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PREVALENCE OF BRUCELLOSIS IN DAIRY COWS IN KRUCIL SUBDISTRICT, PROBOLINGGO REGENCY

Prevalensi Brucellosis pada Sapi Perah di Kecamatan Krucil Kabupaten Probolinggo

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Abstract

Brucellosis is an important zoonotic infectious disease caused by bacteria belonging to the *Brucella* genus. Probolinggo Regency in East Java Province is recognized as one of the regions with a large population of dairy cattle, particularly in Krucil Subdistrict, which holds the highest number of dairy cows in the regency. This study aimed to assess the health status of dairy cattle related to Brucellosis infection in Krucil Subdistrict, Probolinggo Regency. The research employed a random sampling approach involving 35 Friesian Holstein (FH) dairy cows originating from three villages: Tambelang, Bermi, and Krucil. Serological testing was carried out at the BBVet Wates Laboratory using the Rose Bengal Test (RBT) method to identify antibodies against *Brucella abortus*. The results of the study showed that the prevalence of brucellosis in Krucil District was 0%. The findings indicated that all collected samples (100%) tested seronegative for Brucellosis, demonstrating that the area under study remains free from this disease. Ongoing preventive programs, including vaccination, disease monitoring, and the implementation of strict biosecurity, are recommended to preserve the Brucellosis-free status.

Keywords: Brucellosis, dairy cattle, serological test

Abstrak

Brucellosis merupakan penyakit infeksius strategis yang bersifat zoonosis dan disebabkan oleh bakteri dari genus *Brucella*. Kabupaten Probolinggo di Provinsi Jawa Timur diketahui memiliki populasi sapi perah yang cukup besar, dengan Kecamatan Krucil sebagai wilayah dengan jumlah ternak terbanyak. Penelitian ini bertujuan untuk mengevaluasi status kesehatan sapi perah terhadap infeksi *brucellosis* di Kecamatan Krucil, Kabupaten Probolinggo. Penelitian dilaksanakan dengan metode pengambilan sampel acak (*random sampling*) pada 35

Volume 17 No. 6: 1789-1795
December 2025
https://doi.org/10.24843/bulvet.2025.v17.i06.p02

ekor sapi perah jenis Friesian Holstein (FH) milik peternak di tiga desa, yaitu Tambelang, Bermi, dan Krucil. Pemeriksaan serologis dilakukan di Laboratorium Balai Besar Veteriner (BBVet) Wates menggunakan metode *Rose Bengal Test* (RBT) untuk mendeteksi antibodi terhadap *Brucella abortus*. Hasil penelitian menunjukkan bahwa prevalensi *brucellosis* di Kecamatan Krucil yaitu 0%. Dimana seluruh sampel (100%) negatif terhadap *brucellosis*, yang berarti wilayah tersebut tergolong bebas dari penyakit *Brucellosis*. Upaya pencegahan seperti vaksinasi, pengawasan rutin, dan penerapan biosekuriti yang ketat perlu terus dilakukan untuk mempertahankan status bebas penyakit di wilayah penelitian.

Kata kunci: *Brucellosis*, sapi perah, pemeriksaan serologis

INTRODUCTION

The demand for livestock-derived food products continues to increase each year, driving the expansion of the dairy cattle industry in Indonesia. According to the 2024 Livestock and Animal Health Statistics, national milk production has shown an upward trend compared with the previous year (Statistik Peternakan dan Kesehatan Hewan, 2024). As each animal is susceptible to various diseases, farmers must possess adequate knowledge of clinical signs and appropriate control measures to ensure timely prevention and treatment (Winarsih, 2018). East Java Province is recognized as the region with the largest dairy cattle population in Indonesia, thereby carrying a substantial responsibility in maintaining the quality of animal products that are Safe, Healthy, Wholesome, and Halal (ASUH).

One of the infectious diseases that remains a major concern is brucellosis, due to its rapid and efficient mode of transmission. Its spread may be facilitated by animal movement and interregional trade, including illegal distribution activities that occur without proper quarantine oversight. At the farm level, traditional husbandry systems, the shared use of equipment, group housing, contamination of grazing areas, suboptimal vaccination coverage (<70%), and the presence of carrier female cattle further contribute to the persistence and dissemination of this disease (Noor, 2006; Perwitasari, 2010; Lake et al., 2010).

Brucellosis is a zoonotic disease caused by bacteria of the genus *Brucella*, particularly *Brucella abortus* in cattle. The infection can result in abortion, retained placenta, reduced milk production in cows, and orchitis in bulls (Radostits et al., 2017). Beyond its impact on livestock productivity, the disease can also be transmitted to humans through direct contact with infected animals or the consumption of contaminated animal products (Corbel, 2006). To date, Indonesia—especially regions with large dairy cattle populations—has not fully eradicated brucellosis (Novita, 2016). Diagnosis is commonly performed using serological tests to detect specific antibodies against cell wall polysaccharide antigens of certain *Brucella* spp. (Dwi et al., 2018).

According to the 2024 Livestock Statistics published by the East Java Provincial Livestock Service, Probolinggo District ranks fifth in dairy cattle population within the province, with a total of 14,239 head (Dinas Peternakan Provinsi Jawa Timur, 2024). Meanwhile, Krucil Subdistrict recorded the highest dairy cattle population in Probolinggo District in 2023, totaling 5,914 head (Badan Pusat Statistik Kabupaten Probolinggo, 2023). This high population places Krucil as a priority area for brucellosis surveillance and control. Based on these conditions, the present study aimed to detect the presence of brucellosis in dairy cattle in Krucil Subdistrict, Probolinggo District, using the Rose Bengal Test (RBT) serological method.

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Pendidikan Tinggi, Riset, dan Teknologi No. 158/E/KPT/2021

Volume 17 No. 6: 1789-1795
December 2025
https://doi.org/10.24843/bulvet.2025.v17.i06.p02

MATERIALS AND METHODS

Ethical Feasibility for Animal Use

This study did not require ethical clearance, as it involved only the collection of blood samples from the sampled animals.

Study Subjects

The research was conducted in three villages within Krucil Subdistrict, Probolinggo District Tambelang, Bremi, and Krucil during July 2025. Sampling was performed using a random sampling method. A total of 35 Friesian Holstein (FH) dairy cows were sampled, and approximately 5 ml of blood was collected from each animal following standard procedures. All Rose Bengal Test (RBT) analyses were carried out at the Wates Veterinary Center (BBVet Wates), Yogyakarta.

Study Design

A quantitative descriptive design was applied to systematically and factually describe the presence of Brucella antibodies in dairy cattle. Serological examination was performed using the RBT agglutination method to detect antibodies against *Brucella* sp., following the procedure described by Sugiyono (2009).

Blood Collection Procedure

The equipment and materials used for blood collection included venoject tubes, G12 venoject needles, disinfectants, cotton, alcohol, and sterile distilled water. Blood was drawn from the jugular vein or coccygeal vein using silicone-coated vacutainer tubes. After collection, samples were left at room temperature until serum formation, or centrifuged to accelerate separation. Strict aseptic techniques were applied throughout the procedure to avoid bacterial contamination, and all laboratory equipment was maintained in sterile condition.

Rose Bengal Test (RBT) Procedure

Prior to testing, serum samples and Brucella RBT antigen were removed from refrigeration and equilibrated to room temperature for 30–60 minutes. A total of 30 µl of serum was dispensed onto a test plate and mixed with an equal volume of antigen, forming a circle approximately 2 cm in diameter. The mixture was gently agitated using a rotary agitator for about 4 minutes. If Brucella antibodies were present, visible agglutination occurred. The degree of agglutination was classified as follows (Novita et al., 2016):

0 : no agglutination (negative)

\+ : mild agglutination

++ : partial agglutination

+++ : strong and clearly visible agglutination

Data Analysis

RBT results were analyzed quantitatively in a descriptive manner to determine the prevalence of brucellosis in the dairy cattle population. Test outcomes were also interpreted qualitatively based on the observed agglutination reactions. Prevalence was calculated using the following formula (Budiharta, 2002):

$$\text{Prevalence (\%)} = \frac{\text{Number of positive animals}}{\text{Number of samples examined}} \times 100\%$$

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Pendidikan Tinggi, Riset, dan Teknologi No. 158/E/KPT/2021

Volume 17 No. 6: 1789-1795
December 2025
https://doi.org/10.24843/bulvet.2025.v17.i06.p02

The test results were categorized into two distinct groups: seropositive, characterized by the presence of agglutination which indicates detectable antibodies against *Brucella abortus* in the serum, and seronegative, defined by the absence of agglutination, confirming the lack of specific *Brucella* antibodies.

RESULTS AND DISCUSSION

A total of 35 serum samples were collected from six dairy farmers in three villages Tambelang, Bermi, and Krucil within Krucil Subdistrict in July 2025. All samples were examined using the Rose Bengal Test (RBT), as presented in Table 1. The examination showed that all tested samples were negative, resulting in a brucellosis prevalence of 0% in Krucil Subdistrict, including Tambelang, Bermi, and Krucil. A negative RBT result is characterized by the absence of clumping or visible fine granules, indicating that the samples were free from *Brucella* spp. infection.

The RBT method is based on an antigen–antibody reaction involving immunoglobulins present in serum. The presence of immunoglobulin G (IgG) is indicative of infection (Rojas and Alfonso, 2013). The degree of agglutination reflects the antibody concentration in the serum, categorized as negative (0), low (+1), moderate (+2), and high (+3) (Rohyati et al., 2017). Thus, a positive reaction indicates a moderate antibody concentration against *Brucella* spp.

The antigen used in RBT is a smooth-type *Brucella* antigen stained with Rose Bengal and suspended in a buffer solution with a pH of 3.65. Under neutral pH conditions, the test can detect both IgM and IgG; however, at pH 3.65, only IgG reacts because IgM becomes inactivated (Kaltungo et al., 2014; Klein, 1991). Therefore, the agglutination observed in RBT reflects the binding between the antigen and IgG antibodies.

Environmental contamination can act as a trigger for disease occurrence and facilitate transmission between animals through direct contact. Moreover, herd population density and management practices also play major roles in the incidence of brucellosis (Azzahrawani et al., 2018). Based on the findings of this study, dairy cattle in Krucil Subdistrict appear to be managed under favorable environmental conditions, with good husbandry practices and biosecurity measures, supporting the brucellosis-free status of the area.

Nevertheless, continuous brucellosis surveillance remains essential in Probolinggo District. Regular monitoring is necessary to track disease trends in the field, particularly considering the movement of livestock across villages, subdistricts, districts, and provinces, which can be difficult to regulate. Active surveillance and routine monitoring will help maintain the brucellosis-free status and ensure early detection should any reactor animals enter the region.

This 0% prevalence finding aligns with reports from several other regions in Indonesia that have also declared brucellosis-free status through similar serological surveillance. The successful control can be attributed to the cumulative effect of long-term vaccination programs, effective test-and-slaughter policies for infected animals, and heightened farmer awareness regarding biosecurity. However, it is crucial to interpret these results within the limitations of the diagnostic method. While RBT is an excellent screening tool due to its high sensitivity and rapid results, it is not confirmatory. The possibility of false negatives, especially in cases of chronic infection with low antibody titers or very recent exposure, cannot be entirely ruled out. Therefore, a negative RBT result, particularly in a single cross-sectional study, should be seen as a strong indicator of freedom from disease at the population level rather than an absolute guarantee for every individual animal (Abnaroodheleh et al., 2025).

The sustainability of this brucellosis-free status hinges on several critical factors. First, the integrity of animal movement controls must be maintained, as the introduction of an infected

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Pendidikan Tinggi, Riset, dan Teknologi No. 158/E/KPT/2021

Volume 17 No. 6: 1789-1795
December 2025
https://doi.org/10.24843/bulvet.2025.v17.i06.p02

animal is the most significant risk. Second, the financial and operational continuity of government-led surveillance and compensation programs for culled animals is vital for farmer compliance. Any lapse in these measures could lead to a silent re-emergence of the disease. Future studies could strengthen these findings by employing a combination of tests, such as following RBT screening with a more specific confirmatory test like the Complement Fixation Test (CFT) or ELISA on a subset of samples. Additionally, expanding surveillance to include other livestock species that can act as reservoirs for *Brucella* would provide a more comprehensive epidemiological picture of the region (Dadar et al., 2021).

CONCLUSION AND RECOMMENDATIONS

Conclusion

This study demonstrated that the prevalence of brucellosis in dairy cattle in Krucil Subdistrict, Probolinggo District, based on the Rose Bengal Test (RBT), was 0%. Therefore, no cases of brucellosis were detected in the dairy cattle population examined in this study.

Recommendations

Further research with a broader sampling design and more comprehensive diagnostic approaches including confirmatory tests such as the Complement Fixation Test (CFT) and bacterial isolation is recommended to obtain more accurate information regarding the brucellosis status in the study area.

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Volume 17 No. 6: 1789-1795
December 2025
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Volume 17 No. 6: 1789-1795
December 2025
https://doi.org/10.24843/bulvet.2025.v17.i06.p02

Table

Table 1. Brucellosis Test Results in Krucil Subdistrict, Probolinggo District

Village	Owner	Code	Cattle Breed	Test Result
Tambelang	Agus Suoriyono	P1	FH	Seronegative
Tambelang	Agus Suoriyono	P2	FH	Seronegative
Tambelang	Agus Suoriyono	P3	FH	Seronegative
Tambelang	Agus Suoriyono	P4	FH	Seronegative
Tambelang	Agus Suoriyono	P5	FH	Seronegative
Tambelang	Agus Suoriyono	P6	FH	Seronegative
Tambelang	Ismail	P7	FH	Seronegative
Tambelang	Ismail	P8	FH	Seronegative
Tambelang	Ismail	P9	FH	Seronegative
Tambelang	Ismail	P10	FH	Seronegative
Tambelang	Ismail	P11	FH	Seronegative
Tambelang	Ismail	P12	FH	Seronegative
Tambelang	Ismail	P13	FH	Seronegative
Tambelang	Abdul Fatah	P14	FH	Seronegative
Tambelang	Abdul Fatah	P15	FH	Seronegative
Tambelang	Abdul Fatah	P16	FH	Seronegative
Tambelang	Abdul Fatah	P17	FH	Seronegative
Tambelang	Abdul Fatah	P18	FH	Seronegative
Bermi	Sumiyati	P19	FH	Seronegative
Bermi	Sumiyati	P20	FH	Seronegative
Bermi	Sumiyati	P21	FH	Seronegative
Krucil	Adam	P22	FH	Seronegative
Krucil	Adam	P23	FH	Seronegative
Krucil	Adam	P24	FH	Seronegative
Krucil	Adam	P25	FH	Seronegative
Krucil	Adam	P26	FH	Seronegative
Krucil	Adam	P27	FH	Seronegative
Krucil	Adam	P28	FH	Seronegative
Krucil	Adam	P29	FH	Seronegative
Krucil	Adam	P30	FH	Seronegative
Krucil	Adam	P31	FH	Seronegative
Krucil	Adam	P32	FH	Seronegative
Krucil	Adam	P33	FH	Seronegative
Krucil	Suparjiono	P34	FH	Seronegative
Krucil	Suparjiono	P35	FH	Seronegative

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Pendidikan Tinggi, Riset, dan Teknologi No. 158/E/KPT/2021