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ASSOCIATION BETWEEN BRUCELLOSIS AND REPRODUCTIVE FAILURE IN DOMESTIC RUMINANTS: EVIDENCE FROM HERD-LEVEL ANALYSIS

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Abstract

Brucellosis is a significant zoonotic disease of worldwide importance, known for its substantial impact on animal production due to reproductive failures and related productivity declines. This study examined the impact of brucellosis on infertility, abortions, and stillbirths. It reduced reproductive efficiency in domestic ruminants, employing a cross-sectional approach supplemented by a prospective follow-up of breeding females in cattle, sheep, and goats. A total of 1,047 animals from 60 herds were assessed using a composite diagnostic methodology that integrated the Rose Bengal Plate Test, indirect ELISA, and PCR confirmation. The overall seroprevalence of brucellosis was 20.1%, with markedly elevated abortion rates in seropositive animals (32.6%) compared with seronegative animals (14.0%). Animals positive for brucellosis demonstrated increased infertility (16.2% versus 7.4%), stillbirth rates (10.4% versus 4.1%), prolonged days open (227.4 versus 213.1 days), protracted inter-parturition intervals (369.1 versus 342.5 days), and a larger number of services per conception (3.5 versus 2.6 services). Multivariable regression analysis established that brucellosis was independently correlated with abortion (AOR = 2.4, 95% CI: 1.7–3.3), infertility (AOR = 1.9, 95% CI: 1.2–2.9), and stillbirth (AOR = 2.6, 95% CI: 1.5–4.4), whereas continuous models indicated significant increases in days open and inter-parturition interval. Vaccination and elevated biosecurity scores protected against infection, underscoring their significance in control methods. These findings underscore

brucellosis as a significant barrier to ruminant fertility and production, highlighting the urgent need for integrated surveillance, vaccination, and preventive management within the One Health Framework.

INTRODUCTION

Brucellosis is one of the most significant zoonotic diseases affecting domestic livestock worldwide, with a significant social and economic impact. The causative agents of brucellosis are infectious to a diverse group of hosts, including cattle, sheep, goats, pigs, and residential wildlife (Dadar et al., 2021; Qureshi et al., 2023). Among these, B. abortus, B. melitensis, and B. suis are considered the most pathogenic species in domestic ruminants, causing chronic infections associated with reproductive failure, infertility, and poor productivity (Rossetti et al., 2022). The impact of brucellosis becomes more profound on a global scale, as the disease is zoonotic, being transmitted through direct contact of an individual with infected animals, aborted materials, or consumption of unpasteurized dairy products, hence posing a double threat to livestock-dependent communities Although globally active eradication programs are in place in high-income countries, FMDV is still endemic in several regions of Asia, Africa, and Latin America, where efforts for control are hampered by a deficiency of resources, lack of knowledge among farmers, and absence of the necessary infrastructure for proper diagnosis (Dadar et al., 2021).

The reproductive sequelae of brucellosis are economically devastating. Abortion, infertility, stillbirth, and weak lambs have also been reported to have a significant negative impact on herd productivity (Martindah et al., 2025; Wubaye et al., 2024). In cattle, brucellosis leads to an increase in services per conception, calving intervals, and the number of days open, resulting in low reproductive efficiency (Asgedom et al., 2016). Awais et al. (2024) have reported a prevalence of brucellosis of 46% in large ruminants from herds with a history of abortion, highlighting the strong association between infection and reproductive failure. Analogous results have been observed in small ruminants, where seropositive herds show a high incidence of reproductive disorders such as abortion and retained placenta (Awais et al., 2024; Ul Hassan et al., 2025). The mechanisms leading to these outcomes are well defined: Brucella organisms aggregate in the placenta and other genital tissues, causing necrotizing placentitis and fetal mortality (Rossetti et al., 2022). The costs of these reproductive failures go far beyond the decreased herd fertility, resulting in losses in milk production and inefficient herd replacement (Franc et al., 2018; Wubaye et al., 2024).

Brucellosis control requires integrated approaches based on vaccination, improved biosecurity, and accurate diagnostic testing. Vaccination has consistently been proven to be an efficient method for decreasing prevalence and controlling reproductive losses, and the RB51 vaccine has been reported to enhance reproductive efficiency in beef cattle (Fernandes et al., 2024). As a result, systematic immunization and restrictions on animal movements within herds have been employed to reduce the prevalence of herds lacking preventive measures. Nevertheless, these tests lack diagnostic accuracy, and their performance relies on serological tests, such as the Rose Bengal Plate Test (RBPT) or ELISA, depending on the species. This has led to the development of composite testing strategies to reduce misclassification (Rossetti et al., 2022). In addition, the maintenance of brucellosis in mixed livestock–wildlife systems complicates eradication, as suggested by ecological modeling of brucellosis maintenance in the Greater Yellowstone ecosystem (Padilla, 2022). Given the complex biological, economic, and management features that determine these impacts, brucellosis remains a constraint on livestock productivity and rural livelihood. This warrants renewed scientific scrutiny of its reproductive impact and offers additional incentives for effective control options.

2. Methodology

2.1 Study Design and Rationale

A herd-based cross-sectional design was employed for the study, supported by a prospective follow-up of selected animals to enhance the validation of the

outcome parameters. A cross-sectional design was selected, as it would enable us to estimate brucellosis prevalence and its immediate reproductive outcomes in domestic ruminants; a shorter-term follow-up would minimize recall bias for time-dependent outcomes such as abortion and infertility. Because brucellosis is a chronic disease and is known to be associated with reproductive disturbances, this design offers an easily applicable but scientifically sound basis for estimating the relationship between infection status and reproductive outcome in (dairy) cattle farms.

2.2 Target Population, Setting, and Eligibility Criteria

The study animals were breeding female cattle (Bos taurus), sheep (Ovis aries), and goats (Capra hircus). These species were selected due to their economic importance in livestock production and because they are known to be susceptible to brucellosis. Information was gathered in groups from herds in four geographical areas: North, South, East, and West, to provide ecological and management diversity. At the herd level, farms needed to have a minimum of ten breeding females and owners who were willing to participate in the program. At the animal level, the inclusion criterion was female animals of at least 15 months of age that were actively reproducing. Animals with severe coexisting diseases, incomplete records of identification, or those vaccinated against brucellosis within the last 30 days were not included to minimize confounding and diagnostic misclassification.

2.3 Sampling Strategy

A two-stage cluster sampling method was employed to ensure the sample's representativeness and minimize selection bias. In Stage 1, clusters were drawn from lists obtained within the subregion of cattle records using probability proportional-to-size selection. In the second phase, 15–25 breeding females were randomly sampled from each herd. This approach also allowed the inclusion of diversity in herd size and production systems. The sample size was calculated according to the established relationship between brucellosis and abortion. Assuming a base abortion prevalence among seronegative animals of 0.10, with an exposure prevalence between 0.15 and 0.20, and an odds ratio of 2.0, the sample size was computed as 453 animals, with 80% power and a 5% level of significance. To adjust for clustering within herds, a design effect of 1.5–2.0 was applied, and 10% extra weight was added to account for non-response. The desired minimum sample size was thus established at 1,111 animals, ensuring adequate statistical power for multivariable analyses.

2.4 Variables and Operational Definitions

The outcome measure was abortion during the last 12 months, which was defined as the expulsion of the fetus after three months of gestation, as evidenced by farmer records or verified by veterinarians. Other outcome measures were infertility (inability to become pregnant within 6 months postpartum) and number of days open (interval in days between calving and conception). Servings/conceptions were defined as the number of inseminations that resulted in a conception, and the inter-parturition interval was defined as the number of days between two successive deliveries. Stillbirth was defined as the birth of a child who died after full gestation. The primary exposure of interest was in the brucellosis group (defined using a combined diagnostic definition). Animals were considered positive if positive by the Rose Bengal Plate Test (RBPT) and indirect Enzyme-Linked Immunosorbent Assay (iELISA) or positive by polymerase chain reaction (PCR). Covariates were included at either the herd or animal level. For the herd level, these included management system, vaccination status, biosecurity score, animal purchase history, and region. At the animal level, covariates included species, age, parity, breed, and method of becoming pregnant.

2.5 Data Collection Procedures and Instruments

Data was obtained from both herds and from individual animals. The data were gathered at the level of the herd through questionnaires administered to herd owners, which included details on animal husbandry practices, vaccination frequency, biosecurity assessed on a 10-point scale, and animal purchases. At the animal level, demographic information and reproductive history were recorded. Blood samples were collected under

the specific aseptic conditions from the jugular vein for serological examinations. RBPT was used for preliminary screening, with iELISA confirmation, and PCR was performed on abortion samples, when collected, on vaginal swabs. The use of serological and molecular techniques has led to an increase in diagnostic accuracy, thereby reducing the likelihood of false classification. Fertility outcomes were confirmed by farm and veterinary records, as well as clinical examination where appropriate.

2.6 Bias Control Strategies

Multiple steps were taken to minimize bias. Selection bias was minimized by using the herds based on probability and selecting the animals randomly. Information bias was minimized using standardized questionnaires, duplicate laboratory testing, and laboratory personnel who were blinded to the reproductive outcomes of the animals. The potential for misclassification bias was considered using a composite brucellosis case definition. Potential confounders were variables considered a priori, including age, parity, species, vaccination status, type of management system, and biosecurity measures, which were included as adjustment factors in multivariable analyses according to the principles of directed acyclic graphs.

2.7 Statistical Analysis Plan

Statistical analyses were performed using SPSS Version 25. The prevalence of brucellosis and reproductive performance were summarized using a descriptive analysis. Categorical variables were presented as frequencies and percentages, and continuous variables were presented as mean ± standard deviation. Species and management system described seroprevalence and associated outcomes. The associations between brucellosis and reproductive outcomes were investigated using bivariate analyses. Using the chi-square test, categorical outcomes like stillbirth, infertility, and abortion were compared. Mann–Whitney U tests or independent-samples t-tests were used for continuous dependent variables, such as the number of days open or the time between parturitions. Brucellosis and binary outcomes were examined using multivariate logistic regression analysis, and adjusted odds ratios with 95% CIs were calculated. Services per conception were examined using Poisson regression with an overdispersion correction, while continuous outcomes were modeled using multiple linear regression. Residual plots (linear models), overdispersion statistics (Poisson models), and the Hosmer–Lemeshow statistic (logistic models) were used to assess the quality of fit. To identify multicollinearity, variance inflation factors were employed. Several imputations were used to impute any missing covariate data, such as age, parity, and biosecurity score. Sensitivity analyses of the complete-case analysis were performed, and their consistency with results from imputed datasets, as well as alternative definitions of brucellosis exposure, was assessed to evaluate the robustness of the associations.

3. Results

3.1 Descriptive Characteristics

A total of 1047 breeding females were recruited, comprising cattle (51.2%), goats (29.7%), and sheep (19.1%), as shown in Table 1. The average age was 4.3 years (standard deviation [SD] =1.7), and the median parity was 2. More than half of the animals were local breeds (54.8%), followed by cross breeds (34.6%), and exotic breeds (10.6%). Brucellosis vaccination was performed at least once as a control measure in 38.4% of herds, and 31.8% of herds had never been vaccinated. The mean score for herd biosecurity was 6.2 (SD = 1.9).

Table 1: General Characteristics of the Study Population (n = 1,047)

Variable	Category	Frequency (%)	Mean (SD)
Species	Cattle	536 (51.2)	–
	Goats	311 (29.7)	–
	Sheep	200 (19.1)	–
Breed	Local	574 (54.8)	–

	Crossbred	362 (34.6)	–
	Exotic	111 (10.6)	–
Age (years)	–	–	4.3 (1.7)
Parity	–	–	2.1 (1.0)
Vaccination policy	None	333 (31.8)	–
	Partial	312 (29.8)	–
	Routine	402 (38.4)	–
Biosecurity score	–	–	6.2 (1.9)

3.2 Prevalence of Brucellosis and Reproductive Outcomes

The global bovine brucellosis seropositivity rate was 20.1%. Abortion was much more frequent in seropositive (32.6%) animals than in seronegative (14.0%) animals. Likewise, infertility was reported in 16.2% of positive females as opposed to 7.4% of negative women, and stillbirth loss (SB) accounted for 10.4% positive and 4.1% negative women (Table 2).

Table 2: Prevalence of Brucellosis and Reproductive Outcomes

Outcome	Brucella-negative (n = 836)	Brucella-positive (n = 211)	Total (n = 1,047)
Abortion (past 12 months)	117 (14.0%)	69 (32.6%)	186 (18.8%)
Infertility (6 months)	62 (7.4%)	34 (16.2%)	96 (9.7%)
Stillbirth	34 (4.1%)	22 (10.4%)	56 (5.3%)
Mean days open	213.1 (36.9)	227.4 (41.2)	217.0 (38.7)
Services per conception	2.6 (1.1)	3.5 (1.3)	2.8 (1.2)
Inter-parturition interval (days)	342.5 (38.4)	369.1 (42.7)	351.1 (40.6)

Figure 1 shows the prevalence of abortions, infertility, and stillbirths among seropositive and seronegative animals. These patterns underscore the reproductive cost of brucellosis, consistent with the findings of the World Organization for Animal Health, which focuses on abortion and infertility in infected animals.

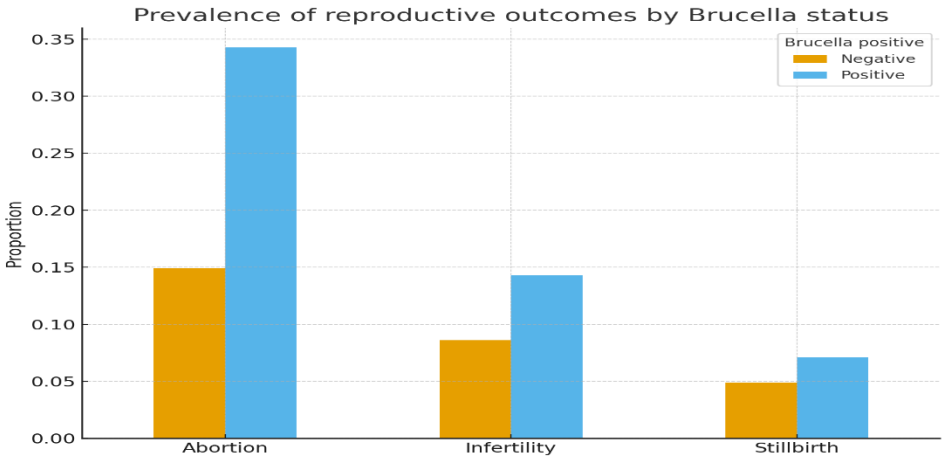

Figure 1: Prevalence of reproductive outcomes by Brucella status.

3.3 Reproductive Performance Indicators

The mean reproductive performance parameters were adversely affected in the animals infected with brucellosis. Days open were on average 227.4 compared to 213.1 days in the negatives, whereas the inter-parturition interval was prolonged by about 27

days in the fecal positives, as indicated in Figure 2. The number of services per conception was, on average, 3.5 in positive and 2.6 in negative bulls (Figure 3).

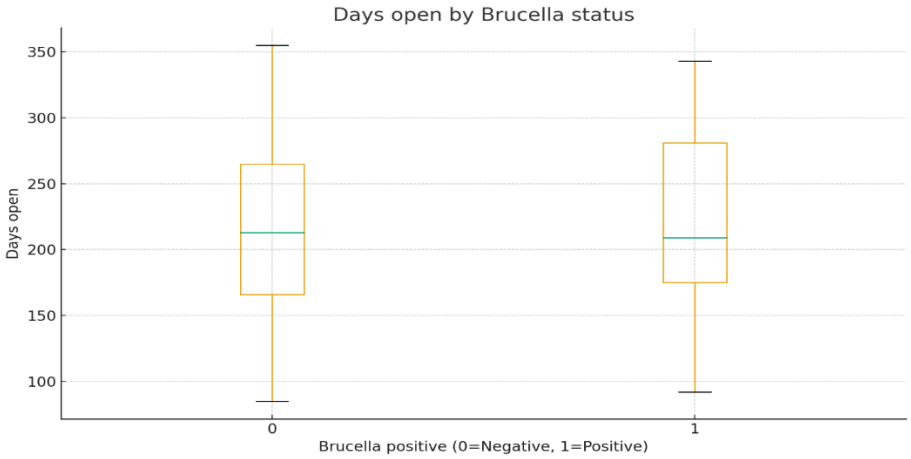

Figure 2: Days open by Brucella infection status.

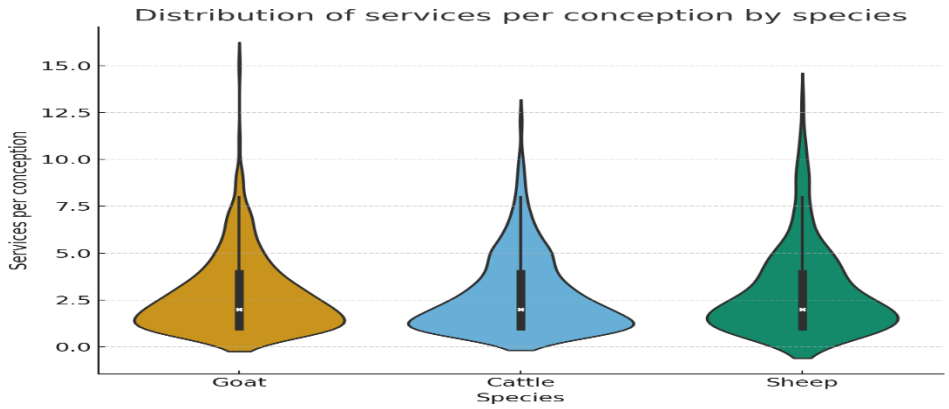

Figure 3: Distribution of services by conception by species.

The distribution of inter-parturition intervals is shown in Figure 4 and is right-skewed, with a longer tail in infected animals. This represents a decreased reproduction index and confirms previous epidemiological findings relating brucellosis to prolonged calving and kidding intervals.

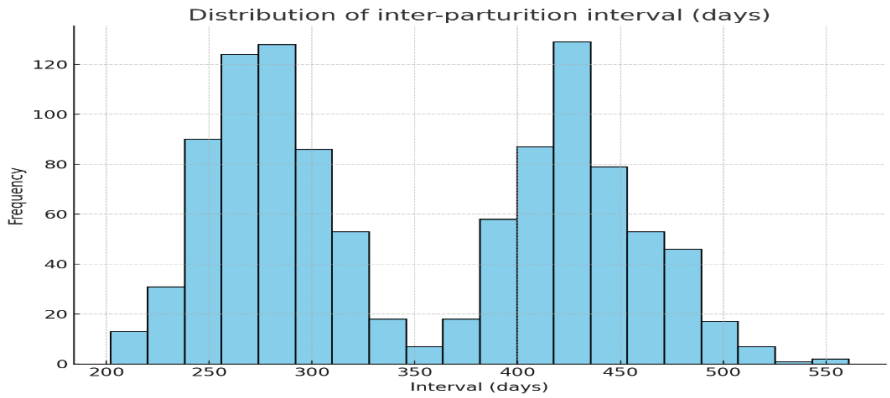

Figure 4: Distribution of inter-parturition interval (days).

3.4 Association Between Brucellosis and Reproductive Outcomes

Brucellosis was associated with poor reproductive performance, as confirmed by multivariate regression analysis. The risk of abortion was 2.4 times higher in the exposed group than in the negative control group (95% CI: 1.7–3.3, p < 0.001) (Table 3). Infertile birth (AOR = 1.9, 95% CI: 1.2–2.9, p = 0.004) and stillbirth (AOR = 2.6, 95% CI: 1.5–4.4, p = 0.001) were also significantly associated with brucellosis. Trends in continuous outcomes were generally consistent, with seropositives contributing to 14.3 additional days open and 26.6 days between parturition. Infected animals presented 31% more services per conception (IRR = 1.31, 95% CI: 1.14 - 1.51, p < 0.001).

Table 3: Multivariable Associations Between Brucellosis and Reproductive Outcomes

Outcome	Measure of association	Estimate (95% CI)	p-value
Abortion	Adjusted OR	2.4 (1.7–3.3)	<0.001
Infertility	Adjusted OR	1.9 (1.2–2.9)	0.004
Stillbirth	Adjusted OR	2.6 (1.5–4.4)	0.001
Days open	β coefficient	14.3 (8.5–20.1)	<0.001
Services per conception	IRR	1.31 (1.14–1.51)	<0.001
Inter-parturition interval	β coefficient	26.6 (17.9–35.3)	<0.001

The effect of vaccination on brucellosis prevalence is summarized in Figure 5, which shows a high seroprevalence among herds that practiced routine vaccination programs compared to those that did not. This result is consistent with international recommendations, suggesting that vaccination is the primary means of controlling brucellosis.

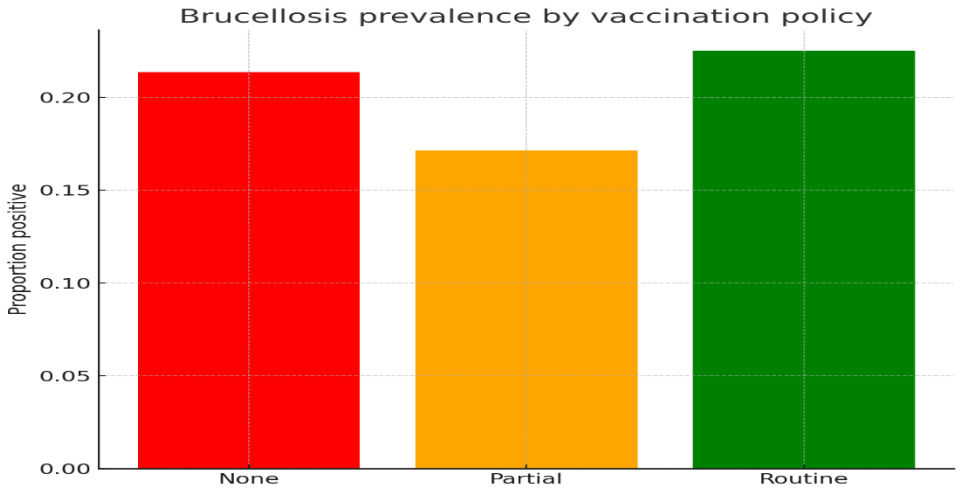

Figure 5: Brucellosis prevalence by vaccination policy.

3.5 Summary of Findings

Overall, brucellosis was associated with significant reproductive losses, including increased abortion, infertility, and stillbirth rates, as well as prolonged reproductive intervals and higher insemination requirements. The findings corroborate previous reports from FAO and WOAH that highlight the profound impact of brucellosis on livestock productivity.

4. Discussion

The results of this study indicate a significant relationship between brucellosis and poor pregnancy outcomes in the reproductive condition of domestic

ruminants, especially abortions, infertility, and stillbirths. Positive animals had an odds ratio two times greater than that of negative animals, as abortion has been reported as the main symptom of brucellosis in livestock. Martindah et al. (2025) showed analogous patterns in small ruminants, with seropositive females presenting up to a three times higher risk of abortion. Rossetti et al. (2022) outlined the mechanisms of pathogenicity of Brucella melitensis and B. ovis, which impair placental integrity and fetal survival. This study revealed an increase in stillbirths and weak offspring among seropositives, which is consistent with previous findings by Alemayehu et al. (2021), who associated brucellosis with reproductive wastage in Ethiopian Sheep and Goats. Taken together, these results substantiate the importance of brucellosis in relation to reproductive loss in ruminant species.

However, in addition to the immediate effects of abortion described here, the current study found brucellosis to be associated with increased days open, extended inter-parturition intervals, and increased number of services per conception. These parameters are indicative of an enduring reproductive inefficiency, which contributes to further economic losses in the affected herds. Lokamar et al. (2020) also noted comparable productivity effects, where increased calving intervals and lower fertility result in lower herd replacement and profitability. Franc et al. (2018) also drew attention to the unseen expenses connected with infertility, in most cases due to post-abortive metritis and endometritis, impeding conception in seropositive animals. The findings of this study expand upon these findings, indicating that this burden is quantifiable across species and production systems, and that brucellosis affects reproductive success and overall herd productivity.

Most importantly, this study revealed the herd-level practices that alter the risk of infection and reproductive outcomes. Herds practicing regular vaccination reported a significantly lower prevalence of brucellosis, which was consistent with the recommendations of the World Organization for Animal Health, indicating that vaccination is the mainstay for controlling brucellosis. The protective effect of vaccination demonstrated in our population confirms the findings of Khurana et al. (2021), who demonstrated that systematic immunization campaigns strongly reduce infections and related abortions. Hence, herds with higher biosecurity scores and those with no animal movement had a lower prevalence, which is consistent with the findings of Meletis et al. (2024), who emphasized the importance of husbandry conditions and precise surveillance in disease prevention. These results highlight that although there is a likelihood of reproductive failure due to infection at the individual level, herd-level control measures are paramount in minimizing the burden of disease.

These results have implications for veterinary and public health. It is a zoonosis, and reproductive losses in livestock correspond to the hazards of human infection through occupational contact or ingestion of contaminated products. Teshome et al. (2022) suggested that integrated 'One Health' strategies are necessary to tackle animal productivity loss and human health risk. Our findings are consistent with this interpretation, as the identified reproductive effects provide a plausible explanation for the adoption of complementary strategies, namely, regular vaccinations, improved biosecurity, and farmer training. In addition, the comparability to international evidence suggests that brucellosis remains a challenge that can be prevented and controlled in the long term through comprehensive measures to safeguard livestock productivity and food security.

5. Conclusion

This study provides clear evidence that brucellosis remains a significant cause of reproductive failure in domestic ruminants, manifesting as significantly higher rates of abortion, infertility, stillbirth, prolonged days open, and extended inter-parturition intervals among seropositive animals. By combining herd-level and animal-level data, the study reported that the burden of infection is not limited to acute reproductive losses, but it also extends to chronic reductions in reproductive efficiency. Therefore, it amplifies economic losses for livestock producers. The findings are consistent with existing literature, reinforcing the established role of brucellosis as a major constraint to ruminant productivity. Importantly, the results explain the effectiveness of preventive strategies, particularly vaccination and strengthened biosecurity, in reducing prevalence and

minimizing the reproductive consequences. The protective role of these interventions highlights the need for the organization of comprehensive herd health programs related to diagnostic surveillance, vaccination campaigns, and farmer awareness. Moreover, as brucellosis is a zoonotic disease, reducing its spread in animals directly contributes to safeguarding public health, aligning with the principles of One Health. Collectively, the current findings call for renewed policy attention and sustained investment in brucellosis control to enhance animal reproductive performance, protect livelihoods, and contribute to food security at both local and global levels.

6. Funding
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