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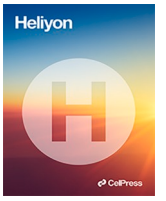

Research article

Seroprevalence of *Coxiella burnetii*, Leptospira interrogans serovar hardjo, and *Brucella species* and associated reproductive disorders in cattle in southwest Ethiopia

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ABSTRACT

In Ethiopia, *Coxiella burnetii*, Leptospira Hardjo, and *Brucella* spp are recognized as the primary factors contributing to cattle reproductive issues. A cross-sectional study was conducted in southwest Ethiopia from October 2020 to October 2021 to assess the risk of reproductive disorders associated with L. Hardjo, *Coxiella burnetii*, and *Brucella* spp. Moreover, the study aimed to identify the factors associated with reproductive disorders. Using an indirect ELISA, antibodies against these pathogens were observed in serum samples collected from 461 cattle. We employed multivariable random effect logistic regression analysis to identify potential risk factors associated with reproductive disorders in cattle. The study areas showed a prevalence of 25.16 % (95 % CI: 21.20–29.12) for cattle reproductive disorders. The presence of Leptospira Hardjo (OR = 2.9, 95 % CI: 1.17–4.02) and *Coxiella burnetii* (OR = 3.0, 1.49–5.94) antibodies was associated to the occurrence of cattle reproductive disorders. Seropositivity to pathogens *B. abortus*, *C. burnetii*, and L. Hardjo, along with co-infection of all three, showed association with cattle abortion. The presence of L. Hardjo seropositivity and co-infection with *C. burnetii* were related to dystocia in cattle. Cattle with retained fetal membranes were associated with co-infection seropositivity to these pathogens. Additionally, *B. abortus* seropositivity was linked to cases of repeated breeding in cattle. Age, breeding practices, and dog access to cattle showed associations with reproductive disorders, with odds ratios of 2.3 (95 % CI: 2.03–4.69), 2.9 (95 % CI: 1.83–4.82), and 6.5 (95 % CI: 1.04–2.53) respectively. This research indicates that *Brucella abortus*, *Coxiella burnetii*, and Leptospira Hardjo, which are responsible for severe zoonotic diseases, have a substantial negative impact on cattle production by causing reproductive disorders. To address the transmission of these diseases, it is essential to implement effective mitigation strategies and enhance public awareness. Additional investigation is necessary to identify and understand the factors contributing to cattle reproductive disorders in the specified area.

1. Introduction

Reproduction plays a crucial role in cattle farming, influencing the productivity of industry and profitability [1]. Cattle production is substantially affected by reproductive problems, which can stem from factors like diseases, nutritional deficiencies, and

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environmental conditions [2,3]. In Ethiopia, dairy farming faces substantial challenges due to reproductive diseases, resulting in economic losses and subpar reproductive outcomes in smallholder dairy farms [4,5]. Reproductive disorders such as abortion, dystocia, stillbirth, retained fetal membrane, endometritis, and pyometra are prevalent [6,7]. Several of these disorders stem from genital infections, some of which may have zoonotic potential [8–10]. Leptospirosis, Q-fever, and brucellosis are infectious diseases that play a significant role in contributing to reproductive problems in cattle [11–13].

Leptospirosis, caused by the bacteria *Leptospira*, is a remarkably transmissible zoonotic disease affect both domestic and wild animals [14]. The condition is associated to reproductive issues such as abortion, stillbirth, and infertility [15–17]. While seroprevalence studies have been conducted globally, there is a scarcity of data in East Africa, particularly in Ethiopia. Nevertheless, research from a neighboring country [18] and certain regions of Ethiopia [16,17] has documented the highest seroprevalence of Leptospira Hardjo in cattle, strongly associated with reproductive complications. Likewise, brucellosis, resulting from *Brucella* bacteria, is a globally prevalent contagious disease in cattle, resulting in reproductive problems [19,20]. Despite numerous serological tests indicating the common occurrence of brucellosis in Ethiopian cattle, the infection persists throughout the country [21–23]. Moreover, Q-fever, which is caused by the bacterium *Coxiella burnetii*, is another zoonotic disease associated to reproductive problems in cattle [24,25]. According to a study conducted by Kiptanui et al. [26], the seroprevalence of *Coxiella burnetii* in cattle was reported to be 64 % in Kenya, underscoring the substantial exposure of animals to this pathogen in East Africa. In Ethiopia, a few studies indicated that the seroprevalence of *C. burnetii* ranged from 8.8 % to 31.6 % [27–29].

Although leptospirosis, Q-fever, and brucellosis have a considerable impact on cattle reproductive health [30], there has been no research exploring their association and synergistic effects in causing reproductive abnormalities in cattle. It is crucial to comprehend the relationship between these diseases and reproductive issues to establish effective prevention and control measures [31]. Additionally, variations in management and environmental factors across areas can affect the occurrence of reproductive diseases. In Ethiopia, where cattle production practices and environmental conditions differ among regions, reproductive disorders pose a significant challenge [5,32,33]. This study aims to explore the potential risk of reproductive disorders associated to L. Hardjo, *Coxiella burnetii*, and *Brucella* spp in southwest Ethiopia. Furthermore, it seeks to identify the factors associated with these reproductive disorders. The hypothesis being tested is that the presence of these pathogens increases the likelihood of reproductive disorders in cattle. Furthermore, factors such as age, breeding practices, and dog access to cattle are also associated with the occurrence of reproductive disorders in cattle.

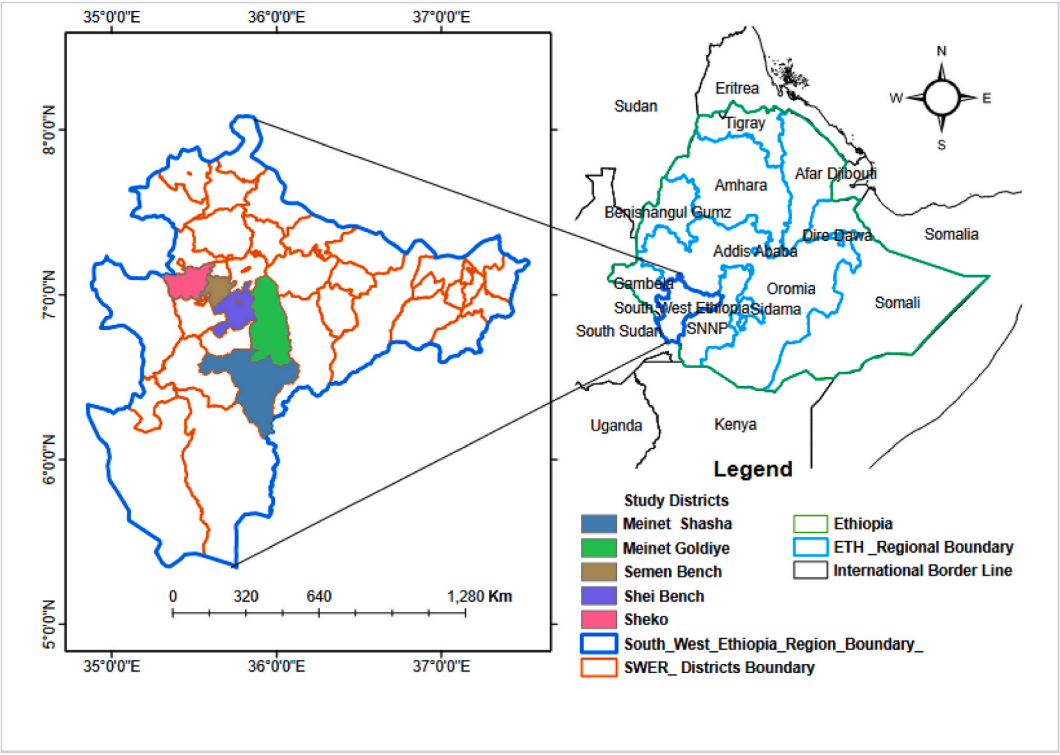

Fig. 1. Map showing study areas.

2. Materials and methods

2.1. Ethical statement

The collection of samples and data for this study was carried out in accordance with ethical guidelines and standards. The Ethiopian Institute of Agricultural Research (EIAR) conducted the study, following approved techniques and procedures that were in line with international standards for animal welfare. The committee on animal welfare and ethical issues provided approval for the study, with the reference number EIAR-2662/2010. Prior to commencing the sample and data collection, appropriate approval and consent were obtained, ensuring compliance with ethical considerations.

2.2. Description of the study areas

The research was carried out in selected districts of Bench-Sheko and West Omo zones, specifically Semen Bench, Shei Bench, Sheko, Meinet Golidiya, and Meinet Shasha, situated in southwest Ethiopia. These zones are positioned between latitudes 5°.88′-7°.21′ N and longitudes 34°.88′-36°.14′ E. The elevations in these zones vary from 500 to 3000 m above sea level. The temperatures experienced annually in these zones span from 15.1 to 27 °C, with annual rainfall ranging from 400 to 2000 mm. Based on the classification of Ethiopian agro-ecology [34], three agro-ecology zones were identified in the study: highland (Semen Bench and Meinet Goldiya), midland (Sheko and Shei Bench), and lowland (Meinet Shasha) (Fig. 1). These zones are characterized as follows: lowland (<1500 masl), mid-altitude (1500–2300 masl), and highland (>2300 masl). In the study areas, the livestock population comprises 1,596,803 cattle, 73,384 sheep, and 71,047 goats [35]. Predominant local cattle breeds include Zebu and Sheko, with some Holstein-Friesian crosses. The management systems employed in the study area encompass extensive (crop-livestock production) and semi-intensive (urban production) practices.

2.3. Study population and design

The research focused on female cattle in the designated districts of Bench-Bench and West Omo zones. These cattle were raised in extensive and semi-extensive production systems. The study involved both local and crossbred cattle aged six months and older. Utilizing a cross-sectional study approach, data was collected in the Bench-Sheko and West Omo zones between October 2020 and October 2021. The research aimed to ascertain the occurrence of reproductive disorders among cattle in the study area, identify their main causes, and investigate related risk factors. Hypothesis: In southwest Ethiopia, there is an association between seropositivity to Leptospira Hardjo, *Coxiella burnetii*, and *Brucella* spp, as well as their co-infection, and an increased risk of reproductive disorders in cattle. Furthermore, factors such as age, breeding practices, and dog access to cattle are also associated with the occurrence of reproductive disorders in cattle.

2.4. Sampling procedure and sample size determination

The sampling process consisted of multiple stages, with random selection being applied at each stage [36]. The study areas were purposefully chosen based on the history of reproductive disorders. The selection procedure involved the use of a simple random sampling technique to choose districts, kebeles (small administrative units within a district), villages, and herds. Six districts were selected by lottery out of the fifteen districts in the zones (Semen Bench, Shei Bench, Sheko, Meinet Shasha, and Meinet Goldiya). Subsequently, 28 kebeles were randomly chosen from these districts. From the selected kebeles, 84 villages were chosen using a simple random sampling method that took into account the number of villages in each kebele. Each cattle in the herds underwent sampling using a simple random sampling method. The reasons for cattle reproduction disorders were ranked based on their potential impact on cattle production, significance as zoonotic diseases in Ethiopia, and accessibility to diagnostic tools, owing to financial and logistical constraints. In consideration of published research and findings from participatory epidemiology in the study areas, three causes of cattle reproduction disorders (*Brucella species*, L. Hardjo, and *Coxiella burnetii*) were chosen for investigation, as indicated by unpublished data. No prior research had been conducted on reproductive disorders specifically related to Leptospira Hardjo, *Brucella* spp, and *Coxiella burnetii* serostatus in the study areas. The study determined the required sample size using Thrusfield's [37] formula, considering an expected prevalence of 50 %, a desired absolute precision of 5 %, and a 95 % confidence interval (CI). The determined sample size was 384 cattle. This calculation assumes no issues with non-response or missing values. To address potential non-response or missing values, it is suggested to oversample by 10 %–20 % [38]. Consequently, 77 additional samples, representing a 20 % increase from the intended sample size, were included. Thus, the study comprised a total of 461 cattle.

2.5. Blood sample collection and serological tests

Aseptic needles and standard vacutainer tubes were utilized to extract about 10 mL of blood from the jugular vein of each cattle. The tubes were appropriately labeled with the cattle's identification number and left in a slant position at room temperature overnight [39,40]. On the subsequent day, the sera were separated, and the corresponding animal codes were added to the cryovial containing the serum. The serum samples were then stored at −20 °C [41] in the Mizan Regional Veterinary Laboratory until they could be transported to the National Veterinary Institute in Bishoftu. This transportation was facilitated using an icebox to maintain the required temperature for laboratory analysis. To assess the presence of positive antibodies against the *Brucella species*, L. Hardjo, and

Coxiella burnetii, serum samples were subjected to testing using a commercial serological kit. Each kit employed in the study adhered to specific instructions for serum sample preparation. Following the methods outlined by OIE [42], the serum samples underwent screening for *Brucella* agglutinins through the Rose Bengal Plate Test (RBPT), with a sensitivity of 98.1 % and specificity of 99.8 % from the Veterinary Laboratories Agency in New Haw, Addlestone, Surrey, KT15 3, UK. Subsequently, the Complement Fixation Test (CFT) from the same agency, with a sensitivity of 96 % and a specificity of 99.8 %, was applied as a confirmatory test for all RBPT-positive sera to identify antibodies against *Brucella abortus*. The estimation of reagent quantity was conducted through titration, adhering to the OIE-recommended method [42]. To identify antibodies against *Coxiella burnetii* in cattle serum samples, we utilized the same indirect ELISA kit. Specifically, the commercial Q-fever antibody ELISA test kit from IDvet, known as the ID Screen®Q fever Indirect Multi-Species kit, located at 310 rue Louis Pasteur-Grabels, France, was employed. This kit demonstrates a sensitivity and specificity of 100 % for detecting *Coxiella burnetii* inactivated phase I and phase II antigens. For cattle sera, a positive threshold value in the iELISA (S/P ratio) was defined as exceeding 50 %. To identify antibodies against Leptospira interrogans sensu lato serovar Hardjo in cattle serum, an indirect ELISA was performed using the PrioCHECK® L. Hardjo Ab kit from Lelystad, Netherlands. This kit boasts a sensitivity of 100 % and a specificity of 97.09 %. The serum samples' corrected OD450 values were presented as a percentage positive (PP) in relation to the reference serum 1 corrected mean OD450 value discovered in wells C1 and D1. A threshold of greater than or equal to 45 % was established as the positive cut-off value (PP) for determining the presence of a specific antibody.

2.6. Data collection

The study considered variables for each cattle, including age, breed, body condition, and parity. Additionally, environmental and management-related factors such as calving season, herd size, herd type, agro-ecology, species composition, introduction of new cattle, contact of cattle with a dog, breeding method used, and management system were documented. Richard [43] identified two types of management systems: extensive and semi-intensive. The assessment of cattle body condition involved classifying it into three groups based on the visibility of ribs and vertebral spinous processes: poor (scores 1 and 2), medium (score 3), and good (scores 4 and 5) [44]. To determine the estimated age at first calving for cattle in tropical environments (ranging from 24 to 36 months), the cattle were segregated into three age brackets: less than three, three to six, and greater than six [45]. Furthermore, herd sizes were divided into three categories: small (<15 heads of cattle), medium (15–30 heads of cattle), and large (>30 heads of cattle). Cattle sharing barns were regarded as a unified herd [46,47]. They were classified into three groups according to their parity: nulliparous (parity 0), monoparous (parity 1), and pluriparous (parity >2) [48]. Cattle with at least one reproductive disorder, such as abortion, dystocia, retained placenta, repeat breeding, stillbirth, estrus problems, vaginal prolapse, or other issues, were identified as cattle positive for reproductive disorders [49].

2.7. Data management and analysis

Microsoft Excel 2010 for Windows was employed to document and store the acquired data, while STATA version 14.0 was utilized for the analysis. The prevalence of reproduction disorders in cattle was determined by dividing the number of cattle with reproduction disorders by the total number of cattle sampled. EpiTools statistical calculators, accessible at https://epitools.ausvet.com.au/trueprevalence, were employed to compute the apparent and true seroprevalence of *Coxiella burnetii*, Leptospira interrogans serovar Hardjo, and *Brucella species*, along with their respective 95 % confidence intervals (CIs). Similarly, the prevalence of reproductive disorders had its 95 % confidence interval determined using the Epitools binomial exact method. Analyzing the connection between reproductive disorders and Leptospira Hardjo, *Brucella* spp, *Coxiella burnetii*, as well as potential risk factors related to reproductive disorders involved utilizing a logistic regression model. Initially, a univariable random effect logistic regression analysis was employed to assess various risk factors (such as age, type of breeding, and cattle-dog contact) associated with reproductive disorders. The identification of risk factors for reproductive disorders and the relationship between Leptospira Hardjo, *Brucella* spp, and *Coxiella burnetii* with reproductive disorders was accomplished through a multivariable random effects logistic regression model. Variables with a p-value of 0.05 or less in the univariable analysis were incorporated into the multivariable logistic model. The backward elimination procedure with an LR-test at a threshold of 0.05 was utilized for variable selection. The strength of associations between variables was assessed using adjusted odds ratios. Before constructing the final model, potential interaction effects and collinearities among variables were examined using cross-product terms and a collinearity matrix index, respectively. The model's validity was assessed through the Hosmer-Lemeshow test, and the predictive power was confirmed using the receiver operating characteristic

Table 1
Distribution of prevalence of cattle reproductive disorder in the southwest Ethiopia.

Study areas	Total animal examined	Total animals positive	Prevalence (%) (95 %CI)
Sheko	171	54	31.58 (24.61–38.55)
Shei Bench	118	21	17.80 (10.90–24.70)
Semen Bench	44	9	20.45 (8.54–32.37)
Meinet Shasha	62	12	19.35 (9.52–29.19)
Meinet Goldiya	66	20	30.30 (19.22–41.39)
Overall	461	116	25.16 (21.20–29.12)

CI: Confidence Interval.

(ROC) curve. A confidence level of 95 % and a p-value of 0.05 were applied to all analyses.

3. Results

In the present study, a thorough examination was conducted on a total of 461 cattle. Of these, 25.16 % (95 % CI: 21.20–29.12) were identified as having at least one reproductive disorder. The Sheko district exhibited the highest prevalence of reproductive disorders at 31.58 %, while the Shei Bench district in southwest Ethiopia had the lowest prevalence at 17.80 %. Notably, a significant disparity ($P < 0.05$) was observed among the various study areas, as indicated in Table 1.

The prevalent reproductive disorder in the studied area was abortion, accounting for 22.56 %. This was followed by the retained placenta at 4.12 %, and repeated breeding at 3.47 %, as depicted in Fig. 2.

The southwest area of Ethiopia exhibited the highest seroprevalence of Leptospira serovar Hardjo at 24.48 %, with *Coxiella burnetii* at 8.68 % and brucellosis at 7.59 %, as indicated in Tables 2–4.

In the study areas, the presence of antibodies indicating infection with *B. abortus*, *C. burnetii*, and L. Hardjo, as well as concurrent infections with these three pathogens, showed a significant correlation with cattle abortion ($P < 0.05$). Furthermore, the likelihood of dystocia in cattle was notably affected by L. Hardjo seropositivity and concurrent infection with *C. burnetii* ($P < 0.05$). Seropositivity for co-infections involving *C. burnetii* and L. Hardjo, *C. burnetii* and *B. abortus*, and L. Hardjo and *B. abortus* were related to the occurrence of retained fetal membranes in cattle ($P < 0.05$). Additionally, only *B. abortus* seropositivity demonstrated an association with repeated breeding in cattle. However, the seropositivity of *B. abortus*, *C. burnetii*, and L. Hardjo, along with their co-infection in cattle, did not exhibit a significant association with reproductive disorders such as stillbirth, anoestrus, and vaginal prolapse (Table 5). Among cattle, those with the highest seroprevalence of L. Hardjo (8.89 %), *C. burnetii* (4.34 %), and *B. abortus* (2.39 %), as well as co-infections of *C. burnetii* and L. Hardjo (3.04 %), displayed the highest prevalence of reproductive disorders in the study areas. Similarly, the seroprevalence of co-infections between *C. burnetii* and *B. abortus* (1.08 %), L. Hardjo and *B. abortus* (0.65 %), and co-infections involving all three pathogens (1.52 %) were documented in the study areas (Table 6).

In a univariable logistic regression analysis, we examined the prevalence of reproductive disorders in naturally bred cattle as compared to artificially bred cattle. The results revealed a higher prevalence of reproductive disorders in naturally bred cattle (25.77 %) compared to artificially bred cattle (23.08 %). The odds of reproductive disorders in naturally bred cattle were approximately three times higher ($OR = 2.9$; $p < 0.05$) than in artificially bred cattle, and this disparity was statistically significant. Moreover, it was noted that cattle exposed to dogs exhibited a higher prevalence of reproductive disorders (28.52 %) in comparison to those not exposed to dogs (20.42 %). This discrepancy demonstrated statistical significance ($P < 0.05$). In terms of reproductive disorders prevalence across different parity groups, monoparous cattle had a prevalence of 21.78 %, nulliparous cattle had a prevalence of 12.73 %, and pluriparous cattle exhibited the highest prevalence at 32.0 %. The variation between these groups was statistically significant ($P < 0.05$) according to univariate logistic regression analysis. Pluriparous cattle displayed an odds ratio (OR) of 3.2, signifying a threefold higher likelihood of reproductive disorders compared to nulliparous cattle. Moreover, the prevalence of reproductive disorders was notably higher (31.11 %) in cattle aged six years and older, demonstrating a statistically significant difference ($P < 0.05$). Older cattle exhibited a twofold elevated risk of experiencing reproductive disorders compared to their younger counterparts. Nevertheless, the univariable logistic regression analysis did not identify any significant associations ($P > 0.05$) between reproductive disorders in cattle and various factors such as agro-ecology, herd size, management system, species composition, co-grazing, breed, season of calving, body condition score, or the introduction of new animals, as detailed in Table 7.

The study suggests that the dependent variable does not exhibit multicollinearity or significant interactions. The final multivariate logistic regression model identifies age, *Coxiella burnetii*, Leptospira Hardjo, breeding practices, and contact between cattle and dogs as

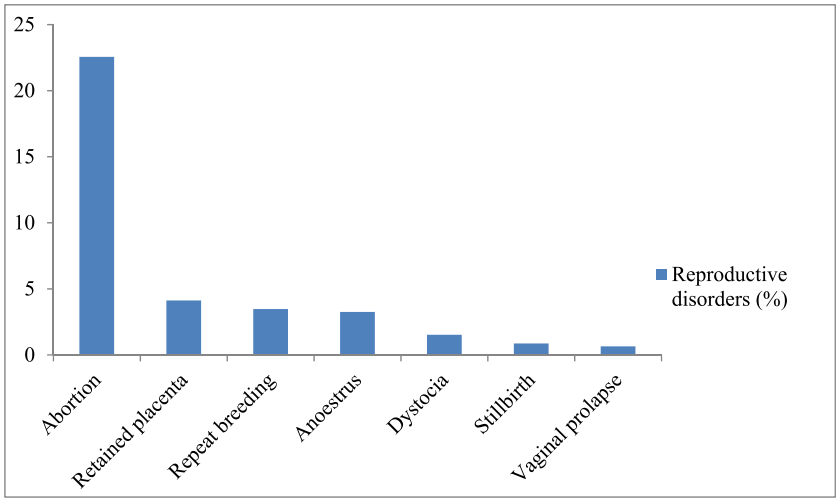

Fig. 2. Major reproductive disorders recorded in the southwest Ethiopia (total sample size were 461 cattle).

Table 2
Apparent and true seroprevalence of brucellosis of cattle in southwest Ethiopia.

Study areas	Total cattle examined	Seroprevalence (%)	95 % CI
Semen Bench	44	4(9.09)	0.60-13.59
Shei-Bench	118	10(8.47)	3.45-13.50
Sheko	171	13(7.60)	3.63-11.57
Meinet Goldiya	62	3(4.84)	0.50-10.18
Meinet Shasha	66	3(4.55)	0.48-9.57
Overall apparent seroprevalence	461	33(7.16)	4.81-9.51
Overall true seroprevalence	461	33(7.59)	5.45-10.48

Table 3
Apparent and true seroprevalence of *Coxiella burnetii* infection of cattle in southwest Ethiopia.

Study areas	Total cattle examined	Seroprevalence (%)	95 % CI
Semen Bench	44	8(18.18)	6.79-29.58
Shei-Bench	118	6(5.08)	1.12-9.05
Sheko	171	15(8.77)	4.53-13.01
Meinet Goldiya	62	7(11.29)	3.41-19.17
Meinet Shasha	66	4(6.06)	0.30-11.82
Overall apparent seroprevalence	461	40(8.68)	6.11–11.25
Overall true seroprevalence	461	40(8.68)	6.44-11.60

Table 4
Apparent and true seroprevalence of Leptospira seroval Hardjo infection in cattle in southwest Ethiopia.

Study areas	Total cattle examined	Seroprevalence (%)	95 % CI
Semen Bench	44	27(61.36)	46.98-75.75
Shei-Bench	118	35(29.66)	21.42-37.90
Sheko	171	34(19.88)	13.90-25.87
Meinet Goldiya	62	12(19.35)	9.52-29.19
Meinet Shasha	66	15(22.73)	12.62-32.84
Overall apparent seroprevalence	461	123(26.68)	22.64-30.72
Overall true seroprevalence	461	123(24.48)	20.48-28.83

Table 5
Relation of seropositivity of *Brucella abortus*, Leptospira Hardjo, *Coxiella burnetii* and mixed infected of those pathogens with cattle reproduction disorders in southwest Ethiopia.

Pathogens	Status	Reproduction disorders						
		Abortion (P-value)	Dystocia (P-value)	Retained placenta (P-value)	Repeat breeding (P-value)	Stillbirth (P-value)	Anoestrus (P-value)	Vagina prolapse (P-value)
Brucella abortus	Positive	0.023[a]	0.699	0.372	0.040[a]	0.899	0.973	0.194
	Negative	–	–	–	–	–	–	–
Leptospira Hardjo	Positive	0.011[a]	0.028[a]	0.556	0.558	0.731	0.626	0.697
	Negative	–	–	–	–	–	–	–
Coxiella burnetii	Positive	0.001[a]	0.097	0.576	0.819	0.396	0.822	0.699
	Negative	–	–	–	–	–	–	–
C. burnetii and L. Hardjo co-infection	Positive	0.004[a]	0.028[a]	0.032[a]	0.612	0.799	0.999	0.987
	Negative	–	–	–	–	–	–	–
C. burnetii and *B. abortus* co-infection	Positive	0.788	0.999	0.035[a]	0.899	0.999	0.799	0.999
	Negative	–	–	–	–	–	–	–
L. Hardjo and *B. abortus* co-infection	Positive	0.593	0.999	0.023[a]	0.999	0.999	0.999	0.999
	Negative	–	–	–	–	–	–	–
Co-infection of three pathogens	Positive	0.025[a]	0.999	0.611	0.999	0.999	0.999	0.999
	Negative	–	–	–	–	–	–	–

[a] Significant difference at 0.05, P-value was obtained from a multivariable logistic regression analysis.

Table 6
Prevalence of reproductive disorders among cattle with single and mixed seropositivity to *B. abortus*, *C. burnetii*, and L. Hardjo in southwest Ethiopia.

Pathogens	Seroprevalence (%)	Reproductive disorder (%)
B. abortus	2.39	9.48
L. Hardjo	8.89	35.34
C. burnetii	4.34	17.24
C. burnetii and L. Hardjo	3.04	12.07
C. burnetii and *B. abortus*	1.08	4.31
L. Hardjo and *B. abortus*	0.65	2.59
Co-infection of three diseases	1.52	6.03

Table 7
Using univariable logistic regression analysis, the risk factors for cattle reproductive disorders in southwest Ethiopia.

Variables	Categories	Total cattle tested	Total cattle positive (%)	Crude OR (CI; 95 %)	P-value
Agro-ecology					0.080
	Lowland	55	10(18.18)	1.3(0.59–2.72)	0.547
	Mid-land	210	63(30.00)	0.7(0.42–1.03)	0.065
	Highland	196	43(21.94)	–	–
Management system	Extensive	366	89(24.32)	1.2(0.75–2.05)	0.412
	Semi-intensive	95	27(28.42)	–	–
Herd size					0.172
	Large	96	17(17.71)	1.7(0.95–3.14)	0.075
	Medium	136	37(27.21)	0.99(0.62–1.60)	0.978
	Small	229	62(27.07)	–	–
Species composition	Mixed	263	64(24.33)	1.1(0.73–1.69)	0.637
	Only cattle	198	52(26.26)	–	–
Co-grazing	Yes	362	84(23.20)	1.6(0.97–2.57)	0.065
	No	99	32(32.32)	–	–
Breed	Cross	39	6(15.38)	1.9(0.79–4.75)	0.148
	Local	422	110(26.07)	–	–
Parity					0.001
	Pluriparous	250	80(32.00)	1.7(0.98–2.91)	0.058
	Monoparous	101	22(21.78)	3.2(1.74–6.00)	0.0001
	Nulliparous	110	14(12.73)	–	–
Age					0.046
	>6 years	135	42(31.11)	2.0(1.56–3.60)	0.013
	3–6 years	188	49(26.06)	1.3(0.79–2.09)	0.320
	<3 years	138	25(18.12)	–	–
Body condition score					0.305
	Poor	89	19(21.35)	1.5(0.83–2.84)	0.172
	Medium	219	52(23.74)	1.3(0.84–2.13)	0.221
	Good	153	45(29.41)	–	–
Type of breeding	Natural	357	92(25.77)	2.9(1.82–4.63)	0.0001
	AI	104	24(23.08)	–	–
Season of calving					0.849
	Autumn	74	20(27.03)	–	–
	Summer	161	43(26.71)	1.0(0.55–1.89)	0.959
	Spring	102	25(24.51)	1.1(0.58–2.21)	0.706
	Winter	124	28(22.58)	1.3(0.65–2.45)	0.480
Introducing new cattle	Yes	176	38(21.59)	1.4(0.88–2.13)	0.166
	No	285	78(27.37)	–	–
Contact of cattle with a dog	Yes	270	77(28.52)	1.7(1.09–2.55)	0.018
	No	191	39(20.42)	–	–

OR: Odds Ratio; CI: Confidence Interval.

significantly associated with cattle reproduction disorders (Table 8). The model adequately explains the data, as evidenced by the Hosmer and Lemeshow's test ($\chi 2 = 6.026$, $P = 0.304$). Additionally, the model demonstrates its predictive accuracy with an area under the ROC curve of 0.766 (95 % CI: 0.617–0.836).

4. Discussion

This research presents data on serological evidence that associates reproductive disorders in cattle in southwest Ethiopia with *Brucella abortus*, *Coxiella burnetii*, and Leptospira Hardjo. The combined effect of these pathogens was also explored in causing such disorders. These agents contribute to severe zoonotic diseases in the country, leading to substantial economic losses attributed to cattle reproductive issues [16,21,28]. The current study revealed a prevalence of 25.16 % for cattle reproduction disorders. Our findings

Table 8
Results of the multivariable logistic regression analysis showing the risk factors for cattle reproductive disorders in southwest Ethiopia.

Variables	Categories	Total cattle tested	Total cattle positive (%)	Adjusted OR (CI; 95 %)	P-value
Age					0.006
	>6 years	135	42(31.11)	2.3(2.03–4.69)	0.002
	3–6 years	188	49(26.06)	1.3(1.65–2.88)	0.032
	<3 years	138	25(18.12)	–	–
Type of breeding	Natural	357	92(25.77)	2.9(1.83–4.82)	0.0001
	AI	104	24(23.08)	–	–
Contact of cattle with a dog	Yes	270	77(28.52)	1.6(1.04–2.53)	0.035
	No	191	39(20.42)	–	–
Coxiella burnetii	Positive	40	20(50.00)	3.0(1.49–5.94)	0.002
	Negative	421	96(22.80)	–	–
Leptospira Hardjo	Positive	123	45 (36.59)	2.9(1.17–4.02)	0.009
	Negative	338	71 (21.01)	–	–

OR: Odds Ratio; CI: Confidence Interval.

indicate higher levels of *Brucella abortus*, *Coxiella burnetii*, and Leptospira Hardjo infections in cattle in the study areas. The higher seropositivity of cattle in the study areas for these pathogens indicates a significant public health risk and substantial economic losses. Factors such as age, breeding practices, and the interaction of cattle with dogs also contribute to an increased probability of reproductive disorders occurring in cattle.

This study helps in the development of effective control strategies for addressing cattle reproduction disorders induced by *Brucella abortus*, *Coxiella burnetii*, and Leptospira Hardjo. While the study did not directly assess specific mitigation measures, it significantly advances the overall comprehension of infections caused by *Brucella abortus*, *Coxiella burnetii*, and Leptospira Hardjo in cattle, particularly in relation to their association with reproductive disorders. The results underscore the significance of adopting suitable control approaches to alleviate these diseases and minimize economic losses in southwest Ethiopia [17]. Furthermore, the results have the potential to contribute to the formulation of interventions, such as vaccination programs, improved breeding practices, and enhanced biosecurity measures. These interventions are aimed at alleviating the impact of *Brucella abortus*, *Coxiella burnetii*, and Leptospira Hardjo on cattle reproductive health in southwest Ethiopia [17,50]. This study, through the identification of risk factors and the raise of awareness regarding zoonotic diseases, establishes a basis for targeted interventions that can enhance cattle health, safeguard public health, and protect the livestock industry in the area. Continued research efforts and collaborations are necessary to develop and assess effective mitigation strategies, building upon the insights gleaned from this study.

The selected studies emphasize infections related to cattle reproductive disorders in East Africa, such as *Brucella abortus*, *Coxiella burnetii*, and Leptospira Hardjo. Factors influencing reproductive disorders encompass age, breeding type, and cattle interaction with dogs [51–54]. Although these studies focus on East Africa, their findings serve as a basis for comprehending the general mechanisms contributing to elevated infections in comparable regions. Furthermore, it is reasonable to extend these risk factors to other areas in East Africa, given the resemblances in climate, livestock practices, and socioeconomic factors. Subsequent research ought to concentrate on exploring the fundamental mechanisms by which these risk factors impact the incidence of infections [55]. This understanding will facilitate the formulation and execution of specific interventions and preventive measures to restrict the spread of *Brucella abortus, Coxiella burnetii*, and Leptospira Hardjo infections in East Africa and potentially elsewhere.

This finding indicates an overall prevalence of 25.16 % for reproductive disorders. The heightened prevalence of reproductive disorders in cattle noted in this study can be related to the higher seroprevalence of *Brucella abortus*, *Coxiella burnetii*, and Leptospira Hardjo in the surveyed areas. The primary contributors to cattle reproductive disorders in Ethiopia are these pathogens [56,57], and one of their clinical manifestations in cattle is reproductive disorders [58]. Comparable prevalence was reported by Abunna et al. [59], and Tigabneh et al. [60] reported a prevalence of 30.1 % in central Ethiopia and 39.8 % in northeast Ethiopia, respectively. In northeast India, a comparable prevalence of 33.9 % was also documented [61]. However, the prevalence identified in our study is less than the figures reported by Ayisheshim et al. [62] and Tolosa et al. [6], who recorded reproductive disorder prevalence of 61.9 % and 66.15 % in northwest and southeast Ethiopia, respectively. Variations in prevalence may arise due to differences in production systems, breeds, causes of reproductive disorders, and environmental factors among various locations.

In the current study, a notable association was observed between L. Hardjo seropositivity and cattle reproduction disorders. Cattle that tested positive for L. Hardjo exhibited about three-fold higher likelihood (OR = 2.9) of experiencing reproduction disorders compared to their L. Hardjo seronegative counterparts. *Leptospira* bacteria persist in active circulation primarily owing to its elevated seroprevalence. The presence of rodents, commonly found in tropical environments without adequate control measures, serves as a source of the disease [63]. This aligns with previous research indicating that leptospirosis significantly contributes to cattle reproduction disorders [62,64–66]. Furthermore, cattle with reproductive issues within herds can potentially act as sources of infection during subsequent parturitions through the discharge from the reproductive tract [67]. Our investigation revealed that among the various infectious diseases that can lead to reproductive issues in cattle, L. Hardjo emerges as a particularly noteworthy factor in the studied areas. In the present study, an association was found between Leptospira Hardjo seropositivity and dystocia, as well as abortion, consistent with the observations made by Tilahun et al. [15], Nthiwa et al. [18], and Desa et al. [16]. Furthermore, veterinary literature, such as Radostits et al. [58], supports these observations by indicating that leptospirosis often manifests clinical features like abortion and other reproductive disorders.

There was a notable association observed between the prevalence of *Coxiella burnetii* and reproductive issues in cattle. Cattle testing

positive for *C. burnetii* antibodies were three times more likely (with an odds ratio of 3.0) to experience reproductive disorders compared to those without the antibodies. This research identified a connection between *C. burnetii* prevalence and reproductive problems in cattle. Infected cattle serve as carriers for the pathogen, posing a potential risk of transmitting the infection to at-risk populations, including farmers, animal handlers, veterinarians, and abattoir workers. Transmission can occur through close contact with infected cattle, involving exposure to their contaminated milk, urine, feces, or reproductive fluids [65,68–70]. The results of this study align with previous research that highlights *C. burnetii* as a notable organism associated to issues in cattle reproduction [25,63,71]. Additional studies have similarly shown a connection between *C. burnetii* and disorders in cattle reproduction [65,69,70,72]. It is important to note that *Coxiella burnetii* is a significant zoonotic pathogen in Ethiopia, but the country tends to overlook infections caused by this organism, considering it a neglected disease [13,28,29].

Mixed infections of *Brucella abortus*, Leptospira hardjo, and *Coxiella burnetii* were found to have a strong association with cattle abortion. The presence of reproductive disorders, including abortion, dystocia, and retained fetal membranes, was also associated to mixed infections of *C. burnetii* and L. hardjo. The study further revealed that mixed infections involving *C. burnetii* and *B. abortus*, as well as those involving L. hardjo and *B. abortus*, significantly correlated with the occurrence of retained fetal membranes. Consistent with this investigation, Okumu et al. [52] illustrated that serological evidence of mixed infections among abortifacient agents in cattle increases the likelihood of their synergistic effects resulting in reproductive disorders in cattle**.** The current study identified various factors contributing to reproductive disorders in cattle, and these findings align with prior research. Specifically, this study found that seropositivity to *B. abortus* was the sole infectious agent related to repeated breeding in cattle, consistent with veterinary literature that highlights infertility as a key clinical characteristic of *Brucella* organisms in cattle [58].

Similar to the previous findings, this study indicates that cattle in contact with dogs face a doubled risk of reproductive disorders (odds ratio = 1.6; $p < 0.05$) compared to those without such contact. This is likely due to the fact that while cattle serve as intermediate hosts for *Neospora caninum*, dogs are its exclusive definitive hosts. Infection in cattle occurs when they consume contaminated feed and water containing oocysts shed from dog feces [73,74]. These results align with earlier research conducted in Ethiopia [75,76] and elsewhere [77,78], demonstrating a significant association between neosporosis and the prevalence of reproductive disorders in cattle with access to dogs. The findings presented by Bahari et al. [79] and Kardjadj [80], indicating frequent contact between cattle and dogs experiencing *Leptospiral* abortion, find additional support in these results. This correlation may be attributed to the prevalence of *Leptospiral* reproductive disorders (canicola infection) in areas where dogs act as the primary reservoir. Additionally, the potential for cross-infection between cattle and dogs exists, with cattle potentially contributing to the natural maintenance of the canicola serovar [81]. Likewise, Soomro et al. [82] noted an increased risk of reproductive disorders in cattle exposed to stray dogs due to the transmission of brucellosis. This transmission occurred through the handling of placentas from birth, deceased or aborted calves, and other bodily fluids, leading to the dissemination of brucellosis [83].

The results of this study reveal a noteworthy association ($P < 0.05$) between the utilized breeding methods and the occurrence of reproductive disorders. Cattle bred through natural means exhibited a 2.9 times higher odds ratio for encountering reproductive disorders when contrasted with those subjected to artificial insemination (AI). One plausible explanation is the heightened susceptibility to disease transmission from an infected bull to a female during natural mating in contrast to artificial insemination. This finding aligns with the findings of Ayisheshim et al. [84] and Tulu and Gebeyehu [49], who observed a higher prevalence of reproductive disorders in naturally bred animals compared to those produced through artificial insemination. Likewise, studies by Regassa and Ashebir [33] and Tolosa et al. [6] reported an increased likelihood of reproductive disorders in cattle bred naturally compared to those bred through artificial insemination.

The present findings reveal a significant association between age and the prevalence of reproductive disorders linked to seropositivity of *C. burnetii*, *B. abortus*, and L. Hardjo. Older cattle exhibited a five fold higher susceptibility (odds ratio = 2.3; $p < 0.05$) to reproductive disorders in comparison to their younger counterparts. This heightened vulnerability among older cattle may be attributed to their comparatively slower recovery from infections and increased susceptibility, stemming from weakened immune systems and prolonged exposure to pathogenic infections [85]. Additionally, older animals are more prone to these pathogens than their younger counterparts due to age-related elevations in sex hormone and erythritol concentrations, fostering the growth and proliferation of bacteria [58]. These findings align with those of Khan et al. [61] and Tesfaye and Shamble [32], highlighting age as a risk factor for reproductive disorders in cattle. Consistent with the observations of Tolosa et al. [6], older cattle exhibit a higher likelihood of encountering reproductive disorders compared to their younger counterparts.

However, various infectious diseases pose a risk of causing reproductive disorders in cattle in the country. Examples include listeriosis, bovine viral diarrhea, neosporosis, mycotic abortion, and others [63]. Based on our research, we have identified leptospirosis, brucellosis, and coxiellosis, along with their interactions, as the primary contributors to cattle reproduction disorders in the study areas. Additionally, potential factors contributing to reproductive problems in cattle encompass endoparasites, nutritional deficiencies, physical injuries, exposure to toxins, seasonal variations, and genetic disorders [58]. Additional research is required to gain a comprehensive understanding of these results. It is crucial to acknowledge that the detection of antibodies in cattle does not necessarily imply current infection at the time of sample collection. To verify the presence of the infecting serovar, it is necessary to conduct isolation and molecular characterization of *Brucella abortus*, *Coxiella burnetii*, and Leptospira Hardjo. Moreover, the study's cross-sectional design restricts the ability to analyze how variations in the incidence patterns of these infections and abortions over time may be represented.

5. Conclusion

This investigation found three pathogens related to reproductive disorders in cattle, underscoring their significant role in causing

production losses in the studied areas. The main reproductive issues stemming from *Brucella abortus*, Leptospira hardjo, *Coxiella burnetii*, and their combined infections encompassed abortion, retained fetal membranes, repeat breeding, and dystocia. Cattle exhibiting positive results for Leptospira Hardjo and *Coxiella burnetii* antibodies were found to be more susceptible to reproductive disorders. Moreover, the study identified older age, natural breeding, and contact with dogs as factors that raise the probability of cattle reproductive disorders. The results emphasize the substantial influence of zoonotic diseases like *Brucella abortus*, *Coxiella burnetii*, and Leptospira Hardjo on cattle production, particularly in relation to reproductive disorders. Raising awareness about the transmission of these pathogens as zoonotic diseases is crucial, and there is a need for the development and implementation of effective control measures. Additionally, conducting further research is essential to pinpoint and isolate potential causes of cattle reproductive disorders in various areas.

Data availability statement

The corresponding author will provide the data used in the current study upon reasonable request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Dereje Tulu Robi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Ararsa Bogale:** Writing – review & editing, Methodology, Investigation. **Beksisa Urge:** Visualization, Supervision, Resources, Project administration. **Melkam Aleme:** Data curation, Methodology, Software, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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