necessary. The probability of a vaccinated or unvaccinated infected animal falling in either of the severity categories was computed using estimated coefficients and the associated cut points of the ordered logistic regression analysis. Proportionality of odds across response categories was tested using the approximate likelihood-ratio test (omodel logit command in STATA version 14).

Multivariable regression analysis using a generalized linear model (GLM) was performed to assess the effect of vaccination on the transmission of LSD by setting LSD infection of animals as binomial (yes/no) dependent variable and vaccination status (yes/no) and fraction of vaccinated among the infected (FracVaccI) as independent variables. The model was fit using the complementary loglog (cloglog) link function and log (number of infected animals/total number of animals per sub-kebele) as offset (Velthuis et al., 2003) using STATA version 14. The susceptibility and infectiousness coefficients obtained from the analysis were used to calculate the transmission parameters by inserting them into the formulae described in Table 1. Note that in this case we observed the total outbreak in the sub-kebele and thus the regression coefficient estimates pertain to the final size of the outbreak and thus we directly estimate the reproduction ratio R rather than the transmission rate parameter β.

Vaccine efficacy for susceptibility (VEs) and infectiousness (VEi) were estimated using formula 1 and 2 as described by Halloran et al. (2010) and Aznar et al. (2011) and for this the four transmission parameters with their expression were defined (Table 1).

$$VEs = 1 - \left(\frac{Ruv}{Ruu}\right) = 1 - \left(\frac{Rvv}{Rvu}\right) \quad (1)$$

$$VEi = 1 - \left(\frac{Rvu}{Ruu}\right) = 1 - \left(\frac{Rvv}{Ruv}\right) \quad (2)$$

A vaccine with an efficacy of 0 was considered as not effective whereas a value of 1 was considered fully efficacious. Values of vaccine efficacies above 0.7 are considered ‘good’, whereas vaccine efficacies in the range of 0.3 to 0.7 are generally considered ‘reasonable’ (Halloran et al., 2010; Lu. et al., 2013). However, this interpretation of vaccine efficacy does not correspond to whether vaccination will reduce R so that R<1, because whether R<1 also depends on the R in the absence of vaccination.

The reproduction ratio in vaccinated animals was calculated by multiplying the effects of vaccination on susceptibility (exp(coefficient of the independent variable Vaccination)), and on infectiousness (exp(coefficient of the fraction of vaccinated among the infected)) and the intercept of the regression model. Whereas R for unvaccinated was calculated from the exponent of the intercept only.

3. Results

3.1. Questionnaire survey

Based on the herd owner’s response, the vaccination coverage at herd level was estimated to be 56.3%. The public veterinary service vaccinated the majority (88.9%) of the herds and more than 95% of the herds did not get routine prophylactic vaccination against LSD but were vaccinated just after the LSD index case was reported in a neighbouring kebele. More than 60% of the herd owners deemed the vaccine to be of low to very low efficacy in protecting against clinical LSD, however, almost all of them responded that the vaccine did not induce any adverse reaction after vaccination (Table 2).

3.2. Follow-up study

3.2.1. Description of LSD occurrence and vaccination

The follow-up study was undertaken in 10 sub-kebeles with 339 infected herds comprising a total of 2053 cattle of which 1304 (63.5%) were vaccinated (Table 3). Herd size varied from 1 (n=6) to 37 (n=1) with an average of 6 and a median of 6 animals. About 95% of the herds had 10 or less animals. The study population consisted of 346 (16.8%) calves, 263 (12.8%) heifers, 227 (11.1%) bulls, 490 (23.9%) cows and 727 (35.4%) oxen. Of the 2053 animals, 526 (25.6%) were diagnosed with LSD, 233 (31.1%) in the unvaccinated group and 293 (22.5%) in the vaccinated group (Chi-square test: $p<0.001$). The PCR results confirmed the LSD infection in all ten sub-kebeles.

The multivariable population averaged model showed that herd did not contribute significantly to the total variance. Therefore multivariable logistic regression without random effects was performed which showed that the estimates and their significance were very similar to the random effects model. All variables remained significant in the multivariable analysis except herdtype but this variable confounded the estimates of location. Results show that vaccination significantly decreased the risk of LSD (OR= 0.49, 95% CI: 0.37; 0.64). Crossbreeds, males and older age were associated with increased risk to be LSD positive compared to their references and the interaction between vaccination and breed was significant. Vaccination is more efficient in crossbreed (OR= 0.49*0.43=0.21) than local breed (OR=0.49) animals (Table 4).

3.2.2. LSD severity and vaccination

The severity of LSD was assessed on a total of 480 clinically infected cattle (264 vaccinated and 216 unvaccinated). In unvaccinated animals, the majority of the affected animals (50.5%) were categorized as severe and 9.7% fell in the mild category whereas in vaccinated animals these figures were 42.8% and 17.1% respectively (Table 5). The results of the multivariable ordered logistic model showed that only vaccination was significantly associated with a different (lower) severity score (Odds Ratio (OR) = 0.68, 95% confidence interval (CI): 0.49; 0.96). The test for the proportional odds assumption was not significant ($p = 0.21$) indicating that it is valid to report the OR as 0.68. Furthermore, the predicted fraction showed that the probability of developing moderate and severe disease was slightly higher in unvaccinated animals (0.89) compared to vaccinated animals (0.84).

3.2.3. LSD vaccine efficacy with respect to transmission

The multivariable GLM analysis showed that both the susceptibility (exp(b) = 0.54, 95% CI: 0.44; 0.66) and infectiousness (exp(b) = 1.83, 95% CI: 1.28; 2.61) effects of the vaccine are significant and thus the effects are a reduction in susceptibility by a factor 2 and an increase in infectiousness by a factor 2 (Table 6).

A 0.46 vaccine efficacy for susceptibility and -0.83 for infectiousness recorded in this study were obtained by inserting the corresponding estimated partial reproduction ratios (Ruu = 1.21, Ruv = 0.65, Rvu = 2.22 and Rvv = 1.19) into formula 1 and 2 (Table 1 and 6).

The estimated reproduction ratios for vaccinated and unvaccinated cattle were almost equal: 1.19 (95% CI: 1.02-1.39) and 1.21 (95% CI: 1.01-1.46). The 0.98 (95% CI: 0.73-1.33) reduction in R by vaccination was not significantly different from 1 ($p = 0.92$).

4. Discussion

LSD vaccine breakdown and a concomitant morbidity are reported in Ethiopian cattle since 1993 (Carn, 1993) while vaccination with KS1 O-180 vaccine is the major control method in the country. However, the efficacy of KS1 O-180 virus strain vaccine against natural LSD infections under field conditions and its impact on the transmission and severity of the disease is largely unknown and both are estimated in this paper.

4.1. Questionnaire survey

The questionnaire survey shows that in almost all study districts no regular vaccination program for LSD is applied. This is related to the long time (5 or more years) interval between LSD epidemics (Woods, 1988) and resource limitation. LSD vaccination is usually initiated by the appearance of an index case in an area. Therefore, vaccination for LSD is commonly carried out at the face of the outbreak to control the disease occurrence. However, vaccinating animals during an outbreak may aggravate the transmission of LSD due to iatrogenic transmission from healthy looking, incubating animals to susceptible animals (Hunter and Wallace, 2001). The survey also showed that most of the vaccinations were provided by the public veterinary service. This clears out the suspect that the vaccine failure might be related to the administration of the vaccine by incompetent practitioners (and that apply LSD vaccination illegally).

Vaccination coverage is an important issue in disease control. Cattle populations with low vaccination coverage are assumed to remain at higher risk for the disease. The 56.3% vaccination coverage at herd level estimated in this study is low given that the vaccine is provided free of charge. The reason for low coverage might be related to owner's belief that the vaccine is not protective. More than 60% of the herd owners interviewed in the questionnaire survey reported low effectiveness of KS1 O-180 vaccine in protecting cattle against clinical LSD confirming the estimated poor performance of the vaccine (Ayelet et al., 2013; Gelaye et al., 2015). However, the low vaccination coverage is not related to vaccine adverse effects as almost all respondents did not indicate any adverse effect. This is in agreement with what Gelaye et al. (2015) reported for the vaccine. However, in other countries adverse reactions in cattle vaccinated with sheep pox and Neethling virus based vaccine have been reported like swelling on the injection site and developing active LSD (Weiss, 1968; Yeruham et al., 1994; Ben-Gera et al., 2015; Abutarbush et al., 2016).

4.2. Follow-up study

The 22.5% morbidity in vaccinated animals recorded in the follow-up study is comparable to 23.8% morbidity reported in central Ethiopia in cattle vaccinated with Kenyan sheep pox vaccine strain (Ayelet et al., 2013). However, a much lower morbidity of 4.7% (Abutarbush, 2014), 11% (Brenner et al., 2009) and 1.6% (Ben-Gera et al., 2015) were recorded in vaccinated cattle of Jordan and Israel. This difference might be attributed to the difference in the quality of the vaccine used, vaccination coverage, management system, environment or climate difference of the areas where the animals are kept.

The factors age group, breed, sex, herdtype and location were included into the logistic regression model to adjust the estimate of vaccination. The adjusted odds ratio for vaccination was 0.49 which indicates that vaccination is protective for LSD. Unvaccinated animals have 2.04 (1/0.49) times higher odds to acquire LSD than vaccinated ones. The interaction between vaccination and breed was significant and it revealed that vaccination was more efficient in crossbreed (OR= 0.21) than local breed (OR=0.49) animals. This might be related with the more susceptibility nature of Holstein-Zebu cross to LSD than pure local Zebu animals (Davies 1991; OIE, 2010). Possible confounding factors which are not measured in this study include movement of animals and vector density. No animal movement restriction was applied in the study area; animals move freely from area to area. This practice was similar in all study kebeles and for both vaccinated and unvaccinated animals. Vector density is also assumed to be similar in all study kebeles because they are located in the same geographical area with similar weather conditions and altitude and on top of that they are all within the range of the insect flight zone.

Vaccination was associated with less severe LSD symptoms. This finding is in agreement with the observation of Abutarbush (2014) who reported a considerable change in feed intake and milk production, fever, and a longer duration of illness in the majority of unvaccinated cattle as compared to vaccinated cattle. Hence, LSD vaccination reduces disease severity and as consequence it may prevent part of the production loss due to LSD. Increased vaccine dose is claimed to improve the protective efficacy of the vaccine. Ben-Gera et al. (2015) reported a low incidence (1.85%) in cattle vaccinated with a 10 fold increased dose of RM65 vaccine. The regular vaccine dose used to immunize cattle against LSD in Ethiopia is 10 fold compared what used to immunize sheep and goat. For cattle, LSD vaccine contains $10^{3.5}$ $TCID_{50}$ attenuated virus per field dose while for sheep and goat it is, $10^{2.5}$ $TCID_{50}$ per dose (NVI, 2010).

The vaccine efficacy of 0.46 as estimated for susceptibility is within the ‘reasonable’ efficacy range of 0.3 to 0.7 (Halloran et al., 2010; Lu et al., 2013).This indicates that vaccination reduces susceptibility to LSD 2.17 times (1/(1-0.54)). However, vaccinated infected animals are 1.83 times more infectious than unvaccinated infected ones. This is contradictory from what is expected from a vaccine. The increased infectiousness might be related with disease management practices. In the usual management practice, diseased animals are isolated and penned separately from healthy animals. However, the situation in vaccinated LSD affected animals is different, they are less diseased (not easily noticed) and thus remain longer in the herd (not isolated or removed) while they are infectious. This condition might be favourable for the transmission of the virus. Therefore, in this regard, animal disease management might contribute to increased infectiousness. However, this finding needs further investigation because the disease management and other factors which can influence the infectiousness were not under control. In general, the gain in decreasing susceptibility in vaccinated cattle is cancelled out by almost the same increment of infectiousness and this indicates that KS1 O-180 vaccine is not effective in controlling LSD in cattle populations. The overall low efficacy of the vaccine substantiates the previous findings that vaccination against LSD does not provide protection from clinical disease (Ayelet et al., 2013; Abutarbush, 2014; Gari et al., 2015). Most LSD vaccines currently available, except the homologous Neethling vaccine, provide poor protection against LSD transmission (Brenner et al., 2009; Somasundaram, 2011; Tuppurainen et al., 2014; Ben-Gera et al., 2015), which is a challenge for the control of the disease.

Although vaccinating cattle against LSD is considered the main control option in resource poor countries like Ethiopia, little is known about the effect of vaccination on the disease dynamics. In the current study, the estimated reproduction ratios were 1.21 and 1.19 for unvaccinated and vaccinated cattle, respectively. In both cases R is greater than 1 and confirms that LSD virus can spread in cattle populations, regardless of their vaccination status, and can cause a major outbreak. This shows that vaccination with KS1 O-180 vaccine alone cannot eliminate the disease from a cattle population. Thus, a more competent LSD vaccine and other additional measures, like movement control, detection and removal of infected animals, are needed to bring the reproduction ratio to below 1.0.

An observational study was chosen for this study because it is less costly and enables to assess the performance of the vaccine under real-life circumstances, including the complex and not easily controllable exposure to LSDV due to the insect vectors involved. Important confounders were measured and equal exposure risk of vaccinated and unvaccinated animals were assumed. Furthermore, the study design avoids the ethical problem of using a placebo when an approved vaccine is available (Torvaldsen and McIntyre, 2002).

Observational studies are prone to potential biases due to its uncontrolled nature. The biases may be related to selection, misclassification of cases, confounding factors, dealing with the impact of unknown or unmeasured factors (Dohoo et al., 2003), missing information, and non-comparability of groups. The distribution of potentially confounding variables among the study groups and other variables which were not considered might also be a source of bias. Another limitation to this study is related to the severity assessment; subjectivity might be somehow involved in allocating affected animals into different categories and on few occasions the observer might have been unblinded for the vaccination status of the animal because the owner might have complained about the poor efficacy of the vaccine. We assumed that exposure to infection was equal in both vaccinated and unvaccinated animals, that all important confounders were measured and adjusted for by the model used. Considering these limitations, the results reported here should be interpreted carefully.

5. Conclusion

The results of our study showed that KS1 O-180 strain vaccine reduces susceptibility of cattle to LSD but it also increases infectiousness by about the same amount, partially because animals with less severe disease signs may remain undetected in the herd for longer periods. Generally, the vaccine has poor efficacy in protecting cattle populations against LSD, neither by direct clinical protection nor by reducing transmission. Therefore, the prevailing situation dictates the urgent need of a competent LSD vaccines development to control LSD in endemic countries and to halt its current spread to free countries and continents.

Conflicts of interest: None

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Reference

Abutarbush, S.M., 2014. Efficacy of vaccination against lumpy skin disease in Jordanian cattle. Vet. Rec. 175.

Abutarbush, S.M., Hananeh, W.M., Ramadan, W., Al Sheyab, O.M., Alnajjar, A.R., Al Zoubi, I.G., Knowles, N.J., Bachanek-Bankowska, K., Tuppurainen, E.S., 2016. Adverse reactions to field vaccination against lumpy skin disease in Jordan. Transbound. Emerg. Dis. 63, e213-219.

APHRD, 2012. Ethiopia animal health yearbook 2011 Animal and Plant Health Regulatory Directorate (APHRD), Addis Ababa, Ethiopia.

Ayelet, G., Abate, Y., Sisay, T., Nigussie, H., Gelaye, E., Jemberie, S., Asmare, K., 2013. Lumpy skin disease: preliminary vaccine efficacy assessment and overview on outbreak impact in dairy cattle at Debre Zeit, central Ethiopia. Antivir. Res. 98, 261-265.

Aznar, I., McGrath, G., Murphy, D., Corner, L.A., Gormley, E., Frankena, K., More, S.J., Martin, W., O'Keeffe, J., De Jong, M.C., 2011. Trial design to estimate the effect of vaccination on tuberculosis incidence in badgers. Vet. Microbiol. 151, 104-111.

Ben-Gera, J., Klement, E., Khinich, E., Stram, Y., Shpigel, N.Y., 2015. Comparison of the efficacy of Neethling lumpy skin disease virus and x10RM65 sheep-pox live attenuated vaccines for the prevention of lumpy skin disease - The results of a randomized controlled field study. Vaccine 33, 4837-4842.

Brenner, J., Bellaiche, M., Gross, E., Elad, D., Oved, Z., Haimovitz, M., Wasserman, A., Friedgut, O., Stram, Y., Bumbarov, V., Yadin, H., 2009. Appearance of skin lesions in cattle populations vaccinated against lumpy skin disease: statutory challenge. Vaccine 27, 1500-1503.

Carn, 1993. Control of capripoxvirus infections. Vaccine 11, 1275-1279.

Dohoo, I., Martin, W., Stryhn, H., 2003. Veterinary Epidemiologic Research. AVC Inc. Charlottetown, Canada. pp. 1-21.

Davies, F.G., 1991. Lumpy skin disease, an African capripox virus disease of cattle. Br. Vet. J 147, 489-503.

De Jong, M.C.M., 1994. Experimental quantification of vaccine induced reduction in virus transmission reduction. Vaccine 12, 761-766.

Diekmann, O., Heesterbeek, J.A.P., Metz, J.A.J., 1990. On the definition and the computation of the basic reproduction ratio R0 in models for infectious diseases in heterogeneous populations J. Math. Biol. 28, 365-382.

Gari, G., Abie, G., Gizaw, D., Wubete, A., Kidane, M., Asgedom, H., Bayissa, B., Ayelet, G., Oura, C.A., Roger, F., Tuppurainen, E.S., 2015. Evaluation of the safety, immunogenicity and efficacy of three capripoxvirus vaccine strains against lumpy skin disease virus. Vaccine 33, 3256-3261.

Gari, G., Bonnet, P., Roger, F., Waret-Szkuta, A., 2011. Epidemiological aspects and financial impact of lumpy skin disease in Ethiopia. Prev. Vet. Med. 102, 274-283.

Gari, G., Waret-Szkuta, A., Grosbois, V., Jacquiet, P., Roger, F., 2010. Risk factors associated with observed clinical lumpy skin disease in Ethiopia. Epidemiol. Infect. 138, 1657-1666.

Gelaye, E., Belay, A., Ayelet, G., Jenberie, S., Yami, M., Loitsch, A., Tuppurainen, E., Grabherr, R., Diallo, A., Lamien, C.E., 2015. Capripox disease in Ethiopia: Genetic differences between field isolates and vaccine strain, and implications for vaccination failure. Antivir. Res. 119, 28-35.

Gelaye, E., Lamien, C.E., Silber, R., Tuppurainen, E.S., Grabherr, R., Diallo, A., 2013. Development of a cost-effective method for capripoxvirus genotyping using snapback primer and dsDNA intercalating dye. PloS One 8, e75971.

Halloran, M.E., Longini, J.I., Struchiner, C.J., 2010. Design and Analysis of Vaccine Studies. Springer, New York. pp. 19-45.

Halloran, M.E., Struchiner, C.J., Longini, J.I., 1997. Study designs for evaluating different efficacy and effectiveness aspects of vaccines. Am. J. Epidemiol. 146,789-803.

Heffernan, J.M., Smith, R.J., Wahl, L.M., 2005. Perspectives on the basic reproductive ratio. J. R. Soc. Interface 2, 281-293.

Hunter, P., Wallace, D., 2001. Lumpy skin disease in southern Africa: a review of the disease and aspects of control. J. S. Afr. Vet. Ass. 72, 68-71.

Kumar, S.M., 2011. An outbreak of lumpy skin disease in a Holstein dairy herd in Oman: A clinical report Asian J. Anim. Vet. Adv. 6, 851-859.

Longini, Jr., I.M., Sagatelian, K., Rida, W.N., Halloran, M.E., 1998. Optimal vaccine trial design when estimating vaccine efficacy for susceptibility and infectiousness from multiple populations. Stat. Med. 17, 1121-1136.

Lu, Z., Schukken, Y.H., Smith, R.L., Mitchell, R.M., Gröhn, Y.T., 2013. Impact of imperfect Mycobacterium avium subsp. paratuberculosis vaccines in dairy herds: A mathematical modeling approach. Prev. Vet. Med. 108, 148-158.

Mebratu, G.Y., Kassa, B., Fikre, Y., Berhanu, B., 1984. Observation on the outbreak of lumpy skin disease in Ethiopia Rev. Elev. Méd. Vét. Pays Trop. 37, 395-399.

NVI, 2010 Product catalogue. User manual for lumpy skin disease vaccine. Issue In: National Veterinary Institute (Ed.), 12.

OIE, 2010. Manual of diagnostic tests and vaccines for terrestrial animals, chapter 2.4.14, Lumpy skin disease. OIE, Paris. http://web.oie.int/eng/normes/MMANUAL/A_Index.htm (accessed February 26, 2016). OIE, Paris.

OIE, 2015. Manual of diagnostic tests and vaccines for terrestrial animals, volume 1, chapter 1.1.6, principles of veterinary vaccine production. OIE, Paris. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/1.01.06_VACCINE_PRODUCTION.pdf (accessed March 8, 2016). OIE, Paris.

OIE, 2016. OIE-Listed diseases, infections and infestations in force in 2016. Available at: http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2016/ (accessed March 8, 2016). OIE, Paris.

Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R., 1999. Clinical Veterinary Microbiology. Elsevier Ltd, London. pp. 486-495.

Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J., Leonard, F.C., 2002. Veterinary Microbiology and Microbial Disease. Blackwell Science, Great Britain. pp. 335-342.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D., 2007. Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. Sounders Elsevier, Spain. pp. 1424-1426.

Smith, P.G., Rodrigues, L.C., Fine, P.E., 1984. Assessment of the protective efficacy of vaccines against common diseases using case-control and cohort studies. Int. J. Epidemiol. 13, 87-93.

Somasundaram, M.K., 2011. An outbreak of lumpy skin disease in a Holstein dairy herd in Oman: a clinical report. Asian J. Anim. Vet. Adv. 6, 851-859.

Tasioudi, K.E., Antoniou, S.E., Iliadou, P., Sachpatzidis, A., Plevraki, E., Agianniotaki, E.I., Fouki, C., Mangana-Vougiouka, O., Chondrokouki, E., Dile, C., 2016. Emergence of lumpy skin disease in Greece, 2015. Transbound. Emerg. Dis. 63, 260-265.

Torvaldsen, S., McIntyre, P.B., 2002. Observational methods in epidemiologic assessment of vaccine effectiveness. Commun. Dis. Intell. Q. Rep. 26, 451-457.

Tuppurainen, E.S., Oura, C.A., 2012. Review: lumpy skin disease: an emerging threat to Europe, the Middle East and Asia. Transbound. Emerg. Dis. 59, 40-48.

Tuppurainen, E.S., Pearson, C.R., Bachanek-Bankowska, K., Knowles, N.J., Amareen, S., Frost, L., Henstock, M.R., Lamien, C.E., Diallo, A., Mertens, P.P., 2014. Characterization of sheep pox virus vaccine for cattle against lumpy skin disease virus. Antivir. Res. 109, 1-6.

Tuppurainen, E.S., Venter, E.H., Shisler, J.L., Gari, G., Mekonnen, G.A., Juleff, N., Lyons, N.A., De Clercq, K., Upton, C., Bowden, T.R., Babiuk, S., Babiuk, L.A., 2017.

Review: capripoxvirus diseases: current status and opportunities for control. Transbound. Emerg. Dis. 64, 729-745.

Tuppurainen, E.S.M., Venter, E.H., Coetzer, J.A.W., 2005. The detection of lumpy skin disease virus in samples of experimentally infected cattle using different diagnostic techniques. Onderstepoort J. Vet. Res. 72, 153-164.

Van der Goot, J.A., Van Boven, M., Koch, G., de Jong, M.C.M., 2007. Variable effect of vaccination against highly pathogenic avian influenza (H7N7) virus on disease and transmission in pheasants and teals. Vaccine 25, 8318-8325.

Velthuis, A.G.J., De Jong, M.C.M., Kamp, E.M., Stockhofe, N., Verheijden, J.H.M., 2003. Design and analysis of an Actinobacillus pleuropneumoniae transmission experiment. Prev. Vet. Med. 60, 53-68.

WAHID, 2016. Summary of Immediate notifications and Follow-ups. World Animal Health Information Database (WAHID).

Weiss, K.E., 1968. Lumpy skin disease virus. Virol. Monogr. 3, 111-131.

Woods, J.A., 1988. Lumpy skin disease- A review. Trop. Anim. Hlth Prod. 20, 11-17.

Yeruham, I., Perl, S., Nyska, A., Abraham, A., Davidson, M., Haymovitch, M., Zamir, O., Grinstein, H., 1994. Adverse reactions in cattle to a capripox vaccine. Vet. Rec.135, 330-332.

Table 1. The fitted model to estimate LSD transmission rates in cattle populations with different levels of vaccination coverage in Mota town and Hulet Ejju Enessie district of Ethiopia.

Partial R value	Log β = c0 + c1*vaccination + c2*fracVaccI[a] Description	Expression[b]
Ruu	Transmission from an unvaccinated to an unvaccinated animal	e^{c0}
Ruv	Transmission from an unvaccinated to a vaccinated animal	$e^{c0 + c1}$
Rvu	Transmission from a vaccinated to an unvaccinated animal	$e^{c0 + c2}$
Rvv	Transmission from a vaccinated to a vaccinated animal	$e^{c0 + c1 + c2}$

[a] Fraction of vaccinated among the infected.

[b] Relation between infection parameters and estimated coefficients of the model, where c0 is the estimated intercept and c1 and c2 are the estimated regression coefficients of the variables vaccination and fracVaccI respectively.

Table 2. Ethiopian herd owners' opinion on LSD vaccine effectiveness and adverse reactions.

Level	Vaccine effectiveness			Vaccine adverse reactions		
	Frequency	Percent	Cum. percent	Frequency	Percent	Cum. percent
Very high	0	0	0	1	0.8	0.8
High	29	23.2	23.2	1	0.8	1.6
Moderate	20	16.0	39.2	0	0	1.6
Low	6	4.8	44	0	0	1.6
Very low	70	56.0	100	123	98.4	100
Total	**125**	**100**	**100**	**125**	**100**	**100**

Table 3. LSD infection and death proportion in vaccinated and unvaccinated cattle population at different localities of Mota town and Hulet Ejju Enessie district of Ethiopia.

Sub-kebele/town	**Population**			**Unvaccinated**			**Vaccinated**		
	Total	**No. (Proportion) infected**	**No. (Proportion) died**	**Total**	**No. (Proportion) infected**	**No. (Proportion) died**	**Total**	**No. (Proportion) infected**	**No. (Proportion) died**
Mota	169	40 (0.237)	2 (0.012)	87	26 (0.299)	2 (0.023)	82	14 (0.171)	0 (0.000)
Akobe	108	22 (0.204)	0 (0.000)	74	14 (0.189)	0 (0.000)	34	8 (0.235)	0 (0.000)
Atetanat	134	50 (0.373)	8 (0.060)	51	19 (0.373)	2 (0.039)	83	31 (0.373)	6 (0.072)
Kesmender	145	35 (0.241)	2 (0.014)	38	9 (0.237)	0 (0.000)	107	26 (0.243)	2 (0.019)
Komma	76	16 (0.211)	0 (0.000)	7	3 (0.429)	0 (0.000)	69	13 (0.188)	0 (0.000)
Semo	220	54 (0.245)	0 (0.000)	214	53 (0.248)	0 (0.000)	6	1 (0.167)	0 (0.000)
Shewaber	187	44 (0.235)	2 (0.011)	108	28 (0.259)	1 (0.009)	79	16 (0.203)	1 (0.013)
Webmariam	432	109 (0.252)	8 (0.019)	127	64 (0.504)	4 (0.031)	305	45 (0.148)	4 (0.013)
Yerez	430	125 (0.291)	7 (0.016)	23	10 (0.435)	2 (0.087)	407	115 (0.283)	5 (0.012)
Zenabach	152	31 (0.204)	0 (0.000)	20	7 (0.350)	0 (0.000)	132	24 (0.182)	0 (0.000)
Overall	**2053**	**526 (0.256)**	**29 (0.014)**	**749**	**233 (0.311)**	**11 (0.015)**	**1304**	**293 (0.225)**	**18 (0.014)**

Table 4. Multivariable analysis of potential riskfactors for LSD infection in Mota town and Hulet Ejju Enessie district of Ethiopia (n=2053) using logistic regression.

Risk factor	**Category**	**No. of animals**	**No. LSD**	**Odds Ratio**	**95% CI**	**p-value**
Vaccination	Vaccinated	1304	293	0.49	0.37-0.64	0.000
	Unvaccinated	749	233	Ref		
Breed	Cross	312	95	3.83	2.25-6.53	0.000
	Local	1741	431	Ref		
Age group	Calf	346	46	Ref		
	Young	490	91	1.50	1.01-2.22	0.043
	Adult	1217	389	3.02	2.14-4.25	0.000
Sex	Male	1120	339	1.79	1.44-2.23	0.000
	Female	933	187	Ref		
Herdtype	Specialized	126	28	0.53	0.22-1.27	0.157
	Mixed	1927	498	Ref		
Location	Ayen Berhan	432	109	1.25	0.60-2.60	0.557
	Beza Bizuhan	564	175	2.12	1.02-4.00	0.044
	Debre Gubae	373	82	1.33	0.63-2.81	0.458
	Hibre Selam	515	120	0.80	0.38-1.66	0.545
	Mota town	169	40	Ref		
Interaction	Vaccinated * cross breed			0.43	0.23-0.81	0.008

Table 5. LSD severity in vaccinated and unvaccinated cattle population (n= 480) of Ethiopia.

Severity level	**Vaccinated**		**Unvaccinated**	
	Number	**Proportion in %**	**Number**	**Proportion in %**
Mild	45	17.1	21	9.7
Moderate	106	40.2	86	39.8
Severe	113	42.8	109	50.5
Total	**264**	**100**	**216**	**100**

Table 6. Analysis of the effect of vaccination on susceptibility and infectiousness of LSD in Mota town and Hulet Ejju Enessie district of Ethiopia (n=2053) using GLM.

Variable	**Susceptibility/ infectiousness**	**Coefficient (b)**	**Effect (exp(b))**	**95% CI**	**p-value**
Vaccination	Susceptibility	-0.62	0.54	0.44; 0.66	0.000
FracVaccI[a]	Infectiousness	0.60	1.83	1.28; 2.61	0.001
Constant		0.19	1.21	1.00; 1.46	0.045

[a]Fraction of vaccinated among the infected in each population (= sub-kebele).

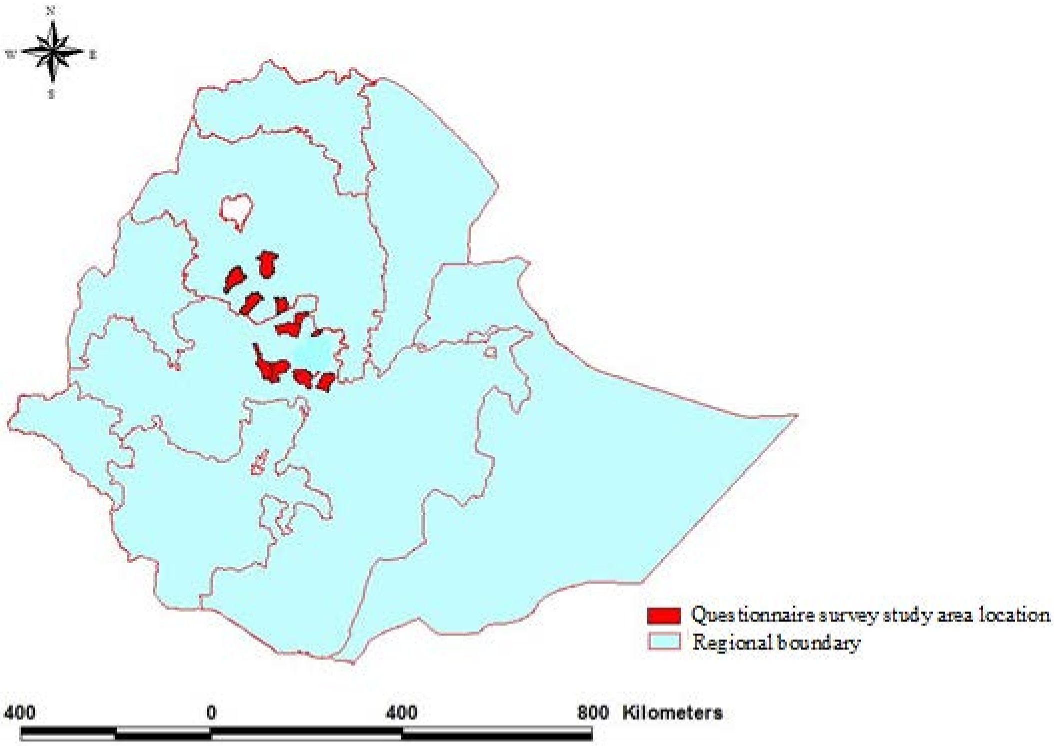

Figure 1. Map of Ethiopia showing the area where the questionnaire survey was performed.

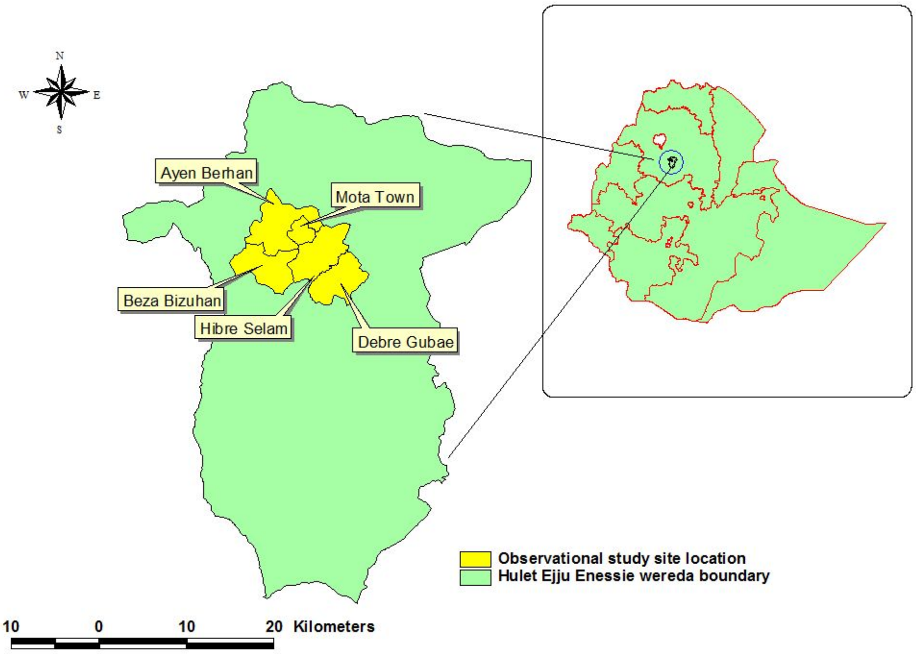

Figure 2. Map of Hulet Ejju Enessie district (Ethiopia) showing LSD vaccine efficacy observational study site.