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Epidemiological Study of Brucellosis in Polewali Mandar Regency, Indonesia

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Abstract
This research aims to determine the prevalence rate of brucellosis in the Polewali Mandar Regency (Polman), examine the risk factors associated with seropositive diagnoses, and determine the brucellosis case model at the farm level. The research was carried out through a cross-sectional study and obtained a sample size of 390 sera. Identification of risk factors is based on questionnaire data conducted on around 113 breeders spread across Polman Regency. The data were analyzed using univariate and bivariate descriptive statistics, chi-square, and odds ratio (OR). The brucellosis model at the farm level was analyzed by multivariate logistic regression. The results of this study indicate that the prevalence of brucellosis in the Polman Regency is 27.95%. Risk factors associated with the diagnosis of brucellosis in Polman Regency included a history of abortion, handling of aborted animals, abortion at the end of pregnancy, knowledge about brucellosis, handling of residual abortions, high grazing density, introduction of new livestock, dirty cage, and grazing method. The model for diagnosing brucellosis in Polman Regency is = -2.48923 + 3.38734 history of abortion + 2.06312 abortions at the end of pregnancy. The model shows that the history of abortion and the incidence of abortion at the end of pregnancy can increase the seropositive diagnosis of brucellosis at the farm level. This study demonstrates that Polman is an endemic area for brucellosis with a high prevalence (> 2%) and has the potential to continue increasing with a history of abortion at the end of pregnancy.

Keywords: *Bovine brucellosis; seroprevalence; Polewali Mandar*

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Introduction

Polewali Mandar (Polman) is a regency in West Sulawesi that was previously a part of South Sulawesi until 2004. The Polman Regency is directly adjacent to the South Sulawesi Province. Polman Regency has considerable potential for livestock development with available grazing land (pasture). According to data from the Agriculture and Food Security Office of Polman Regency, the cattle population in Polman Regency was 30,141 heads in 2019.

Brucellosis is a disease related to miscarriage in the final semester of pregnancy and inflammation in the male reproductive organs (1). Brucellosis can affect animals of all age groups but generally persists longer in sexually mature animals (2). *Brucella* bacteria can be found in high concentrations in the uterus of pregnant animals. The abortion of the fetus, the placental membrane and uterine fluid become the primary sources of infection (3).

The main principles of brucellosis eradication include case detection, slaughter of all reactors, vaccination, traffic control, and

disease tracking. Case detection and disease tracing can be accomplished by understanding the various factors associated with the transmission and spread of the disease. Brucellosis in cattle is highly contagious and caused by the *Brucella abortus.* Infection with brucellosis is characterized by miscarriage (abortion) at the end of pregnancy and high infertility rates on farms (4). The primary transmission of brucellosis occurs through ingesting materials or livestock products contaminated with the bacteria *B. abortus*, such as fetuses, placentas, fetal fluid, milk, and more. Bovine sperm infected with brucellosis may also contain the bacteria *B. abortus* (5). Other risk factors include introducing animal reactors for brucellosis, management systems that facilitate livestock interaction, and livestock vaccination rates below 70% (6). Traditional extensive farming systems also facilitate the spread of this disease (7). Transmission through mating can occur due to artificial insemination with semen from infected males with brucellosis (8).

Most farmers in the Sulawesi region, including those in the Polman Regency, still adhere to traditional livestock management practices, employing an extensive or semi-extensive grazing system. Specifically concerning the Polman Regency, in recent years, no vaccinations have been conducted, despite the ongoing seropositive diagnoses for brucellosis in this area. Many farmers opt to retain livestock even after a history of abortion, as abortions are typically limited to the initial pregnancy, and subsequent offspring are typically healthy. Determining brucellosis prevalence and associated risk factors is vital in establishing effective control measures for the disease.

This study aims to determine the prevalence rate of brucellosis in the Polman Regency, examine the risk factors associated with seropositive diagnoses, and determine the brucellosis case model at the farm level. The results of this study are expected to provide information concerning the prevalence and epidemiological characteristics of brucellosis in the Polman Regency. This information can be utilized as a basis for deliberation in establishing control and eradication strategies for brucellosis.

Materials and Methods

The research was conducted from March to May 2023 in the Polman Regency, West Sulawesi, where the total cattle population is 30,141, predominantly consisting of Bali cattle, spread across 16 districts (Figure-1). Prevalence determination was based on a cross-sectional design, with farmers as the epidemiological units. The sampling technique employed a multistage random sampling approach. District-level sampling was determined in relative proportions according to the population in each district. Village and farmer samples were selected through simple random sampling. The sample size calculation followed the formula n = 4PQ / L^2 (10), assuming an estimated brucellosis prevalence in Polman of 8.14% in 2019 (11) with a confidence level of 95%. The sample size tripled to minimize bias and ensure robustness, resulting in 369. Serum collection was carried out in each district proportionate to the cattle population. A 5-milliliter blood sample was collected from each cattle via the jugular vein using a 10 mL syringe. The blood was left at room temperature for 24 hours, after which the serum was extracted using cryovials. Each cryovial containing serum was labeled, and pertinent risk factors such as age, breed, and sex were recorded concurrently during blood collection. The collected serum samples were stored at −20°C until further testing, which involved the Rose Bengal Test (RBT) and the Complement Fixation Test (CFT), conducted at the Disease Investigation Center of Maros (DIC Maros). A serum sample was deemed positive if it tested seropositive in the CFT test.

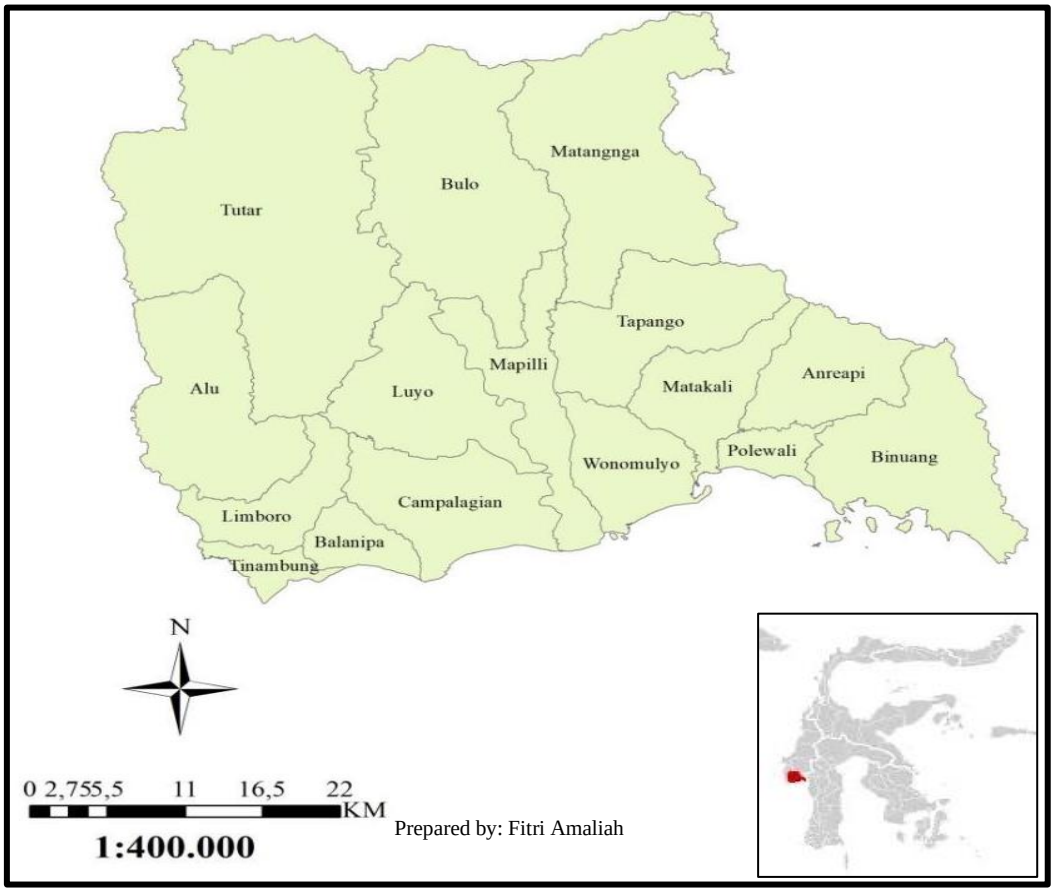

Figure 1. Map of Polman Regency
(Source: Map prepared by the corresponding author)

Risk factors were identified based on data collected through direct interviews with farmers using questionnaires. The validity and reliability of the questionnaire were assessed using IBM SPSS Statistics Version 25.0. The validity of the questions was evaluated using the SPSS Pearson Correlation tool, and the reliability was assessed using the reliability analysis tool. The validity measurement results for the variables indicated a significance level of >0.05, and Cronbach's Alpha value was 0.630, exceeding the threshold of 0.600, confirming the validity and reliability of the variable.

The data obtained through questionnaires in this study were subjected to statistical analysis, including descriptive, univariate, and bivariate analyses using Chi-square and odds ratio (OR) in IBM SPSS Statistics version 25.0. Additionally, multivariate logistic analysis was conducted using Statistix for Windows and IBM SPSS Statistics version 25.0 to establish a statistical model with a significance level of $p>0.05$ and a confidence level of 95%. Concurrently, the model was formulated through multivariate logistic regression analysis, employing a significance level of $p=0.05$ and a 95% confidence level. The resulting model took the form of $Y=\alpha+\beta1X1+\beta2X2 +\ldots\ldots +\beta nXn +e$.

Results and Discussion

Seroprevalence of brucellosis

The RBT and CFT tests on serum samples yielded 109 samples with a seropositive diagnosis out of the 390 samples tested. Consequently, the seroprevalence value for brucellosis in Polman Regency was determined to be (as shown in Table-1): Seroprevalence = 27.95%.

Table-1. Prevalence of seropositivity of brucellosis Polewali Mandar Regency

No.	Districts	Serum samples	RBT		CFT	
			Seropositive	%	Seropositive	%
1	Allu	20	0	0.00	0	0.00
2	Anreapi	12	3	25.00	6	50.00
3	Balanipa	8	1	12.50	1	12.50
4	Binuang	22	1	4.55	0	0.00
5	Bulo	0	0	0.00	0	0.00
6	Campalagian	104	64	61.54	54	51.92
7	Limboro	23	1	4.35	0	0.00
8	Luyo	40	24	60.00	20	50.00
9	Mapili	29	19	65.52	15	51.72
10	Matakali	18	1	5.56	1	5.56
11	Matangnga	0	0	0.00	0	0.00
12	Polewali	6	1	16.67	1	16.67
13	Tapango	32	3	9.38	2	6.25
14	Tinambung	18	0	0.00	4	22.22
15	Tutar	35	1	2.86	3	8.57
16	Wonomulyo	23	2	8.70	2	8.70
	Total	390	121	31.03	109	27.95

RBT: Rose Bengal Test; CFT: Complement Fixation Test

Since none of the cattle in this study received vaccination against brucellosis, seropositivity was attributed to natural exposure. A seropositive diagnosis via CFT was identified in 11 out of 16 districts (68.75%), with the highest proportion of seropositivity observed in districts such as Mapili, Anreapi, Campalagian, Luyo, and Tinambung (Figure-2).

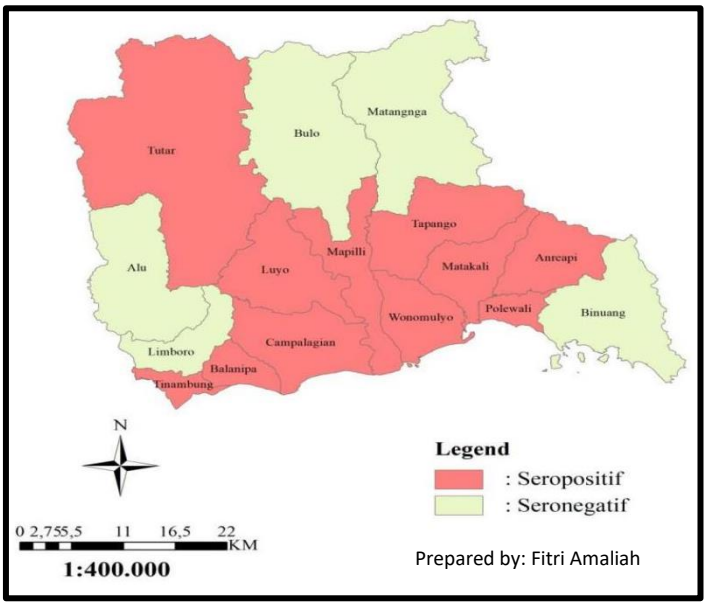

Figure-2. Map of seropositive brucellosis distribution in Polewali Mandar Regency (Source: Map prepared by the corresponding author)

The seroprevalence of brucellosis in Polman Regency was 27.95%. As outlined in the Road Map for Brucellosis Control and Eradication by the Directorate General of Animal Husbandry and Health, Ministry of Agriculture (2015), regions with a prevalence exceeding 2% are classified as heavily infected. West Bandung Regency has also earned this classification, exhibiting a prevalence of 5.10% (12). Similar findings were reported by Wahyuni *et al.* (13), who observed a prevalence of 7.5% in dairy cattle. Notably, Tagueha *et al.* (14) documented a relatively high prevalence (21.74%) in Letti District, Southwest Maluku.

Effective control and eradication measures are imperative, given its classification as a heavily infected endemic area. In this context, recommended strategies include vaccination and the culling of reactor animals.

Univariate and bivariate analysis of risk factors for brucellosis

The results of the univariate and bivariate analysis of brucellosis risk factors in cattle within Polman Regency are presented in Table-2. Variables that exhibit significant influence are indicated by a p-value of <0.05 and a high odds ratio (OR). These influential variables encompass a history of abortion (p=0.000; OR=128), handling of aborted animals (p=0.000; OR=94.667), abortion at the end of pregnancy (p=0.000; OR=62.333), knowledge about brucellosis (p=0.000; OR=9.841), handling of residual abortions (p=0.003; OR=8.471), high grazing density (p=0.000; OR=6.861), introduction of new livestock (p=0.006; OR=5.833), dirty cage (p=0.003; OR=3.600), and grazing method (p=0.005; OR=3.167). Conversely, other variables demonstrated no significant impact on brucellosis (p>0.05), including management systems (p=0.861), breeding (p=0.228), free and open access (p=0.699), farmer's age (p=1.000), breed (p=1.000), and hygroma (p=1.000).

Table 2. Univariate and bivariate analysis of risk factors associated with brucellosis in Polewali Mandar Regency

Variable	Category	Frec.	Seropositif CFT		Chi-square	p-value	OR
			Sample	%			
History of abortion	1. No	35	3	2.80	72.191	0.000	128.00
	2. Yes	78	72	67.29			
Handling of aborted animal	1. Culling	36	4	3.74	68.695	0.000	94.667
	2. Maintained/sold	77	71	66.36			
Abortion at the end of pregnancy	1. Early trimesters	43	9	8.41	60.975	0.000	62.333
	2. Late trimesters	70	66	61.68			
Knowledge about *brucellosis*	1. No	59	27	25.23	21.602	0.000	9.481
	2. Yes	54	48	44.86			
Handling of residual abortions	1. Buried	87	51	47.66	8.724	0.003	8.471
	2. Thrown away	26	24	22.43			
High grazing density*	1. No	44	18	16.82	19.105	0.000	6.861
	2. Yes	69	57	53.27			
Introducing new livestock	1. No	85	50	46.73	7.445	0.006	5.833
	2. Yes	28	25	23.36			
Grazing method	1. Alone	37	18	16.82	6.606	0.010	3.167
	2. Mixed	76	57	53.27			
Dirty cage	1. No	74	56	52.34	7.152	0.005	3.600
	2. Yes	39	19	17.76			
Management systems	1. Intensive	5	4	3.74			
	2. Extensive	108	71	66.36			
Breeding	1. Natural breeding	86	54	50.47			
	2. AI**	27	21	19.63			
Free and open access	1. No	9	7	6.54			
	2. Yes	104	68	63.55			
Farmers age	1. ≦ 40 years	21	14	13.08			
	2. > 40 years	92	61	57.01			
Breed	1. Bali	108	72	67.29			
	2. Mixed breed	5	3	2.80			
Hygroma	1. No	106	70	65.42			
	2. Yes	7	5	4.67			

*>5 farmers on 500 m^2 grazing area

**AI : Artficial insemination

The risk factor associated with a history of abortion exhibits robustness with an OR of 128.00, signifying that livestock having experienced an abortion possess a 128-fold increased likelihood of being diagnosed with seropositive brucellosis compared to cattle without such a history. Research conducted by Terefe *et al.* (18), Aulakh *et al.* (19), Mugizi *et al.* (15), and Asmare *et al.* (20) have collectively demonstrated the significant impact of a history of abortion as a risk factor in influencing the presence of brucellosis. Similarly, Yanti *et al.* (12) reported that the history of abortion significantly contributes to brucellosis in West Bandung (p=0.00; OR=9.9). Abortion is one of the prominent clinical manifestations of brucellosis, alongside retained placenta, stillbirth, infertility, and extended calving intervals (21).

The risk factor of handling aborted animals significantly impacts the seropositivity of brucellosis (p=0.000; OR=94.667). The choice between selling or maintaining cattle with a history of abortion carries a 94.667 times greater risk of elevated brucellosis incidence compared to the decision to cull them. Infection with *B. abortus* in livestock results in abortion, typically transpiring once during the initial pregnancy and becoming recurrent in subsequent pregnancies; however, the livestock remains infected for the duration of its life (26). The occurrence of abortion towards the end of pregnancy was similarly identified as a significant factor impacting the seropositivity of brucellosis (p=0.000; OR=62.333) in this study. Insights drawn from dairy cattle practitioners in Eastern Ethiopia have affirmed that abortions occurring late in gestation correlate significantly with brucellosis incidences on farms (18). The highest proportion (70.97%) of abortions occurs during the 6-9 months of gestation. Additionally, a noteworthy correlation (p=0.000; OR=32.560) was observed between brucellosis and abortions in the third trimester of pregnancy. The notable occurrence of abortions during this stage may be attributed to increased uterine susceptibility due to erythritol, which favors bacterial development (22,23).

Farmers' understanding of brucellosis, specifically *B. abortus*, dramatically influences their awareness and vigilance against the disease. In this study, the risk factor of farmer knowledge demonstrated significant relevance to brucellosis seropositivity (p=0.000; OR=9.841). Farmers with limited knowledge about brucellosis face a risk of 9.841 times higher risk of contributing to brucellosis occurrences than those with sufficient knowledge on the subject. Shome *et al.* (24) similarly highlighted that farmers' awareness regarding brucellosis significantly impacted brucellosis cases (p=0.004; OR=8.224) in India. According to Terefe *et al.* (18), approximately 91% of dairy farmers with inadequate awareness about the disease were associated with causing abortions late in pregnancy.

The handling of residual abortion material also significantly impacts the seropositive diagnosis of brucellosis (p=0.003; OR=8.471). Disposing of abortion material in rivers or pastures carries an 8.471 times higher risk of causing seropositive brucellosis compared to burying the abortion material in the ground. *B. abortus* can be isolated from aborted fetuses (including entrails, spleen, and lungs), placenta, vaginal smears, or milk (24). Consequently, inattentive handling of abortion material can lead to the dissemination of *B. abortus* bacteria. As Terefe *et al.* (18) indicated, discarding aborted material or fetuses in the vicinity contributes to brucellosis infections within herds.

Herds with high grazing density were at a 6.861 times greater risk of being diagnosed with seropositive brucellosis than those with low grazing density. Livestock rearing practices in Indonesia tend to be semi-intensive to extensive, particularly with cattle in the eastern regions. The heightened prevalence of brucellosis on farms can be attributed to several factors, including livestock density, which leads to increased interactions among livestock and, thus, a higher likelihood of disease transmission (28, 29). As highlighted by Terefe *et al.* (18), research also indicated that herd density (>20 livestock) significantly elevates the risk of brucellosis occurrence (p=0.00; OR=9.13).

Introducing new livestock significantly impacts the seropositive diagnosis of brucellosis as a risk factor. An area's vulnerability to diagnosed brucellosis increases by 5.833 when new animals are introduced from other regions. This risk escalates further when no information about the brucellosis status in that area is available. Similar research conducted by Mugizi *et al.* (15) underscored the significance (p=0.027) of introducing new livestock to the seropositivity of brucellosis in Soroti City, Uganda. The introduction of new livestock also emerged as a noteworthy factor with a significant impact on the prevalence of brucellosis in the Fulani population (16) as well as in Portugal (p<0.001) (17).

Dirty cage conditions also significantly impact the incidence of brucellosis (p=0.005; OR=3.600). Inadequate fecal disposal and subpar cage hygiene demonstrated a significant effect (p=0.042; OR=2.87) on brucellosis incidence, as did unclean livestock drinking water containers (p=0.001; OR=3.05) (17).

The grazing system is a significant risk factor for the seropositivity of brucellosis (p=0.010; OR=3.167), with livestock grazing alongside other animals presenting a 3.167-fold greater risk than individual grazing. The findings of the same study also highlighted that communal grazing had a considerable impact on brucellosis incidence (p<0.001) (27). The management system involving communal grazing has been positively linked to an elevated prevalence of brucellosis (6).

Multivariate analysis of risk factors and brucellosis models

Multivariate analysis utilizing logistic regression was applied to brucellosis based on the outcomes of CFT seropositive diagnosis, yielding a case model of -2.489 + 3.387 for the history of abortion and +2.063 for abortions at the end of pregnancy (Table-3). The model indicates that both the history of abortion (β=+3.387; OR=29.588) and abortion at the end of pregnancy (β=+2.205; OR=7.871) contribute to an elevated seropositive diagnosis of brucellosis within the Polman Regency (Table-4).

Table-3. Logistic regression analysis of brucellosis models in Polman Regency

Predictor Variable	Coefficient	Std Error	Coef/SE	P
Constant	-2.48923	0.61888	-4.02	0.0001
Abortion history	3.38734	0.88735	3.82	0.0001
Abortion at the end of pregnancy	2.06312	0.84522	2.44	0.0146
Deviance	57.63			
P-value	1.000			
Degrees of freedom	110			

Table-4. Odds ratio values in the brucellosis model at farm level in Polman Regency

Variable	OR	95% CI	
		Lower	Upper
Abortion history	29.588	5.192	168.604
Age of gestation at abortion	7.871	1.496	41.403

CI = Confidence Interval

The model equation obtained from the multivariate logistic regression analysis is Y = -2.489 + 3.387 for the history of abortion + 2.06312 for gestational age at abortion. This model is deemed feasible as it satisfies the omnibus test value feasibility (p=0.000). Utilizing the Nagelkerke R-square value, an explanatory power of 0.762 was achieved, indicating that the independent variables can account for 76.2% of the variance in the seropositive diagnosis of brucellosis. The model mentioned above exhibited a reasonable degree of accuracy, as demonstrated by the Hosmer-Lemeshow goodness-of-fit test with a sensitivity of 97.33% and specificity of 84.21%. The outcomes of the multivariate analysis revealed that the history of abortion variable was associated with an increase in the seropositive diagnosis of brucellosis (β=+3.38734), as well as a rise in the incidence of abortion towards the end of pregnancy also corresponded to an elevation in brucellosis cases (β=2.06312), both with a significance value of p=0.001 (p<0.05). Clinical manifestations of brucellosis engender issues in the reproductive organs, leading to problems such as abortion, weak birth calves, and infertility (30). The seroprevalence of livestock positively correlated with the incidence of abortion (31).

The findings of other studies also demonstrated that a history of abortion significantly influenced the seropositivity of brucellosis in cattle (p-value <0.001; OR 4.7) (32). Abortion, or miscarriage, refers to the premature release of a nonviable fetus from the womb. Demise occurring at 1-2 months of gestation is called early embryonic death. Both non-infectious and infectious factors can lead to abortion in livestock. Non-infectious factors include physical trauma to the cattle's body, ingestion of plants containing toxins (such as mycotoxins and nitrates), deficiency in vitamins (A and E) and minerals (selenium), heat stress resulting in fetal hypoxia, hypotension, and acidosis. Infectious agents known to induce

abortion in livestock encompass bluetongue caused by Orbivirus, BVD (Bovine Viral Diarrhea), brucellosis due to the bacteria *B. abortus* in cattle, campylobacteriosis due to infection with *Campylobacter fetus veneralis*, Chlamydiosis caused by *Chlamydophila abortus*, epizootic bovine abortion, IBR (Infectious Bovine Rhinotracheitis), leptospirosis due to *Leptospira interrogans*, Listeriosis caused by *Listeria monocytogenes*, mycotic abortion (*Aspergillus, Mucor, Absidia, Rhizopus*), Neosporosis due to *Neospora caninum* infection, Trichomoniasis caused by *Tritrichomonas fetus*, *Truperella pyogenes*, and *Ureplasma diversum* (33, 34).

Abortion attributed to brucellosis predominantly transpires during the mid to late stages of gestation (34). As Putra (35) reported, nearly 97% of miscarriages due to brucellosis transpire in pregnancies exceeding three months (48.5% of miscarriages manifest in pregnancies spanning 4-6 months, and 48.5% in pregnancies spanning 6-9 months). Various researchers have indicated that herds with a history of abortions are correlated with seropositivity (36,37).

Conclusion

The findings of this study reveal a brucellosis prevalence of 27.95% in the Polman Regency. Univariate and bivariate analyses have demonstrated significant associations between seropositive brucellosis diagnosis and various risk factors, including the history of abortion, handling of aborted animals, abortion occurring towards the end of pregnancy, knowledge about brucellosis, handling of residual abortions, high grazing density, introduction of new livestock, unsanitary cages, and grazing methods. The multivariate analysis highlights that the history of abortion and abortion at the end of pregnancy are risk factors that notably increase the likelihood of seropositivity for brucellosis.

Acknowledgments

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Conflict of Interests

The authors declare that they have no competing interests.

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