Sero-epidemiology and associated risk factors of brucellosis among sheep and goat population in the mid southern Nepal: A comparative study

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Research article

Keywords: Brucellosis, zoonotic disease, sheep and goat, livelihood, seroepidemiology, one health approach

Posted Date: July 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-39025/v1

Version of Record: A version of this preprint was published on March 25th, 2021. See the published version at https://doi.org/10.1186/s12917-021-02835-8.

Abstract

Background

Brucellosis is a zoonotic disease of animals and humans caused by the Brucella spps. In Nepal, the presence of brucellosis in small ruminants, e.g., sheep and goats, has impacted the farmers' livelihood and people's food safety. A cross-sectional study was conducted at Rupandehi districts of Nepal to the discover the seroepidemiology and associated risk factors of brucellosis in sheep and goat population. Altogether, 19 sheep and 60 goat farms located in the districts' local units were visited, and the owners were interviewed to get the information on animal characteristics, management, and movement patterns. Three hundred fifty-seven serum samples (80 sheep and 277 goat's samples) were collected from selected herd based on the probability proportional to their sizes. Each serum sample was tested for Rose Bengal Plate Test (RBPT) and ELISA to estimate the seropositivity. Bivariate analysis followed by multivariable logistic regression was applied to calculate corresponding odds ratios of each variable associated with the brucellosis.

Results

Out of 80 sheep samples, 12 (15%; 95%CI: 8.79%-24.41%, P<0.001) and out of 277 goat samples 3 (1.1%; 0.37%-3.14%, P<0.001) were tested positive to brucellosis. Age of greater than 1.5 years (OR= 6.39, 95%CI: 1.23, 54.67, p= 0.04) was identified as the significant risk factor for brucellosis in sheep population. While in the goat population, none of the variables were identified as the significant risk factors in multivariable regression analysis. However, the goat from the frequent grazing herds had borderline significance (OR = 8.81, 95%CI: 0.44, 174.56, p<0.15). It might be because of the regular movement of sheep herds that get mixed up with the goat populations.

Conclusion

The study provides evidence that the burden of brucellosis in sheep is significantly higher than goats. The brucellosis control program in sheep should be applied immediately, as the contiguous herds of sheep and goats keep mixing while grazing and selling. Also, the strict biosecurity and biosafety measures should be implemented among the sheepherders to prevent infection of Brucella in them. We suggest further study on both small ruminants and the sheep owners to reveal the transmission dynamics through one health approach.

Background

Brucellosis is an economically important zoonotic disease of both animals and humans caused by the gram-negative bacteria of Brucella species [1–2]. People contract Brucellosis by the consumption of unpasteurized dairy products [3–5] occupational exposures through handling aborted fetus or placenta of infected animals [3], and inhalation of contaminated aerosol while processing of the animal products[7–8]. Brucellosis creates significant economic losses to the livestock industry worldwide

because it usually results in abortion, infertility, and a decrease in milk and meat production [5]. The disease has been successfully managed or eradicated from several developed countries, but it is still endemic in livestock and humans in resource-poor countries [9–10].

The small ruminants such as goat and sheep production is related to the livelihood of Nepalese farmers, and the goat is considered as the poor man's cow [11–12].They are one of the principal commodities of the livestock production system in Nepal. There is an estimate of 11 million goats and 0.8 million sheep population in Nepal [9]. Nepalese sheep supports the local carpet industry [8]. On the other hand, goat meat provides the second (20.36%) most substantial volume of meat consumption after the buffalo meat (54.34%) in Nepal [9].

Moreover, the demand for the goat and sheep meat would be highest during September to November annually, because of the two large festivals falls within this period [14–15]. With the import and the rapid movement of small ruminants during festival seasons [15–16], there could be the highest risk of livestock diseases transmission between the ruminant's population. Also, there may be a public health threat due to diseases like Brucellosis when people choose to slaughter goat and sheep at their homes for the festivals. On the other hand, the sheep and goat herds have transhumant migration than large ruminants in Nepal, and they are more likely to contract diseases during the movement [12].

Though there are some of the studies related to the seroprevalence of animal and human brucellosis in Nepal [18–20], there are not any risk factors related studies with the animal brucellosis in Nepal. Identifying risk factors and implementing prevention and control programs could lead to a decrease in the disease burden in the small ruminants. We aimed to estimate the comparative seroprevalence and risk factors of goat and sheep, as they generally found in contiguous herds or mixed farming systems. The effective control and preventive measures can be applied once the risk factors are identified.

Methods

Study sites

This study was conducted in the Rupandehi district located in the mid-southern region of Nepal. (Fig 1). The population of goat and sheep in the Rupandehi district was 185,332 and 4,024, respectively, that supply around 73,556 metric tons of meat annually in the nation [14]. This district was selected for study because it shares the border with India from where Nepal periodically used to import around 400,000 goats through international border quarantines check posts [15]. Because of this, there was a risk of disease introduction among the small ruminant population in the country by formal and informal livestock trade. Also, there is a risk of disease spread due to the internal movement of goats and sheep between other adjacent districts.

Study design

A cross-sectional study was conducted on the goat and sheep population of Rupandehi district between January to March 2020. The semi-structured questionnaire was administered to collect the information on each herd's animal characteristics, management status, and animal movement system. The survey was initially designed in English and later translated to the local Nepali language. Next, blood samples were collected during the time of interviewing the sheep and goat herd owners. The written consent from the owners was obtained during the questionnaire and sample collection processes.

Sampling and sample size calculation

A sampling frame was constructed to list of all the registered goat and sheep farms in the districts. There were sixteen local levels in Rupandehi districts with 106 accessible commercial goat and sheep herds [14]. Next, the total number of the flock was calculated by assuming that the prevalence of the disease in the congregation was 50% at 95% confidence interval (CI) with 5% desired precision by using the following formula (Equation i)

The formula is based on:

$$N = \frac{1.96^2 P_{exp} (1-P_{exp})}{d^2} \quad \text{.....(i)}$$

where N = sample size, Pexp = expected prevalence, and d = absolute precision [16].

The total number (N) of the herd to be selected by this method was 84.

Finally, we visited a total of eighty-four farms in the district, but five farms did not agree to participate. So, we were able to collect samples from only 79 farms (60 goat farms and 19 sheep farms). Out of these, we collected 357 samples in total (277 goat and 80 sheep samples) to include them in the analysis.

Laboratory Analysis

The collected samples were stored in the ice pack and transported to the labs for further analysis. First the Rose Bengal Plate tests were performed for screening brucellosis. And all the serum samples were subjected to ELISA tests for Brucellosis. All the tests were performed at Central Veterinary Laboratory (CVL), the national reference lab for animal diseases.

Rose Bengal test (RBPT)

Rose Bengal Antigen from ID vet, France used for the tests. It was a rapid test to screen the Brucella spps antibody with a sensitivity of 87.2% [17], and the specificity is 99.6% [18].The test serum (0.03 ml) was mixed with an equal volume of RBPT antigen on a glass slide to produce a zone of approximately 2 cm in

diameter. The mixture was agitated gently for four minutes at ambient temperature, and then observed for agglutination. We considered the tests was positive when any visible reaction or agglutination on it. However, we suggest other tests to confirm the results of RBPT due to its low sensitivity in chronic cases and low specificity in endemic areas.

ELISA (Enzyme-linked immunosorbent assay)

Indirect multispecies ELISA kit manufactured by ID vet France was used in the test. The kit could detect antibodies of various species of brucella such as Brucella abortus, Brucella mellitensis, and Brucella suis. The sensitivity and specificity of this test were 96.8% and 96.3%, respectively, according to the Bayesian estimation approach [19]. All the testing procedures were performed strictly based on the protocols provided by the manufacturer. The test plates were read under the ELISA reader (“Multiskan™ FC Microplate Photometer”) at optical density (OD) 450nm within 15 minutes.

Validation and interpretation of the test

The test was validated if;

i) The mean OD value of the positive control (ODpc) was higher than 0.350. i.e., OD positive control ≥ 0.350.

ii) The ratio of the mean OD values of the positive and negative control (ODpc and ODnc) was higher than 3., i.e., OD positive control / OD negative control ≥ 3.

Interpretation

For each sample, the S/P percentage (S/P%) was calculated as follows using the sample and control OD values.

i.e. S/P% = (OD sample - OD nc / OD pc- OD nc) x100

Samples with an S/P%, less than or equal to 20%, are considered negative, and greater than 20% are considered positive.

Data management and statistical Analysis

The raw data collected from the paper-based questionnaire was manually entered on the MS Excel spreadsheet and converted to CSV files. The data was analyzed using open-source epidemiological software Open epi and R version 3.6.1 [20]. The descriptive data analysis was performed to investigate the population characteristics of both the species.

The animal level prevalence of goat and sheep was calculated to estimate the overall species wise disease prevalence in the district. The Q-Q plot for the normality assessed the data distributions of each continuous variable. These continuous variables, such as herd size, age category, and parity number, were converted into binary categorical variables using the quartile of distributions (e.g., median) to manage the problem linearity [21].

Each of the independent factors were examined with the response variables (seropositivity) by bivariate analysis of two by two table contingency table. The fisher's exact tests and chi-square tests (when appropriate) were used to examine associations between response variables and explanatory variables.

The empty or zero cells in the two by two table analyses were corrected by data modification methods by Argesti (2002) in Open epi to calculate corresponding odds ratios and p values [29–30].In this case to rectify the maximum likelihood estimation of the logistic model suffer from small-sample bias, firth logistic regression was used by logistif function in R and firth logistic in STATA in multivariable regression analysis [29–34].

The correlation between the continuous variables such as herd size, age category, parity number was performed. Since there were strong correlations (r = 0.63) between the age and parity number detected, the parity category was dropped from the model because age was a biologically plausible risk factor [23]. The variable's potential confounding effects were checked with the changes in the point estimates of the variables that remain in the model [36–37]. Any changes in the coefficient with >20% were included in the final model.

The potential risk factors with the significance level $p \leq 0.2$ following the bivariate analysis were manually entered in the final multivariable model [26].A backward stepwise variable selection was used to add the variable with the lowest p-value to construct a final model with a significance level of $p \leq .05$. Any variables with p-value <0.05 were considered statistically significant risk factors. The above process was performed separately for each set of risk factors of goat and sheep herd for the valid comparison.

Results

3.1 Descriptive study of animal population

The study included 60 (75.94%) goat farms and 19 (24.1%) sheep farms; among them a total of 277 (77.59%) goat samples and 80 (22.41%) sheep blood samples tested for brucellosis. The median herd size of the goat and sheep farms were 48 and 100, respectively. The median age of goat and sheep were 1.5 and 2 years, respectively. The individual species wise herd characteristics of goat and sheep are depicted in table 1. About 17.65% (12/68) sheep were either purchased from nearby herds or brought from India, and 61% (105/172) of the goat were introduced from the neighboring districts or abroad. The median age of the sheep owner was 45, and that of the goat farms was 35. Only 55 of the sheep herd were registered while 90% of the sheep herd were registered. A Terai indigenous community maintained most of the sheep herds, and that was one of the sources of their livelihood.

Sero-prevalence of goat and sheep population

Of the total of 80 sheep samples tested, 12 (15%; 95% CI: 8.79–24.41), and among 277 goat samples tested 3 (1.1%; 95%CI: 0.37–3.14) were seropositive to Brucella. The brucellosis was detected only in female goats, but in the sheep populations, higher proportion of males 18.75% (3/16) were positive to Brucella than female 14.1% (9/64). The local goats, such as Khari, were positive to Brucella. Lampuchre breed is indigenous sheep that has the highest burden of disease. The detailed illustrations of the sex-wise and breed wise comparison of seroprevalence of Brucella among goats and sheep are described in table 1.

Table 1:Comparison of seroprevalence of Brucella among goats and sheep by sex and breed wise classification

Variables	Category	Total number (%)	RBPT positive (%)	ELISA positive (%)	Overall Prevalence (95%CI)
Species					
Goat	Male	65 (18.21)	0.00	0.00	1.1% (0.37-3.14)
	Female	212 (59.38)	1.81% (5/277)	1.1 % (3/277)	
Sheep	Male	16 (20)	18.75 % (3/16)	18.75 % (3/16)	15% (8.79-24.41)
	Female	64 (80)	12.5% (8/64)	14.1% (9/64)	
Breeds					
Breed of Goat	Local (Khari, Terai)	135 (48.74)	2.22 % (3/135)	2.22 % (3/135)	1.1% (0.37-3.14)
	Exotic (Boer, Jamunapari)	142 (51.26)	1.41 % (2/142)	0.00	
Breed of Sheep	Lampuchre	75(93.75)	14.67% (11/75)	16% (12/75)	15% (8.79-24.41)
	Baruwal	5 (6.25)	0.0	0.00	

Univaraible regression analysis

The bivariate analysis of the sheep and goat data was depicted in Tables 2 and 3, respectively. The sheep of age greater than 1.5 years had significantly 3.29 higher odds of brucellosis than the sheep of age ≤ 1.5 years (OR = 4.29, 95%CI: 1.16, 20.63, p = 0.0406). There were significantly higher odds of brucellosis among sheep herd size of >100 than the sheep herds of ≤100. The sheep that had parity greater than 1.5 were 4.11 more likely to be detected with brucellosis compared to sheep ≤ 1.5, but the result was statistically borderline significant (OR = 4.11, 95%CI: 0.98, 21.29, p = 0.055).

On the other hand, there were some empty cells in the two by two contingency tables in the goat bivariate analysis. Correction was made with the addition of 0.5 and calculated the odds ratios [22, 27–28] (Table 3). In bivariate analysis, the goats that were taken for grazing had significantly higher odds (OR = 14.5, 95% CI: 1.1, 283.9, p = 0.003) of detecting brucellosis compared to goats stall-fed at farms (Table 3).

Multivariable logistic regression analysis

The variables that qualified from the sheep data for multivariable analysis (p<0.20) were age category, gender, common grazing system, disinfection process applied at the farm entry point. Parity was dropped from the model because it had a higher correlation with the age category (r = 0.63). Similarly, for the goat data, the same sets of the variables were included in final firth multivariable logistic regression based on the cut off criteria of p<0.20.

In the multivariable regression analysis, sheep of older age (>1.5 years) had significantly higher odds OR = 6.39, 95%CI: 1.23, 54.67, p = 0.046) of Brucella compared to the younger sheep (≤1.5 years) (Table 4).

On the other hand, none of the variables were identified as the significant risk factors for the brucellosis in goat population after firth logistic regression [27]. But, the goats from the frequent grazing herds had higher odds (OR = 8.81, 95%CI: 0.44, 174.56) of Brucella than goats from isolated herds. However, the finding was at borderline level of significance (p<0.153) (Table no 5).

Table 2: Univariable analysis results of potential risk factors associated with sero-positivity of sheep population against Brucella spps.

Determinants	Total no of sheep	Brucella positive	Brucella negative	Odds ratio (OR)	95% CI	P value
Animal Origin						
Purchased	12	3	9	2.19	(0.85, 2.21)	0.32
Home Breed	68	9	59	Ref		
Age (median=1.5 years)						
>1.5	37	9	28	4.29	(1.16, 20.63)	0.041*
<=1.5	43	3	40	Ref		
Herd size (median= 100)						
>100	24	7	17	4.2	(1.19,15.91)	0.026*
<=100	56	5	51	Ref		
Parity (Median=1 year)						
>1	24	6	18	4.11	(0.98,21.29)	0.055
<=1	40	3	37	Ref		
Gender						
Male	16	3	13	1.41	(0.28,5.53)	0.646
Female	64	9	55	Ref		
Common Grazing herds						
Yes	74	12	62	2.6	(0.12, 49.16)	0.154
No	6	0	6	Ref		
Repeat breeding						
Yes	11	3	8	2.94	(0.62,2.63)	0.199
No	53	6	47	Ref		

*P value<0.05 means statistically significant

Table 3: Univariable analysis results of potential risk factors associated with sero-positivity of goat population against Brucella spps.

Determinants	Total no of goats	Brucella Positive	Brucella Negative	Odds Ratios (OR)	95% CI	P value
Animal orgin						
Purchased	105	2	103	1.2	0.11, 26.11	0.87
Home bred	172	1	171	Ref		
Age (median=2 years)						
<=2	194	3	191	3.1	0.16, 59.74	0.12
>2	83	0	83	Ref		
Herd size (median= 48)						
<=48	140	3	137	7	0.36, 136.8	0.06
>48	137	0	137	Ref		
Parity (Median=1)						
<=1	113	3	110	6.5	0.33, 127.2	0.04*
>1	102	0	102	Ref		
Gender						
Female	211	3	209	2.19	0.112, 42.9	0.34
Male	65	0	65	ref		
Grazing system						
Yes	92	3	89	14.5	1.1, 283.9	0.01*
No	185	0	185	Ref		
Disinfection at the farm entry point						
No	135	3	132	7.5	0.39, 147.1	0.06
Yes	142	0	142	Ref		
Repeat breeding						
Yes	42	1	41	2.1	0.18, 23.28	0.29

No	171	2	169	Ref

Table 4: Multivariable analysis results of risk factors (p<0.05) associated with sero-positivity of sheep population against Brucella spps.

Determinants	Category	Coefficient	Standard Error	Odds ratio (OR	95% CI	P value
Age (median=1 year)	>1.5	1.86	0.9309	6.39	(1.23, 54.67)	0.04*
	<=1.5					
Herd size (median=100)	>100	-1.5016	0.9176	4.49	(0.03, 1.29)	0.09
	<=100					

*P value<0.05 means statistically significant

Table 5: Multivariable analysis results of risk factors (p<0.05) associated with sero-positivity of goat population against Brucella spps

Determinants	Category	Coefficient	Standard Error	Odds ratio (OR)	95% CI	P value
Age (median=1 year)	>1.5	0.98	1.55	2.66	(0.13, 55.69)	0.53
	<=1.5					
Herd size (median= 48)	>48	1.4112	1.559	4.10	(0.19, 87.01)	0.36
	<48					
Grazing System	Yes	2.1757	1.524	8.81	(0.44, 174.56)	0.15 [a]
	No					

a: This variable is a borderline significant and could be a potential risk factor

Discussion

Seroprevalence of brucellosis between sheep and goats

We conducted a comparative study on the epidemiology of Brucella among goat and sheep herds in Rupandehi district. The burden of brucellosis was highest among the sheep 15% (8.79–24.41) compared to goats (1.1%(0.37–3.14). A seasonal study by Singh et al.[28] estimated that 6.6% (n = 212) of sheep and 3.4% (n = 774) of goat were seropositive by Indirect multispecies ELISA from of various districts of Nepal. It also justifies that the prevalence of brucellosis is higher in sheep than goats. Also, Nepal has a transhumant rotational sheep grazing system in many parts of Nepal, and the sheep's migratory pattern could contribute to the highest transmission rate [8]. This finding was also supported by a study conducted by Rajala et al. [23] in Tajikistan. However, some literature suggests that goats are more susceptible to B. melitensis infection than sheep[29]. It might depend upon the variation in geographical settings and differences of management system of livestock productions. There were low numbers of seropositive goats in this study (3/227); however, if we had conducted our study in September to October, the prevalence of the disease would increase. It is because the highest movements of goats occur around this time for the ritual slaughter in Nepal. As, as none of the sheep and the goats' herds were vaccinated, this was the evidence of natural infection transmission of brucellosis within the small ruminants in the study areas.

Significant risk factors

It is the first risk factor study for brucellosis among small ruminants in Nepal to the best of our knowledge. Brucellosis is one of the priority zoonosis documented in the papers by the Government of Nepal. There might be some differences with the local risk factors identified with those identified elsewhere, but the effective disease management lies in localized ways of managing the diseases.

The sheep population of age greater than 1.5 years had significantly higher odds of detecting brucellosis. It might be because the older sheep remained in the flock for a long time, and they had a longer duration of exposure [23,30]. It is supported by many other studies by [23,27,31]. who mentioned that biologically younger animals are more resistant to infection than adult animals. However, age was not a significant risk factor for the brucellosis for the goats in the district. It may be because that goat herds were mainly maintained for the meat production in Nepal, and they are sent to slaughter within a year.

The herd size of sheep significant ($p = 0.026$) at univariable analysis was borderline significant ($p = 0.093$) in multivariable analysis. The sheep herds were larger than goat herds, and there were chances of disease transmission as the number of animals moved around in the big crowds. Also, the sheep herds we visited were closer to one another such that there is transmission of diseases to the contiguous herds as they mixed up during grazing.

None of the variables related to the goats were significantly associated with the Brucella antibody detection. However, the goats managed for the grazing system had the borderline significance of detecting brucellosis ($p<0.2$). So, the goats that were deemed positive to anti-brucella antibody might be due to accidental exposure to nearby sheep herds. As in fig, the sheep and goat herds were clustered

separately, but there was a probability of intermingle when taken for grazing in the pasture or moved to live markets.

The findings from this work provide better epidemiological insight that could be utilized to manage such a significant disease in small ruminant's production in Nepal. Discovering such a substantial burden of brucellosis in small ruminants and mostly in sheep should concern the livestock department. The farmworkers' safety issue, on the other hand, was possible facets that we could not address in this study.

Conclusion

Though the brucellosis was documented in the papers by the Government of Nepal as one of the priority zoonosis, the active disease surveillance and reporting is almost absent. It is an economically important disease for small ruminants, and the occupational risk among the people. The goat and sheep being the valuable commodities related to the Nepalese farmer's livelihood, the prevention and the control and brucellosis is crucial. This study estimated the burden of the disease and risk factors related to brucellosis among the goats and sheep population of Nepal. We suggest prospective such studies on the national level to get the bigger picture of the epidemiology of animal brucellosis in Nepal.

List Of Abbreviations

NVC: Nepal Veterinary Council

CVL: Central Veterinary Laboratory

RBPT: Rose Bengal Plate Agglutination Test

ELISA: Enzyme Linked Immunosorbent Assay

OD: Optical Density; PC: Positive Control, and NC: Negative Control

Declarations

Ethical approval and consent to participate

An ethical statement for animal subjects was approved by the Nepal Veterinary Council Nepal (NVC), the official statutory body of animal health, with the Ref. No 279(TG)/2076–77. A written consent from the cattle owners were obtained before the interview. The experienced veterinarian handled all the animals during sample collections taking into account minimal pain in the animals. The owners agreed to receive the test reports of their cattle herds maintaining confidentiality.

Consent of publication

Not applicable.

Availability of the data materials

The data for this project can be accessed from the personal data repository link: https://github.com/tulsiramgompo/sheep_and_goat_brucellosis

Competing interests

The author declares that they have no competing interests.

Funding

Authors received no special funding for this work.

Authors' Contributions

TRG: Conceived the idea, perform lab tests, conducted data analysis, wrote the original draft, review the final draft.

RS: Collected data, collected samples, assisted in lab tests, assisted in literature review.

IT: Collected data, collected samples

YG: Supervision

Acknowledgments

We want to acknowledge Dr. Diker Dev Bhatta for providing the resources to the research,

Dr. Ram Chandra Sapkota, and Dr. Luna Gongal for their contributions in the laboratory work.

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Figures

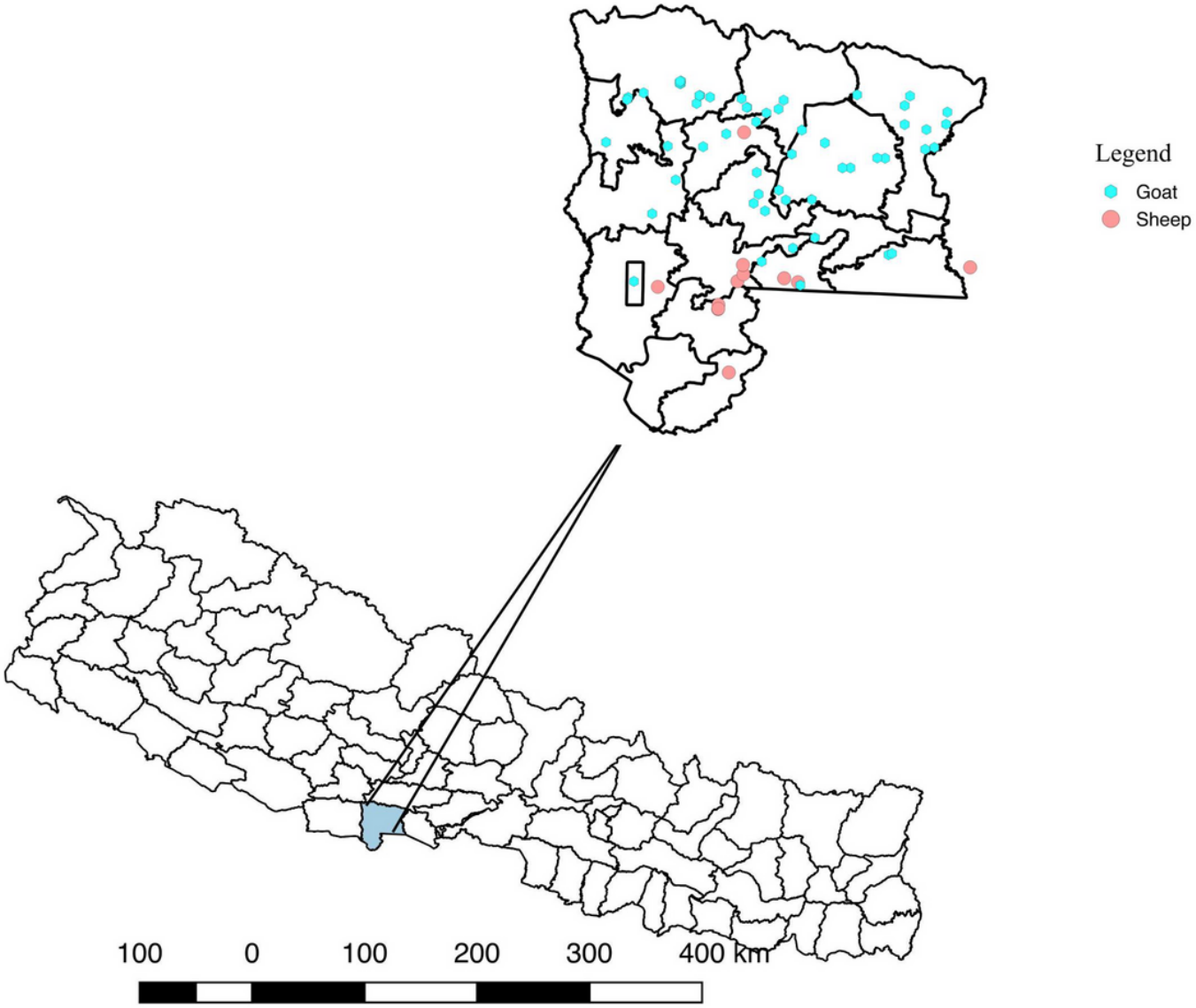

Figure 1

A map of Nepal with the study district indicated, and locations of sheep and goats farms in it (generated using QGis 2.18).