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Relationship Between Contact with Cattle That Experienced Abortions and Rose Bengal Test Results

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ABSTRACT

Background: Most provinces in Indonesia have yet to be declared brucellosis-free and knowledge of brucellosis infections transmitted to humans is insufficient. Therefore, the spread of *Brucella* infection will be more dominant in community groups with close contact with cattle as disease carriers. **Methods:** This study used an analytical observational design with a cross-sectional approach. The sample was cattle breeder's blood taken from the median cubital vein, as much as 5 ml. The number of samples used was 40. The *Brucella* examination was carried out with the Rose Bengal Test (RBT) and confirmed with Polymerase Chain Reaction (PCR) examination. The samples were grouped based on the contact with cattle that experienced abortions. The relationship between contact and RBT results was analyzed using the Chi-square test. **Results:** (1) There were no cattle breeders that tested positive for brucellosis; (2) As many as 7 (17.50%) cattle breeders had contact with cattle that experienced abortions; (3) 10 breeders showed positive (+) RBT results; (4) PCR results from all breeders' blood samples were negative; (5) The Chi-square test obtained p = 1.000. **Conclusion:** No statistically significant relationship was found between contact with cattle that experienced abortions and RBT results.

Keywords: brucellosis, cattle abortion, cattle breeder

INTRODUCTION

Zoonotic diseases can be caused by viruses, bacteria, parasites, and fungi which cause various types of clinical symptoms in humans and animals, ranging from mild to severe symptoms and even resulting in death. Animals can sometimes appear healthy even when they carry disease germs and can unknowingly infect the human population (CDC, 2021). The incidence of zoonoses is expected to increase in the future. Currently, global society is facing the increasing impact of zoonotic diseases due to environmental degradation, global warming, and progressive urbanization (Zamanian et al., 2020)(Suraya & Mardhiati, 2018). Zoonotic diseases in the United States and the world are prevalent, both emerging and remerging. Scientists estimate that more than six out of every ten infectious diseases found in humans originate from animals, and three out of every four new or emerging infectious diseases in humans come from animals (CDC, 2021).

According to the World Health Organization (WHO) (2020), one of the most widespread zoonotic diseases in the world is brucellosis, ranked as one of the seven neglected diseases. As many as 500,000 incidents of brucellosis in humans are reported each year, but the actual incidence is estimated at 5,000,000 to 12,500,000 cases each year. In developing countries, *Brucella abortus, Brucella melitensis*, and *Brucella suis* are the main causes of animal and

human brucellosis. Meanwhile, in developed countries, the incidence rate of brucellosis is low, for example in the USA, there are only 0.4 cases per 1,000,000 people (Hull & Schumaker, 2018). Several countries, including Syria, are reported to have the highest incidence (1,603.4 cases per 1,000,000 people) compared to other countries that report statistics to WHO, followed by Mongolia, Iraq, Tajikistan, Saudi Arabia, Iran, China, Greece, and Brazil. Brucellosis remains an uncontrolled problem in areas of high endemicity, such as the Mediterranean, Middle East, Africa, Latin America, and parts of Asia (Gupte & Kaur, 2016). With the development of the livestock industry in every country, the threat of zoonoses cannot be avoided; the brucellosis epidemic is one of the zoonoses that is a public health problem in the world. Common routes of infection in humans are the gastrointestinal tract (swallowing infected milk), skin (contact with infected animal tissue), and mucous membranes (droplets), which occur in laboratory workers by inhalation (Gupte & Kaur, 2016). Apart from affecting the development of animal husbandry, for humans themselves, brucellosis often becomes a chronic problem due to misdiagnosis because it has non-specific symptoms and signs, which results in treatment errors and repeated attacks. Misdiagnosis will cause an economic burden related to the cost and length of treatment for brucellosis in humans (Wang et al., 2022).

Indonesia itself has not been declared free of brucellosis, especially in the central areas of dairy farming due to a lack of awareness; most cattle breeders cull cattle that are proven positive for brucellosis so that infected cattle become lifelong carriers in those locations (Primatika et al., 2021). The epidemic of brucellosis among animals in economically developing countries still poses a constant threat to humans due to hygiene factors. In contrast, in developed countries, due to international travel, imports of animal feed, and other products, this disease continues to emerge (Wang et al., 2022). Based on the Decree of the Minister of Agriculture Number 4026/KPTS/OT.140/4/2013 concerning the determination of types of Strategic Infectious Animal Diseases (PHMS), brucellosis is identified as one of the diseases categorized under this regulation (Primatika et al., 2021). Livestock brucellosis in Indonesia is increasing in prevalence, reaching 40%, and spreading across almost all regions of Indonesia. This condition also plays a role in influencing the spread of brucellosis from animals to humans, in other words, as a risk factor for brucellosis in humans. The number of cases of brucellosis in humans cannot be fully confirmed. Still, previous studies showed the emergence of antibody titers from workers in slaughterhouses, cattle and pig farms. In Indonesia, brucellosis cases have not been properly detected because the publicity of brucellosis as a zoonotic disease is still minimal, so the public, especially livestock breeders, does not fully understand that brucellosis is a disease that can be transmitted to humans (Novita, 2016).

According to data from the West Nusa Tenggara Province Animal Husbandry and Animal Health Service, in 2019-2020, no cases of brucellosis were found in the livestock population (Dinas Peternakan dan Kesehatan Hewan Nusa Tenggara Barat, 2020). The cattle population in Gerung District is spread across fourteen villages, and the largest number of cattle is in Banyu Urip village, while the number of people working as livestock laborers in West Lombok Regency is 225 people (BPS Lombok Barat, 2021). Through observations in the field, there are still livestock suspected of being infected with brucellosis, especially those with a history of repeated abortions, so it does not rule out the possibility of transmission of brucellosis infection to breeders. On the other hand, brucellosis infection in humans can develop into a chronic form, resulting in several serious organ disorders, including endocarditis, paralysis, enlargement of the liver and spleen, arthritis, and abortion in the first trimester of pregnancy. It can result in epididymitis and orchitis (Bosilkovski et al., 2020). Brucellosis disease in humans can be diagnosed using serological laboratory tests (Rose Bengal Test, Complement Fixation Test, ELISA) and molecularly using PCR using the specific primer BSCP31 for *Brucella sp*. and IS711 species *Brucella abortus*, which causes abortion in cattle (Garshasbi et al., 2014; Luna et al., 2016).

RESEARCH METHOD

This study used an analytical observational design with a cross-sectional study approach. According to Novita (2016), 7.02% of breeders had brucellosis. A total of 26 breeders were obtained with the Lemeshow formula**.** This study increased the dropout proportion, so the number of samples became 30. However, to improve the sample representativeness, 10 additional respondents were included, resulting in a total of 40 participants. The inclusion criteria were breeders aged 18-70 years. The target population in the study were cattle breeders domiciled in West Lombok Regency, West Nusa Tenggara Province, while the accessible population was cattle breeders residing in the

village of Gerung District. The examination was conducted at the West Lombok Veterinary Laboratory and the Udayana Biomedical Laboratory. The ethical clearance No. 111/EC-03/FK-06/UNIZAR/XI/2022 was obtained from the Faculty of Medicine, Universitas Islam Al-Azhar.

RESULTS

This study used an analytical observational design with a cross-sectional approach. The samples were 40 cattle breeders in the Gerung sub-districts, all of whom were male. There were 25 breeders from the Gerung Selatan sub-district, 10 from the Gerung Utara sub-district, and 5 from the Dasan Geres sub-district.

Table 1. Contact with Cattle that Experienced Abortions

Contact	Frequency	Percentage
Positive (+)	7	17.50%
Negative (-)	33	82.50%

Based on Table 1, it was found that 7 breeders (17.50%) had contact with cattle that experienced abortions, while the other 33 breeders (82.50%) had no such contact.

Table 2. Age of the Cattle Breeders

Age	Frequency	Percentage
15-64 years old	38	95.00%
≥ 65 years old	2	5.00%

Based on Table 2, the age of cattle breeders is mostly in the 15–64 years old range (95.00%). Meanwhile, cattle breeders with an age range of ≥ 65 years have the lowest frequency (5.00%).

Blood samples were collected from 40 people. As much as 5 cc of median cubital vein blood was taken for the laboratory examination. The blood was collected in an EDTA tube, and then 1 cc (buffy coat) was taken to prepare for the PCR process. Blood with a volume of 4 cc in the EDTA tube was then centrifuged to obtain the serum layer needed for the Rose Bengal Test Serology examination. The RBT examination was conducted at the West Lombok Veterinary Laboratory, West Nusa Tenggara. In the Rose Bengal Test, 25 µL was required for each breeder's serum sample to be placed on the plate. Next, 25 µL of *Brucella* antigen was added to each plate filled with serum. On the designated area of the plate, positive and negative controls were placed. Finally, they were homogenized using a rotatory agglutination for 4 minutes.

The Rose Bengal Test showed negative results from 30 breeder samples and a mild agglutination reaction from 10 breeder samples. This mild agglutination reaction was classified as positive reaction 1 which is fine agglutination with somewhat clear edges and the liquid remains homogeneous (KEMENPAN RI, 2020). The results of this RBT examination would then be confirmed with a PCR examination.

Two commonly used primer pairs for detecting the *Brucella* genus are B4/B5, which targets the gene encoding a specific 31 kDa immunogenic membrane protein, and IS711, which identifies a unique sequence in *Brucella* abortus, the causative agent of brucellosis in cattle (Garshasbi et al., 2014; Luna et al., 2016; Zamanian et al., 2020). Primers were assessed using bioinformatics software including Basic Local Alignment Search Tool (BLAST).

Base on Table 3, The PCR examination was conducted using a 1 mL blood sample (buffy coat) after the DNA isolation stage. Next, the primer was mixed with the DNA from each sample, followed by the PCR process, which included the following stages.

Table 3. *Brucella* Primary

Product	Gene	Sequence 5' – 3'	Primary
223bp	*BSCP31*	TGGCTCGGTTGCCAATATC	B4
		CGCTTGCCTTTCAGGTCTG	B5
498bp	*IS711*	TGCCGATCACTTAAGGGCCTTCAT	F
		GACGAACGGAATTTTTCCAATCCC	R

Table 4. PCR Process

Gene Targets	Cycle Conditions			Number of Cycles
	Step	Temperature	Time	
BSCP31	Initial Denaturation	90^0C	5 minutes	1
	Denaturation	90^0C	1 minute	35x
	Annealing	50^0C	1 minute	
	Extensions	72^0C	1 minute	
	Final Extension	72^0C	10 minutes	1
IS711	Initial Denaturation	90^0C	5 minutes	1x
	Denaturation	90^0C	1 minute	35x
	Annealing	50^0C	1 minute	
	Extensions	72^0C	1 minute	
	Final Extension	72^0C	10 minutes	1

Base on Table 4, Sample pooling was performed for PCR examination preparation wherein several individual samples were combined into a single tube and tested as one composite sample (Rizal, 2021). Each PCR tube contained DNA from five different breeders, resulting in eight pooled samples, coded A1-H1 for the BSCP31 gene and A2-H2 for the IS711 gene. Notably, this study did not include a positive control in the PCR examination.

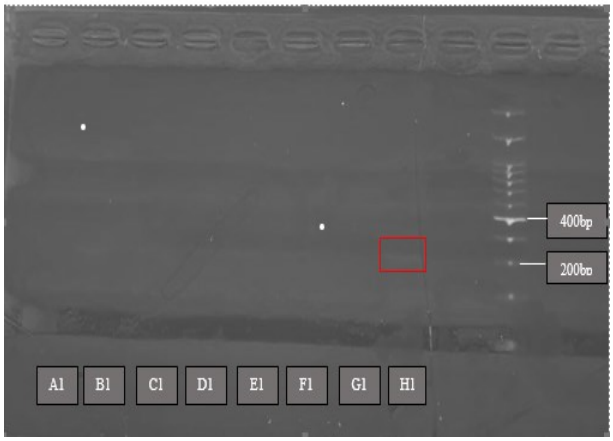

Figure 1. Pooling Electrophoresis Results for BSCP31 Gene Target Samples (223bp)

Figure 1 shows results of PCR examination on the BSCP31 gene pooling sample showed that sample H1 (consisting of sample numbers 9, 40, 41, 42, 43) showed a band slightly above 200bp. In samples A1 to G1, no bands were found.

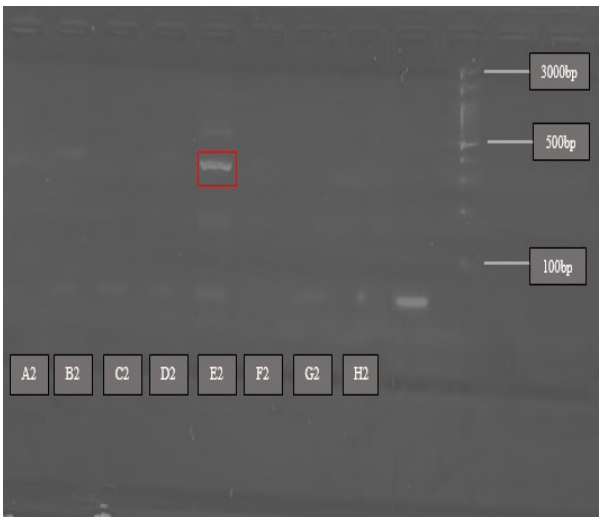

Figure 2. Pooling Electrophoresis Results for IS711 Gene Target Samples (498bp)

Figure 2 shosw the PCR examination results of the pooled sample for the IS711 gene showed a band close to the target of 498 base pairs in the E2 pooled sample (consisting of sample numbers 17, 19, 23, 24, and 25). No band images were obtained in samples A2, B2, C2, D2, F2, G2, and H2.

Next, the samples were processed for PCR examination of the BSCP31 gene, using sample H1 (which consisted of sample numbers 9, 40, 41, 42, and 43). Two samples formed bands near the target of 223 base pairs, specifically samples 43 and 4. The PCR process did not include a positive control, and DNA sequencing was necessary to correctly identify the results.

Sequencing of samples 43 and 4 was performed, and the target gene BSCP31 was edited using MEGA version 11 software. Two bands were observed in sample 43, while sample 4 displayed one band, both of which were close to the target BSCP31 gene at 223 bp. The editing results were analyzed using nucleotide BLAST to identify the sequence. The BLAST results indicated *Homo sapiens*.

Based on the PCR examination results with the universal *Brucella sp.* primer (BSCP31), the pooled sample results for the IS711 gene (samples 9, 40, 41, 42, and 43) showed that the decomposition process did not continue.

Table 5. Prevalence Ratio of Brucellosis Based on Contact with Cattle that Experienced Abortions

Contact	Brucellosis (+)	Brucellosis (-)	Total
Positive (+)	0	7	7
Negative (-)	0	33	33
Total	0	40	40

Based on Table 5, the prevalence ratio was < 1. It can be concluded that contact with cattle that experienced abortions was not a risk factor for transmitting brucellosis to cattle breeders.

Table 6. Table of the Relationship between Contact and RBT results

Contact	RBT (+)	RBT (-)	Total	OR	95% CI	p-value
Positive (+)	2	5	7	1.25	1.20 to 7.74	1.000
Negative (-)	8	25	33			
Total	10	30	40			

Table 6 shows that there is no statistically significant relationship between contact with cattle that experienced abortions and RBT results (p = 1.000).

DISCUSSION

This study shows that all samples of breeders in the Gerung sub-district were men. Previous studies conducted by Muslimin et al. (2017) and Novita (2016) showed that most samples were men. Working as a cattle breeder requires physical activity and energy, not just for providing food but also for managing the pen, giving food, bathing livestock, cleaning livestock manure, maintaining livestock health, etc. This can also be the basis for explaining why brucellosis infection occurs more predominantly in men. This situation can also explain that men are more at risk of contact with livestock as a *Brucella* reservoir.

The age of breeders in this study showed that most study subjects (95.00%) were 15-64 years old. A study conducted by Muslimin et al. (2017) showed that the dominant age range of the study subjects was 18-50 years old. Meanwhile, according to Kustiningsih et al. (2023), 68.90% of people were 25-50 years old. According to the Decree of the Ministry of Health of the Republic of Indonesia in 2021, the age range of 15-64 years is a productive age for society in carrying out work and producing goods and services.

In this study, 7 breeders had contact with cattle that experienced abortions (17.50%), while 33 people (82.50%) did not. A total of 2 breeders (29.00%) who had contact showed a positive RBT result (+), and 5 breeders (71.00%) showed a negative RBT result. Meanwhile, 8 people (24.00%) of 33 breeders who did not have contact showed positive RBT results (+) and 25 people (76.00%) showed negative RBT results. Abortion is one of the typical symptoms that can be observed directly and is suspected as brucellosis in female cattle by veterinary officers. Abortion that occurs spontaneously in animals occurs due to the development of large numbers of *Brucella* bacteria in the bovine placenta, which contains a lot of erythritol, an important bacterial growth factor. Bacteria that influence cross-reaction to the RBT *Brucella* serological examination can also cause abortion, including *Yersinia enterocolitica* infection, which can cause placental damage and abortion.

The Rose Bengal Test serology is used in diagnostic laboratory examinations for brucellosis in breeders. A total of 10 breeders (25.00%) showed a positive reaction (+). The presence of a minimal agglutination reaction indicates the presence of IgM and IgG antibodies in the patient's body against *Brucella* bacteria. Serodiagnosis of brucellosis detecting the presence of antibodies against the smooth-Lipopolysaccharide (S-LPS) component is generally carried out using antigens extracted from the *Brucella abortus* S19 strain. Therefore, the serodiagnosis test based on the introduction of S-LPS cannot avoid cross-reactions against other bacteria. LPS molecules carry epitopes that cross-react with a variety of Gram-negative organisms, including *Yersinia enterocolitica* O:9, *Salmonella abortusovis*, *Francisella tularensis, Escherichia coli* O116 and O157, and *Vibrio cholerae*. For this reason, the results of serodiagnosis tests targeting S-LPS should be interpreted cautiously and correlated with the clinical manifestations of the disease and epidemiological data. The anti-S-LPS antibodies responsible for this nonspecific reaction are mostly of the IgM isotype (Yagupsky et al., 2019). In the acute phase of *Brucella* infection, there will be an increase in IgM antibodies until it peaks up to 3 months after the infection period, followed by an increase in IgG and IgA antibody levels. As chronic *Brucella* infection progresses, there is a sharp decline in IgM and IgA antibody titers, while IgG can still be detected, although accompanied by a decrease. Thus, the agglutination test tends to perform better for diagnosing acute cases, apart from decreasing sensitivity in detecting cases of chronic brucellosis or neuroborreliosis. In patients suspected of having chronic cases, it is recommended that ELISA be used to detect the presence of IgG (CDC, 2021).

Several previous studies showed high sensitivity in the results of RBT examinations. A study by Ekiri et al. in 2020 showed the sensitivity of RBT for patients with brucellosis, 98.00% were categorized as acute brucellosis, 84.00% as subacute, 61.00% as chronic, and 22.00% as neuroborreliosis. A study by Ruiz Mesa et al., in 2005 had RBT sensitivity results of 89.90% for patients whose symptoms lasted < 2 weeks, 95.70% for those with symptoms lasting 2 weeks to 1 month, 94.10% for those with symptoms for 1-3 months, and 88.10% for those with symptoms for > 3 months. A study by Salih et al. in 2007 showed that acute infections were 73.10% higher than patients with a history of infection, 26.80%, which was caused by increased antibody titer levels in patients with previous infections (Salih et al., 2007).

A previous study in Indonesia by Novita et al., in 2017 which was conducted in the Cilawu sub-district, Garut, reported that 7.02% of breeders were positive through the RBT examination and the confirmation of the Complement Fixation Test. Likewise, in a study by Sahayati et al. (2019) in Sleman district, Yogyakarta, it was found that 0.80%

of breeders were positive through the RBT examination and also were confirmed positive by conventional PCR. This difference in results may be attributed to the presence of infected livestock in the study area, thereby increasing the risk of brucellosis transmission to breeders. In this regard, it is important to emphasize that the diagnosis of brucellosis in humans must be made based on appropriate symptoms, clinical findings, and a thorough history so as not to rely solely on weak positive results in the S-LPS serological test. The weakness of the Rose Bengal Test is that it detects the presence of antibodies, but not the causative agent. This examination cannot be used as a diagnostic guide. Therefore, it requires additional serological examinations or molecular examinations, and the prozone phenomenon can form as a result of high levels of antibodies coating the antigen particles which can interfere with the formation of agglutination (Tankeshwar, 2022).

The results of the conventional PCR examination showed negative results. In contrast to a study conducted in Sulawesi by Ahzan et al. (2021), which showed that 3 samples of breeders with contact gave positive results (+++) while 1 sample had positive results (++) from the RBT examination. Furthermore, only 2 samples were positive through the conventional PCR confirmation test. The PCR method is currently very useful in diagnosing brucellosis infection in humans, especially in supporting serological examination because of the frequent suspicion of cross-reactions. Several studies on patients, including one conducted by Garshasbi et al. in 2014, explained that as many as 180 patients suspected of suffering from active brucellosis were examined for DNA extracted from serum samples using a commercial kit. PCR amplification was performed to detect *Brucella* DNA using the target gene BCSP31 and the IS711 locus. The PCR test using a 223 bp amplicon obtained 73.80% (133/180) of the sera tested using primers (B4/B5) derived from the gene encoding the 31-kDa *Brucella* abortus antigen. Meanwhile, a 498 bp amplicon was obtained in 63.80% (115/180) of samples using specific primers for *Brucella* abortus derived from a locus adjacent to the 3' end of IS711. Meanwhile, a study by Hasan et al. in 2022 used real-time PCR to diagnose acute brucellosis from 68 patient samples who were declared seropositive with symptoms of fever of unknown origin. Then, using real-time PCR for the BSCP31 gene, 57 samples (83.30%) were positive for acute brucellosis.

In this study, breeders were not known to experience signs and symptoms suspected of brucellosis, this was related to the presence of bacteria in the blood (bacteremia) which caused the PCR examination to show negative results. The weakness of the PCR examination is that most of these methods were developed using DNA of *Brucella sp*., which is made directly from bacterial culture as a positive control which requires BSL3 laboratory standards, high costs, and ideal procedures to avoid contamination of the specimen and is very dependent on the sample used based on the phase of bacterial infection (Yu et al., 2024).

From the results of the PCR examination, a picture of the bands in sample no. 43 and no. 4, targeting the BSCP31 223bp gene, was obtained. Because there was no positive control in the PCR examination, the sequencing process should be carried out in a reference laboratory. The results of sequencing editing with MEGA v11 software and identification using BLAST resulted in *Homo sapiens* results. The conclusion is that the bands formed in the two samples are variations in human genes.

Based on the results of the RBT serology examination, which was confirmed by PCR testing from 40 samples of cattle breeders, 30 breeders were declared not infected with *Brucella*. Meanwhile, 10 samples from cattle breeders showed a positive RBT result of 1 (minimal antibodies present); it can be concluded that the 10 breeders had a history of previous exposure to *Brucella* or other bacteria that cause abortion in cattle which affects the cross-reaction in RBT. This study did not assess the presence of signs and symptoms suspected of brucellosis so it could not assess the phase of infection that occurred.

In this study, the relationship between contact with cattle that experienced abortions and RBT results was assessed using the Chi-Square test, which obtained $p = 1.000$. These results conclude that there is no significant relationship between contact and RBT results. In this case, several gram-negative bacteria that have the same antigen as *Brucella* can cause abortions in cattle, and consumption of processed food products from infected cattle is the dominant risk factor for *Brucella* bacterial infection in humans.

This study concluded that contact with cattle that experienced abortions is not always a risk factor for transmitting brucellosis to cattle breeders. From this study, 7 breeders had contact, of which 2 breeders showed positive RBT results, while 5 breeders had negative RBT results. It is understood that abortions in cattle can be caused by factors other than *Brucella* infection (Garshasbi et al., 2014; Luna et al., 2016; Primatika et al., 2021; Qureshi et al., 2023; Zamanian et al., 2020).

CONCLUSION

In this study, no breeders with brucellosis were identified through either RBT serological examination or conventional PCR using blood samples. No statistically significant relationship was found between contact with cattle that experienced abortions and RBT results. Future studies with larger sample sizes are recommended.

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CONFLICT OF INTEREST STATEMENT

This study declares that there is no conflict of interest.

REFERENCES

Ahzan, N. A., Muhasirah, M., Nurhayati, N., & Rosmiaty, R. (2021). Zoonotic Surveilance (Brucellosis) in Enrekang District South Sulawesi Province. *Pancasakti Journal Of Public Health Science And Research*, *1*(2), 122–127. https://doi.org/10.47650/pjphsr.v1i2.256

Bosilkovski, M., Stojovski, M., Siskova, D., Ridov, A., Kostoska, E., & Krstevski, K. (2020). Brucellosis in pregnancy: case reports with different outcomes in an endemic region. *Acta Clinica Croatica*, *59*(2), 338–343. https://doi.org/10.20471/acc.2020.59.02.18

BPS Lombok Barat. (2021). *Kecamatan gerung Dalam Angka 2021: BPS Kabupaten Lombok Barat*. https://lombokbaratkab.bps.go.id/publication/2021/09/24/ 60be559a8 8506531c36ee8e6 /kecamatan-gerung-dalam-angka-2021.html.

CDC. (2021). *Zoonoses Diseases: Centers for Disease Control and Prevention*.

Dinas Peternakan dan Kesehatan Hewan Nusa Tenggara Barat. (2020). *Penyakit Hewan Menular Strategis Tahun 2020*. https://data.ntbprov.go.id/dataset/kasus-penyakit-hewan-menular-strategis/resource/c22c56fa-73f9-4f7c-96b2-4920b55853f1

Garshasbi, M., Ramazani, A., Sorouri, R., Javani, S., & Moradi, S. (2014). Molecular detection of Brucella species in patients suspicious of Brucellosis from Zanjan, Iran. *Brazilian Journal of Microbiology : [Publication of the Brazilian Society for Microbiology]*, *45*(2), 533–538. https://doi.org/10.1590/s1517-83822014005000048

Gupte, S., & Kaur, T. (2016). Diagnosis of Human Brucellosis. *Journal of Tropical Diseases*, *04*. https://doi.org/10.4172/2329-891X.1000185

Hull, N. C., & Schumaker, B. A. (2018). Comparisons of brucellosis between human and veterinary medicine. *Infection Ecology & Epidemiology*, *8*(1), 1500846. https://doi.org/10.1080/20008686.2018.1500846

Kustiningsih, H., Sudarnika, E., Saleh, A., Basri, C., & Sudarwanto, M. (2023). The Role of Dairy Breeders in Surveillance and Control Program of Brucellosis in Bogor Regency. *Jurnal Sain Veteriner*, *41*(1), 51. https://doi.org/10.22146/jsv.77617

Luna, L., Chávez, G., Mejía Castañeda, L., Barragan, V., & Trueba, G. (2016). Molecular Detection of Brucella Species in Ecuador. *Journal of Applied Research in Veterinary Medicine, The*, *14*, 185–189.

Muslimin, L., Bangsawan, A. T., & Utami, S. (2017). Brucellosis identification on breeders in Pinrang District. *Nusantara Medical Science Journal*, *2*(1), 33–37. https://doi.org/10.20956/nmsj.v2i1.2258

Novita, R. (2016). Brucellosis : Penyakit Zoonosis Yang Terabaikan. *Jurnal Litbang Pengendalian Penyakit Bersumber Binatang Banjarnegara*, *12*(2), 135–140. https://doi.org/10.22435/balaba.v12i2.4625.135-140

Primatika, R. A., Sumiarto, B., Nugroho, W. S., Widiasih, D. A., Drastini, Y., Yudhabuntara, D., & Susetya, H. (2021). Penyebaran Penyakit Brucellosis di Wilayah Koasistensi Administrasi Dinas dan Kesmavet. *Jurnal Sain Veteriner*, *39*(2), 145. https://doi.org/10.22146/jsv.51253

Qureshi, K. A., Parvez, A., Fahmy, N. A., Abdel Hady, B. H., Kumar, S., Ganguly, A., Atiya, A., Elhassan, G. O., Alfadly, S. O., Parkkila, S., & Aspatwar, A. (2023). Brucellosis: epidemiology, pathogenesis, diagnosis and treatment-a comprehensive review. *Annals of Medicine*, *55*(2), 2295398. https://doi.org/10.1080/07853890.2023.2295398

Sahayati, S., Nugroho, W., & Pramono, D. (2019). Risk Factor of Degree of Agglutination in Rose Bengal Test : Study in Dairy Breeder Suspected Brucellosis in Sleman , Daerah Istimewa Yogyakarta. *1st International Respati Health Conference (IRHC) Proceeding*, 61–66.

Suraya, I., & Mardhiati, R. (2018). Edukasi Hiv/Aids Pada Remaja Siswa Smk. *Buletin Udayana Mengabdi*, *17*(3), 31–34. https://doi.org/10.24843/bum.2018.v17.i03.p06

Tankeshwar, A. (2022). *Sulfide Indole Motility (SIM) Medium: Principle, Procedure, and Results*. Microbeonline. https://microbeonline.com/sulfide-indole-motility-sim-medium/

Wang, X., Zhao, Y., Liu, S., & Gu, B. (2022). Analysis of Brucellosis Cases Between 2010 and 2020 in Guangdong Province, China. *The American Journal of Tropical Medicine and Hygiene*, *106*(6), 1610–1612. https://doi.org/10.4269/ajtmh.21-0870

WHO. (2020). *Zoonotic disease: emerging public health threats in the Region*. World Health Organization. https://www.emro.who.int/about-who/rc61/zoonotic-diseases.html

Yagupsky, P., Morata, P., & Colmenero, J. D. (2019). Laboratory Diagnosis of Human Brucellosis. *Clinical Microbiology Reviews*, *33*(1). https://doi.org/10.1128/CMR.00073-19

Yu, H., Gu, X., Wang, D., & Wang, Z. (2024). Brucella infection and Toll-like receptors. *Frontiers in Cellular and Infection Microbiology*, *14*, 1342684. https://doi.org/10.3389/fcimb.2024.1342684

Zamanian, M., Jahani, E., & Mahmoudi, H. (2020). Multiplex PCR Assay for the Simultaneous Detection of the Brucella Genus in Human Whole Blood and Serum. *The Open Microbiology Journal*, *14*(1), 242–246. https://doi.org/10.2174/1874434602014010242