# RISK FACTOR OF DEGREE OF AGGLUTINATION IN ROSE BENGAL TEST: STUDY IN DAIRY FARMER SUSPECTED BRUCELLOSIS IN SLEMAN, DAERAH ISTIMEWA YOGYAKARTA

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**Abstract**
The incidence of brucellosis in humans in Indonesia has not been reported as well as zoonotic disease. It made most people do not know if brucellosis can be transmitted to humans. On March 2015, there was reported three farmers at one dairy farm got brucellosis. So it needed deep and specific research. This study aims to determine the prevalence and factors associated with suspected brucellosis, based on the degree of agglutination of dairy farmers. The population was all dairy farmer in Cangkringan. This study was conducted by cross sectional design; individual interviewed using a questionnaire containing questions about disease Brucellosis. Blood samples were tested using Rose Bengal Test (RBT) in laboratorium. 249 people joined by randomly sampling in 650 farmers. Data was proceed by Stata using Spearman's test to look at the correlation of each factor. There were two positive serological Brucellosis (0,8%). Spearman's correlation analysis between degrees of agglutination of RBT with risk factor showed using of protective equipment ($\rho$=-0,0243 P=0,7024); using of disinfectants ($\rho$=-0,0896 P=0,1586); time of breed ($\rho$=0,0100 P=0,8758); cattle vaccination status ($\rho$= -0,0273 P=0,6680) were not associated with degree of agglutination. Have a history of brucellosis in humans ($\rho$=0,4399 P=0,0000); direct contact with the infected cow ($\rho$=0,8149 P=0,0000); history of infected cattle ($\rho$= 0,5727 P=0,000) were associated with degree of agglutination. The prevalence of brucellosis incidence on dairy farmers was 0,8%. There was a strong relationship between having a history of brucellosis in humans, direct contact with infected animals, history of animals infected with the degree of agglutination at RBT on dairy farmers.

**Keywords: Brucellosis, factors, risks, agglutination, cows, humans**

## 1. INTRODUCTION

*Brucellosis* is an infectious disease caused by bacteria of the genus *Brucella* and categorized by the OIE as zoonotic diseases. *Brucellosis* in animals infected female usually asymptomatic, whereas in pregnant animals can cause plasentitis which results in abortion in the fifth month of pregnancy until the ninth. If it does not happen abortus, Brucella can be excreted into the placenta, fetal fluids and vaginal mucous. Mammary gland and lymph nodes may also be infected and this microorganism is excreted into the milk [1].

*Brucellosis* is a disease that is easily transmitted to humans, causing acute febrile illness, which may turn out to be more chronic form that also produce serious complications affecting the musculo-skeletal, cardiovascular, and central nervous system. Infection is often due to exposure and basically obtained through oral, respiratory, or conjunctival, but consumption of dairy products is a major risk for the general population in endemic areas.

Morbidity of the disease depends on the infecting species of Brucella. The incidence of *Brucellosis* in cattle has spread to almost all provinces in Indonesia except the Bali and Lombok were declared free of *Brucellosis* in 2002 with the rate varying from 1% to 40% [2].

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*Brucellosis* is a very high risk disease, therefore the tools that have been contaminated with Brucella bacteria should not be in direct contact with humans. This disease can be transmitted from animals to humans and is difficult to cure or treat, so *Brucellosis* is an important zoonotic diseases. As a note, meat from the infected one can be consumed by human (in a good condition and cook well).

There're no cases of human Brucellosis repoted in Sleman. The Department of Agriculture, Fisheries reported that workers at one dairy farm in Cangkringan positive for *Brucellosis*. This farm has RBT routine for the cows, but this cases in human were unpredictable.

The existence of cases of *Brucellosis* in humans that occurs in Sleman district and unprecedented that more studies about *Brucellosis* in humans such as the prevalence and factors associated with the occurrence of *Brucellosis* and testing the degree of agglutination at RBT test is necessary.

## 2. METHODS

This study was observational using Cross Sectional Study. Subjects were dairy farmers in Cangkringan. The sample size in this study was 249 people and taken by simple random sampling. The instrument used was a questionnaire that was adopted and modified from research [3].Univariable data analysis using frequency distribution and bivariable using Spearman ($\rho$) correlation test.

Blood samples were tested by RBT in laboratorium. The concept of examining using RBT was looking at the agglutination formed by the serum reaction and reagents used. Agglutination is a viral or bacterial serological test that is suspended in a unified solution into lumps if the suspension is treated with an antiserum that contains specific antibodies against the virus or bacteria. Every form given score start from 0 if no agglutination occurs, the mixture of antigen and serum remains homogeneous and reddish purple. Score +1 to +3 if agglutination occurs from smooth to perfect with edges surrounded by particles forming lines from broken to thick or lumps of particle look rough. See fig. 1
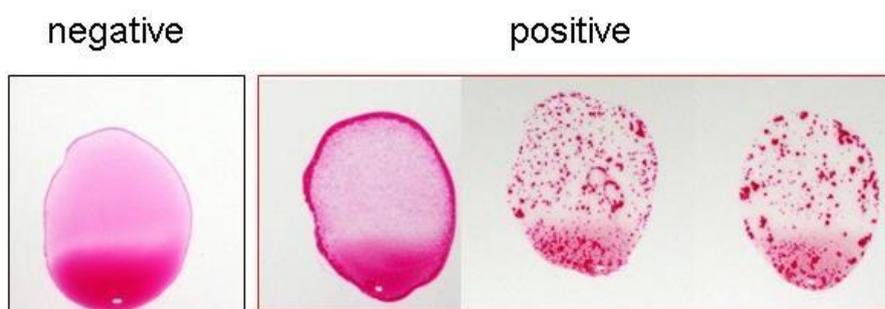


Figure 1. Degrees of agglutination

## 3. RESULTS AND DISCUSSION

Positive serological test results Brucella 2 (100%) in men. Two people are in different age groups: group aged less than 45 years old and between 45-75 years old. Education of farmers most is the elementary school level (48.4%).

Based on the results 44.5% of farmers have less knowledge of the brucellosis in humans. Instead the farmer who has a good knowledge of only 27.3%; 157 (62.9%) farmer used personal protective equipment whenever doing activities at home; use disinfectant or detergent has been done by 124 farmers (49.8%) to clean the cage every day. In this study also showed 228 farmers (91.6%) had never given the vaccine in cattle, since it was first maintained; three farmers (1.2%) answered

had direct contact with animals infected *Brucella*. Animals treated cattle are owned or help deal with the property of others. Total positive cattle infected with Brucella was 6 (2.4%) cows that come from different breeders.

Spearman correlation of test results against all the variables studied, there are five variables that is positive, the use of disinfectants ($\rho$= 0.0941, P=0.1398); time of farming ($\rho$= 0.0100, P=0.8758); direct contact with the infected cow ($\rho$= 0.8149, P=0,000); history of brucellosis in human ($\rho$= 0.4399, P=0,000); a history of infected cattle ($\rho$= 0.5727, P=0,000).

Table 1. Respondent's charater

| Variable | Degree of agglutination score 0 | | Degree of agglutination score +1 | | Degree of agglutination score +2 | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| Sex | | | | | | |
| Male | 115 | 46,6 | 1 | 0,2 | 1 | 0,2 |
| Female | 132 | 53,1 | - | - | - | - |
| Age group | | | | | | |
| <45 years | 95 | 38,4 | 1 | 0,2 | - | - |
| 45-75 years | 151 | 61 | - | - | 1 | 0,2 |
| >75 years | 1 | 1,3 | - | - | - | - |
| Education | | | | | | |
| Not educated | 22 | 8,6 | - | - | - | - |
| Elementary school | 122 | 48,4 | 1 | 0,2 | 1 | 0,2 |
| Junior school | 51 | 21,8 | - | - | - | - |
| Senior school | 51 | 19,9 | - | - | - | - |
| University | 1 | 0,2 | - | - | - | - |

In this study two respondents who have a positive value were male and according to Spearman's test has no relation with the degree of agglutination at RBT test ($\rho$= -0.0956 P=0.1326). According to research [2] brucellosis in men is more common than in women by a ratio of up to 5: 2 to 5: 3 in endemic areas. The same conditions on age ($\rho$=0.0210 P=0.7418), one respondent has a positive value antibody titers were in the age group of less than 45 years. These results are supported by research Noor that the incidence of brucellosis occurred at the age of 30-50 years. It is probably due mostly engaged in the farm were male and age [2].

Cleaning the cage by spraying disinfectant is one effort to prevent the spread of brucellosis or transmission to humans. The use disinfectant to clean the cage has been done by 115 people (46.1%) and has a value of -0.0896 Spearman's Rho (P=0.586) when interpreted do not have a relationship with the degree of agglutination at RBT test. Two respondents, who have a degree of antibody titer positive, do not use disinfectant to clean the cage. In fact, according to research results [7] that the elimination, vaccination and purification process is the most appropriate strategy to control the disease brucellosis in humans.

Direct contact with infected animals have a correlation value of 0.8148 (P=0,000), which means to own a strong correlation with the degree of agglutination at RBT test. This is supported by research [5] which states that the handling in animals such as cleaning, flushing and treating and also helped give birth to be a risk factor for the disease brucellosis with OR 1.8 (95% CI 1.5-2.2; p 0.0001). There were a strong relationship between the handling of fetal cow that died of abortion and contact with the placenta of infected animals with the incidence of brucellosis in humans [4].

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Table 2. Result test of RBT

| Variables | Degree of agglutination score 0 | | Degree of agglutination score +1 | | Degree of agglutination score +2 | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| **Using protective equipment** | | | | | | |
| No | 91 | 36,8 | - | - | 1 | 0,2 |
| Yes | 156 | 63,1 | 1 | 0,2 | - | - |
| **Use disinfectant** | | | | | | |
| No | 123 | 49,8 | 1 | 0,2 | 1 | 0,2 |
| Yes | 124 | 50,2 | - | - | - | - |
| **Origin** | | | | | | |
| Bandung | 3 | 1,2 | - | - | - | - |
| Blitar | 13 | 5,2 | - | - | - | - |
| Boyolali | 4 | 1,6 | - | - | - | - |
| Yogyakarta | 5 | 2 | - | - | - | - |
| Klaten | 3 | 1,2 | - | - | - | - |
| Aid | 151 | 60,6 | 1 | 0,2 | 1 | 0,2 |
| Unknown | 68 | 28,4 | - | - | - | - |
| **Number of cattle** | | | | | | |
| ≤2 | 130 | 53,1 | - | - | - | - |
| >2 | 117 | 46 | 1 | 0,2 | 1 | 0,2 |
| **Time for farming** | | | | | | |
| ≤1 years | 9 | 3,7 | - | - | - | - |
| 2-4 years | 124 | 49,7 | 1 | 0,2 | - | - |
| >5 years | 114 | 45,8 | - | - | 1 | 0,2 |
| **Vaccine status** | | | | | | |
| Not yet | 226 | 91,5 | 1 | 0,2 | 1 | 0,2 |
| Yes | 21 | 8,5 | - | - | - | - |
| **Direct contact with cattle** | | | | | | |
| No | 246 | 98,8 | - | - | - | - |
| Yes | 1 | 0,2 | 1 | 0,2 | 1 | 0,2 |
| **History of brucellosis in human** | | | | | | |
| No | 239 | 95,8 | - | - | - | - |
| Yes | 8 | 3,2 | 1 | 0,2 | 1 | 0,2 |
| **History of brucellosis in cattle** | | | | | | |
| No | 243 | 97,5 | - | - | - | - |
| Yes | 4 | 1,6 | 1 | 0,2 | 1 | 0,2 |
| **Days of storage** | | | | | | |
| 9 | 2 | 1 | - | - | 1 | 0,2 |
| 10 | 8 | 3,2 | 1 | 0,2 | - | - |
| 12 | 12 | 3,2 | - | - | - | - |
| 13 | 26 | 10,44 | - | - | - | - |
| 14 | 26 | 10,44 | - | - | - | - |
| 15 | 22 | 8,84 | - | - | - | - |
| 16 | 23 | 9,24 | - | - | - | - |
| 18 | 18 | 7,23 | - | - | - | - |
| 19 | 22 | 8,84 | - | - | - | - |
| 20 | 19 | 7,63 | - | - | - | - |
| 21 | 21 | 8,43 | - | - | - | - |
| 22 | 20 | 8,03 | - | - | - | - |
| 23 | 19 | 7,63 | - | - | - | - |
| 25 | 9 | 3,61 | - | - | - | - |

Tabel 3. Correlation beetween risk factor and dgrees of agglutination using Spearman's Rho

| Variables | ρ | p |
|---|---|---|
| Age | 0,0210 | 0,7418 |
| Sex | -0,0956 | 0,1326 |
| Education | -0,0547 | 0,3900 |
| Using          protective equipment | -0,0243 | 0,7024 |
| Using desinfectant | -0,0896 | 0,1586 |
| Number of cattle | 0,0941 | 0,1389 |
| Time for farming | 0,0100 | 0,8758 |
| Vaccine status of cattle | -0,0273 | 0,6680 |
| Days of storage | -0,1508 | 0,0173 |
| Direct    contact    with infected | 0,8149 | 0,0000* |
| History of brucellosis in human | 0,4399 | 0,0000* |
| History  brucellosis  in  cattle | 0,5727 | 0,0000* |

The increasing spread of brucellosis in cattle can be due to the movement of livestock that less can be monitored by the farm workers, the cost of replacement cattle compensation positive reactors are expensive and lack of awareness and knowledge of farmers [2]. Lack of observed movement of livestock farmers do not know the result of bovine origin were maintained. Cows were coming from government assistance 153 (61.45%), Bandung 3 (1.2%), Blitar 13 (5.22%), Boyolali 4 (1.61%), Yogyakarta 5 (2%), Klaten 3 (1.2%) and do not know where it comes from 68 (27.3%). Confirmation has done that cows that were mentioned comes from aid, imported from East Java. Respondents also stated that they did not know where it came from, in the sense that when buying cattle that are not tracked from where. This shows a lack of awareness of the possibility of disease transmission.

Total cattle positive Brucella is 6 cows (2.41%) derived from breeders who were respondents. Based on Spearman test obtained value of 0.5727 (P=0,000), which means that this variable is related to the degree of agglutination at RBT test. These results are consistent with research [5] obtained results seroprevalen cows had an OR of 2.7 (95% CI 2.1-3.4; p 0.000), strongly associated with their seroprevalens in humans.

The duration of raising different for each individual farmer. In this study were divided into three groups: less than a year, 2-4 years and more than five years. The largest group is the group of 2-4 years and one of the farmers who had antibody titer is positive there is in this group. One other breeder was in a group of more than five years. In the calculation of Spearman's Rho result which means a long breeding 0.0100 unrelated to the degree of agglutination at RBT test. In univariate can be seen that the longest breeding group still shows the degree of antibody titer is positive, that is contrary to research that old work (over 20 years) reduces the risk factor of contracting brucellosis, with reason when people have already worked for decades, the task would become lighter or reduced contact with cattle (eg as supervisor).

Another attempt to prevent transmission of zoonotic diseases in humans include: control of zoonoses in animals with the eradication or elimination of animal positive serologically and through vaccination, monitoring the health of livestock and governance farms at the farmer level, disseminating early clinical symptoms of zoonotic diseases at the farm or slaughterhouse animals and immediately report and take action on livestock and workers who contracted the disease. Then tighten supervision of livestock traffic by implementing a strict quarantine system, especially from infected countries, banned imports of beef and its products, animal feed, hormones, bone meal, and

gelatin derived from cattle from countries that are not free of infectious diseases. Use protective equipment such as gloves, face mask, goggles, boots that can be disinfected, and a head covering when taking care of sick animals, maintain hygiene by washing hands before food processing after handling raw meat, handling carcasses or tending livestock [8]

## 4. CONCLUSION

In this study it can be concluded that there is a relationship between the direct contacts, history of infected cattle (seroprevalence in cattle), and knowledge of the degree of agglutination at RBT tests on dairy farmers in Sleman. Based on the conclusions that have been drawn then suggested to the community in anticipation of brucellosis transmission between animals by knowing the history of the cattle purchased or acquired and to the protection of yourself when handling infected cows Brucella. To the Health Department for Conducting surveillance against the disease brucellosis in humans and provide counseling to farmers and other workers about the disease brucellosis. The Department of Agriculture, Fisheries and Forestry to oversee livestock traffic in an effort to control the disease brucellosis in cattle and continue cutting program conditional if found positive cattle brucellosis.

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