



Risk Factor Identification and Incidence Rate Measurement on One High-Risk Farm as Brucellosis Precautions in Metro City Lampung

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 28 Nov 2023	<p>Since 2011, Lampung is one of the <i>Brucella</i>-free areas, but April 2021 reported abortion case in a cow farm at Metro city Lampung Province. The purpose of this study was to identify risk factors and measure incidence levels as vigilance re-emerging Brucellosis. Sample of cow serum, taken in a census from whole cattle cow in a farm at risk, as much as 2 times with a distance about 1 month. In the first census, 703 serum samples were taken from the stables and 25 samples from the adjacent. Whole sample tested to <i>Brucella</i> spp with rose bengal test (RBT) and positive result confirmed with complement fixation test (CFT). The second census was conducted on samples without positive CFT results in the first census. The positive RBT at second sensus were followed by CFT and c-Elisa tests. Incidence rate of brucellosis in high-risk housing in Banjarsari Village, North Metro District, Metro City 1,8% or 18 brucellosis per 1000 cows a month. <i>Brucella</i> spp. seropositivity was significantly related to the type of species and sex of the animal; animals with Ongole breed 4.2 times and female animals 4.5 times have a higher level of exposure compared to non-Ongole breed animals and males.</p> <p>Keywords: Brucellosis, census cattle, incidence, prevalence, risk factors.</p>
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1. Introduction

Brucellosis is generally caused by several species of the *Brucella* genus, namely *Brucella abortus*, *Brucella militensis*, and *Brucella suis* (OIE, 2018). Brucellosis in cattle is highly contagious and is caused by *Brucella abortus*. The main clinical symptoms in domestic livestock are characterized by abortion in old gestation, reproductive failure, orchitis, epididymitis, and hygroma (Nicoletti, 2013). The Central for Disease Control (CDC) (2019) says that more than 500,000 new cases of Brucellosis in humans have been reported worldwide in recent years.

Brucellosis is a disease found in many parts of the world. In Indonesia, it was first reported in 1935 and is still happening today. The islands of Java, Sulawesi, and East Nusa Tenggara are areas that are very prone to Brucellosis. The Directorate General of Livestock and Animal Health wants Brucellosis to be completely eradicated by 2025. Eradication of Brucellosis in an area is carried out based on the prevalence rate. The method of eradicating Brucellosis is by case detection and the slaughter of all reactors, vaccinations, traffic control, and disease tracking. Traffic control is carried out by establishing Brucellosis-free cattle status as the main requirement. Several official regulations strengthen this effort, including Regulation of the Minister of Agriculture Number 16/Permentan/OT.140/1/2010 concerning Guidelines for the Identification and Control of Large Ruminants which mentions Brucellosis as a disease that is important to monitor in every large ruminant livestock and the Decree of the Head Agricultural Quarantine Agency Number 853/Kpts/KH.020/L/5/2011 concerning Technical Guidelines for Animal Quarantine Measures for Cattle Traffic (Import and Between Areas) states that detention measures can be taken if laboratory test results are positive, one of which is Brucellosis . and then carried out conditional cuts (extermination). Several provinces and districts/cities in Indonesia also have the same rules to prevent the entry and spread of Brucellosis in their respective regions (Dirkeswan, 2015).

Lampung Province is one of the Brucellosis-free areas according to the Decree of the Minister of Agriculture Number: 5681/Kpts/PD.620/12/2011 dated December 30, 2011, concerning Statements of the Provinces of South Sumatra, Bengkulu, Lampung and the Bangka Belitung Islands Free from Infectious Animal Diseases (Brucellosis) in cattle. In April 2021 cases of miscarriage/abortion began at a cattle farm in Metro Lampung City in Banjarsari Village Metro North District Lampung Province. In June 2021 a census of RBT and CFT testing was carried out on all cattle on the farm and cattle belonging to the community directly adjacent to the farm, and positive results for brucellosis were found in 74 (seventy- four) cows, where positive cows were all from one farm. Seventy-four of the reactor's cows have been conditionally slaughtered. In July 2021, 18 positive cases were found again and they were conditionally cut again. On In February 2022, 3 positive cases were found again and in June 2022, 5 positive cases were found. All reactors have been conditionally slaughtered however cases of Brucellosis continue to be found until November 2022 in the same cage with a total finding of 110 positive cases of Brucellosis. Risk factors for the incidence of brucellosis occur more frequently in female cattle than in males, many attack PO and non-PO cattle, as well as adult age. Meanwhile, none of the dairy cows tested positive for Brucella. Meanwhile, all cow pens use communal/group pens, where each group of pens contains approximately 50 cows and is grouped according to age. The feed given is also the same in the form of forages and oil palm cake. At the time before the incident, Brucellosis used a natural mating system, but after the Brucella incident, neither natural mating nor artificial insemination was carried out. This study aims to identify risk factors and calculate the incidence rate for awareness of re-emerging Brucellosis in Metro City, Lampung Province.

Table 1. Development of Brucellosis cases in Lampung Province after being declared a Brucella-free area in the last 5 years:

Year	Number of Cases Brucellosis	Regency	Information
2017	0	-	-
2018	0	-	-
2019	2	Lampung Middle	New cattle imported from West Java
2020	3	Bandar Lampung	New cattle imported from West Java
2021	92	Metro	Deep cow 1 cage in Metro Lampung
2022	18	Metro	Case in Pen the same in Metro Lampung

Brucella Disease Map 2017-2021, Lampung Veterinary Center/Balai Veteriner Lampung

2. Materials and Methods

Sample

The entire population of cattle, namely 703 heads in a high-risk cattle pen in Metro City and 25 cows in the vicinity which are directly adjacent to the stables were blood drawn. Cows status is that some cows have a history of vaccination with Active Brucellosis Brucivet Strain 19 produced by Pusvetma and some are not vaccinated.

Time and Place of Research

Sampling was carried out in Banjarsari Village, North Metro District, Metro City in the work area of the Lampung Veterinary Center. Sample testing was carried out at the Bacteriology Laboratory of the Lampung Veterinary Center. The research will be carried out from November 2022 – March 2023.

Test Method

Tests were carried out by researchers without knowing the status (positive or negative) of each sample that tested (double-blind). Serological testing to determine brucella abortus infection was carried out with the Rose Bengal test (RBT), complement fixation test (CFT), and competitive ELISA (c-ELISA). The RBT antigen used came from Pusvetma Surabaya and the CFT antigen came from ID Vet. The principle of the RBT test is the presence of homologous antigen and antibody bonds marked by the presence of agglutination labeled with Rose Bengal dye. A positive result is indicated by the presence of agglutination and a negative result is if there is no agglutination. The complement fixation test works by detecting anti-Brucella antibodies that can activate complement. Results were read by observing erythrocyte deposition at the bottom of the well plate. A negative reaction will not show erythrocyte

precipitate and the supernatant will be red due to hemoglobin. A positive reaction is indicated by the presence of erythrocyte precipitates with a supernatant that looks clear (colorless).

In the c-ELISA method, the antibody in the sample competes for binding to the antigen it is bound to a well microplate with A little marker antibody. c-ELISA can reduce the reaction yang caused by the production antibody as a response to vaccination. Very possible to increase the specificity test however will lower c-ELISA sensitivity compared with i-ELISA.

3. Results and Discussion

A total of 703 cows had their serum taken and then examined by the Lampung Veterinary Center/Balai Veteriner Lampung for Brucellosis and the results can be seen in Table 2.

Table 2. Summary of Brucellosis Serological Test Results with RBT and CFT in the First Census

Location	Serum Amount	RBT		CFT	
		Positive	Negative	Positive	Negative
Pen	703	80	648	74	6
Around the Cage	25	0	0	0	0

In the RBT test at the barn, 11.38 % (80/703) of cows showed seropositive results and 92.2 % (648/703) were seronegative. The seropositive results in the RBT test were continued to the CFT test, and the results were 10.5 % positive samples (74/703) and the rest were CFT negative. This shows that the brucellosis reactor was still found in the cage. While in the surrounding environment, no cases of brucellosis were found. Under the definition of a positive case of brucellosis, if the RBT and CFT tests are both positive. So it is known that 74 cows were showing seropositive results on the RBT and CFT tests. The 74 cows were removed from the population and then the serum was collected again after 1 month in the remaining cows.

Table 3 Summary of Brucellosis Serological Test Results with RBT, CFT, and C-ELISA in the Second Census

Location	Serum Amount	RBT		CFT		cELISA	
		Positive	Negative	Positive	Negative	Positive	Negative
Pen	616	24	592	19	5	11	13
Around the Cage	21	2	0	2	0	2	0

In the second census, an additional test method was added, namely the c-ELISA because there was information that the brucellosis vaccination had been carried out in the cage since April 2022 or 7 months earlier. The c-ELISA test is expected to be able to distinguish between seropositive due to natural infection or due to vaccination. In the 2nd census, 3.8 % (24/616) of the RBT tests showed seropositive results and 9.61 % (592/616) were seronegative. Then the seropositive results in the RBT test were continued to the CFT and c-ELISA tests, and the results were 3% (19/616) CFT positive samples and the rest were CFT negative. The seropositive results of the c-ELISA test were 1.7 % (11/616) and the rest were c-ELISA negative. So that it is known that there are 11 cows in the stable that show seropositive results on the RBT, CFT, and c-ELISA tests. While the results of testing on cows in the environment around the stables showed seropositive results to the RBT, CFT, and c-ELISA tests of 9.5% (2/21) and the rest were negative.

Table 4. Summary of Brucellosis Serological Test Results with RBT, CFT and c-ELISA in Census 1 and Census 2

Testing	Serum Amount	RBT		CFT		c-ELISA	
		Positive	Negative	Positive	Negative	Positive	Negative
Census 1	703	80	648	74	6	-	-
Census 2	616	24	592	19	5	11	13

The number of new cases added is calculated as an incidence rate, which is a way of measuring how often new cases occur. From the data, it is known that the increase in cases from 1st census to 2nd

census within 1 month increased from 11 cases of brucellosis. So the cumulative incidence rate of brucellosis in the cage is 1.8% or 18 brucellosis per 1000 cows a month. The distribution of 11 additional cases based on risk factors in 2nd census can be seen in Table 5 as follows:

Table 5. Recapitulation of brucellosis cases based on risk factors

Brucellosis Testing		Census 1			Census 2		
No	Risk factor	Positive	Negative	Amount	Positive	Negative	Amount
I	Female	58	246	304	8	221	229
	Male	16	383	399	3	384	387
	Total I	74	629	703	11	605	616
II	Wean (0-4 months)	0	10	10	0	13	13
	Young (4-18 months)	37	494	531	10	496	506
	Mature (>18 months)	37	125	162	1	96	97
	Total II	74	629	703	11	605	616
III	Dairy	6	36	42	1	37	38
	Ongole/PO breed	26	278	304	8	272	281
	Non Ongole/Non PO	42	315	357	2	296	298
	Total III	74	629	703	11	605	616
IV	Vaccine	72	535	607	11	474	485
	No Vaccine	2	94	96	0	131	131
	Total IV	74	629	703	11	605	616

Lampung Province has been declared free of Brucellosis since 2011, however, cases of brucellosis were still found in Metro City in 2020 and tests and slaughter have been carried out on all cases of brucellosis in Metro City. Therefore, surveillance is carried out to ensure that brucellosis-free conditions can be maintained by implementing Risk Based Surveillance, by taking census samples from sub-populations that have a high risk of transmission of Brucellosis.

Final surveillance results in high-risk pens showed a prevalence rate of 1.7 % of the 616 animals tested and an incidence rate of 1.8% or 18 brucellosis per 1000 cows a month. The cages that were censused were cages that had a history of previous cases of brucellosis. This shows that transmission is still occurring in the cage even though the previous brucellosis reactor has been cut. Some of the reactors had a history of miscarriages in pens, the reactors were kept in pens with other livestock and ate from the same food and water bowls. the possibility of transmission occurs directly, namely orally (feed and cage equipment) or contaminated by abortion. Brucella bacteria are known to survive in a variety of different environmental conditions at certain times. Brucella can survive 2 days in manure or some manure at relatively high temperatures. In drinking water, bacteria can live 5 - 114 days, and 30 - 150 days in wastewater (Sudibyo, 1995). This is in line with Brubaker, 1985 which states that cattle can contract brucellosis after eating or drinking food and drink contaminated with abortifacients. In addition, the effects of weather such as the rainy season, humidity, low temperatures, and little sunlight cause these organisms to be found in water, aborted fetuses, wool, straw, mud, tools, and clothing (Budiharta, S. and Widiasih, AD 2012).

Table 6. Results of Bivariate Analysis of Risk Factors for Brucellosis (95% Confidence Interval)

No.	Variable	Ket	Brucellosis			Chi Square	p-Value	RR	OR
			Positive	Negative	Amount				
1	Gender	Female	8	221	229	6,061	0.007	4.50	4.62
		Male	3	384	387				
2	Age	Wean	0	13	13	0.262	NA	NA	NA
		Young	10	496	506				
		Young	10	496	506	0.406	NA	NA	NA
		Mature	1	96	97				
		Wean	0	13	13	0.135	NA	NA	NA
		Mature	1	96	97				
Weaning+Young	10	509	519	0.374	NA	NA	NA		
Mature	1	96	97						
3	Species	PO	8	272	280	4,057	0.021	4,25	4.35
		Non PO	2	296	298				
		PO	8	272	280	0006	NA	NA	NA
		Dairy	1	37	38				
		Non PO	2	296	298	1,464	NA	NA	NA
		Dairy	1	37	38				
		PO+ non-PO	10	568	578	0.1652	NA	NA	NA
		Dairy	1	37	38				
4	Vaccination	Vaccine	11	474	485	3.025	NA	NA	NA
		No Vaccine	0	131	131				

From the data in Table 6, it is known that the strength of the association of risk factors for the incidence of brucellosis is as follows: Risk factors for gender (male and female). Based on the chi-square analysis, it is known that χ^2 is $6.061 > \chi^2 \text{ tabel} = 3.84$ ($\alpha = 0.05$, $df = 1$) so that it is significant, that is, H_0 is rejected, while H_a is accepted. It can be concluded that there is a relationship or association between Brucellosis and gender. With a Risk Ratio/RR value of 4.5, it means that $RR > 1$ or a positive effect. This means that the possible risk of being infected with brucellosis in cows is 4.5 times greater when compared to bulls. While the Odds Ratio/OR value is $4.6 > 1$ or positive effect. The interpretation is that the probability of brucellosis occurring in cows is 4.6 times faster than in bulls.

Age of cattle risk factors (weaning, young, and adults). Based on the chi-square analysis of comparisons between weaning and young cattle compared to adult cattle, it is known that χ^2 is $0.374 < \chi^2 \text{ tabel} = 3.84$ ($\alpha = 0.05$, $df = 1$) so that it is not significant, H_0 is accepted, while H_a is rejected. It can be concluded that there is no relationship or association between Brucellosis disease and weaning age between young and adults.

Between weaning and young cows, it is known that χ^2 is $0.262 < \chi^2 \text{ tabel} = 3.84$ ($\alpha = 0.05$, $df = 1$) so that it is not significant, H_0 is accepted, while H_a is rejected. It can be concluded that there is no relationship or association between Brucellosis and the age of weaning and young cows. Whereas in the chi-square analysis of the comparison between weaning cattle and adults, it is known that χ^2 is $0.135 < \chi^2 \text{ tabel} = 3.84$ ($\alpha = 0.05$, $df = 1$) so that it is not significant, ie H_0 is accepted, while H_a is rejected. It can be concluded that there is no relationship or association between Brucellosis and the age of weaning and adult cows. Based on the chi-square analysis of the comparison between heifers and adults, it is known that χ^2 is $0.406 < \chi^2 \text{ tabel} = 3.84$ ($\alpha = 0.05$, $df = 1$) so that it is not significant, ie H_0 is accepted, while H_a is rejected. It can be concluded that there is no relationship or association between Brucellosis and the age of young and adult cattle. According to Amna et al., (2020), age has a significant effect on brucellosis infection. Age and cattle rearing patterns are risk factors for brucellosis infection based on research by Sari and Mulyani (2015). Cattle kept together with other livestock or

kept extensively have a greater chance of being infected with brucellosis. However, in this study, it was not proven that age had a significant effect on the incidence of brucellosis because cattle were kept intensively in pens without being grazed.

Risk factors for the type of species (dairy, Ongole/PO breeds, and non-Ongole/PO breeds). Based on the chi-square analysis, the comparison between dairy cows and beef cattle (Po and non-PO) shows that χ^2 is $0.165 < \chi^2 \text{ tabel} = 3.84$ ($\alpha = 0.05$, $df = 1$) so that it is not significant, ie H_0 is accepted, while H_a is rejected. It can be concluded that there is no relationship or association between Brucellosis and the types of dairy cattle and beef cattle. Based on the chi-square analysis, the comparison between Ongole/PO cross-breed cattle and non-Ongole/non-PO cross-breed cattle is known that χ^2 is $4.057 > \chi^2 \text{ tabel} = 3.84$ ($\alpha = 0.05$, $df = 1$) so that it is significant, that is, H_0 is rejected, while H_a is accepted. It can be concluded that there is a relationship or association between Brucellosis and PO and non-PO cattle species. With a Risk Ratio /RR value of 4.2, it means that $RR > 1$ or a positive effect. This means that the possible risk of being infected with brucellosis in PO cattle is 4.2 times greater when compared to non-PO cattle. While the Odds Ratio/OR value is $4.3 > 1$ or positive effect. The interpretation is that the probability of developing brucellosis in PO cattle is 4.3 times faster than non-PO cattle.

Vaccination using *B. abortus* S19 which is known to interfere with the diagnosis of brucellosis can also exaggerate the prevalence due to the persistence of antibodies after vaccination (Dorneles et al. 2015). This is supported by data on vaccination risk factors in Table 3 census 1 as follows, based on chi-square analysis it is known that χ^2 is $11.5 > \chi^2 \text{ tabel} = 3.84$ ($\alpha = 0.05$, $df = 1$) so that it is significant, that is, H_0 is rejected, while H_a is accepted. It can be concluded that there is a relationship or association between Brucellosis and vaccination. With a Risk Ratio /RR value of 7.17, it means that $RR > 1$ or a positive effect. This means that the possible risk of being infected with brucellosis in vaccinated cows is 7.17 times greater when compared to non-vaccinated cows. While the Odds Ratio/OR value is $8.007 > 1$ or a positive effect. The interpretation is that the incidence of brucellosis in vaccinated cows is 8.007 times faster than in unvaccinated cows. Some of the samples tested came from cattle that had been vaccinated with the active brucella Brucivet Strain 19 produced by Pusvetma. The sensitivity of the RBT is very high but the specificity of the test is low, and this test is not suitable for use in animals with a history of vaccination (Smirnova et al., 2013). A diagnosed positive serum sample should then be confirmed by a definitive test. Several countries in the world use the RBT as a screening test followed by the CFT test as a confirmatory test in diagnosing Brucellosis (Khan et al., 2017). Complement Fixation Test is highly specific but less sensitive than RBT and ELISA. The CFT test will show positive results in post-vaccinated ruminants of *B. abortus* S19 or *B. militensis* Rev. 1 is the same as other serological tests (Saavedra et al., 2019). Based on the above, it can be concluded that the RBT and CFT tests alone are not effective enough to determine the diagnosis of brucellosis in areas with a history of vaccination. For this reason, it is necessary to carry out further tests to distinguish whether the seropositivity detected in the RBT and CFT tests originates from natural infections or vaccinations with the c-ELISA test. From Table 3 data in the 2nd census, it is known that the CFT test yielded 19 seropositive results, while the c-ELISA test yielded 11 seropositive results. Because after testing a test and slaughter policy will be implemented, of course, this difference is very helpful for farmers because they can save their 8 cows.

However, in this study, positive cases were still found in cattle that had not been vaccinated both in the pen (census data 1) and in the environment around the pen (census data 2). This shows that there is still a brucellosis reactor. According to Enaro (2020), bacteria can survive for more than 4 months in milk, urine, water, and soil. Areas that are positive for brucellosis are areas that have a previous history of brucellosis, areas with a high number of cows, and high mobilization of livestock sales between regions and without inspection by authorized veterinarians.

Risk factors for vaccination status (vaccinated and not vaccinated). Based on the chi-square analysis, the comparison between vaccination and not vaccination is known that χ^2 is $3.025 < \chi^2 \text{ tabel} = 3.84$ ($\alpha = 0.05$, $df = 1$) so that it is not significant, ie H_0 is accepted, while H_a is rejected. It can be concluded that there is no relationship or association between Brucellosis and vaccination status.

The survey results showed that *Brucella* spp. seropositivity is significantly related to the type of species and sex of the animal; Animals with Ongole breed and female animals have a higher level of exposure compared to non-Ongole breed animals and males. Female animals can have repeated exposure to *Brucella* spp. because they are more likely to stay longer in a flock than males because they are kept for breeding purposes (Kairu et al., 2019).

4. Conclusion

The prevalence of brucellosis in high-risk cages in Banjarsari Village, Metro Utara District, Metro City is 1.7 % and the incidence rate is 1.8% or 18 brucellosis per 1000 cows a month. The possible risk of being infected with brucellosis in cows is 4.5 times greater than in bulls. The probable risk of being infected with brucellosis in Ongole cattle is 4.2 times greater when compared to non-Ongole cattle. There is no relationship or association between brucellosis and age. The vaccination program that has been carried out in the pen has failed and does not protect the livestock.

Recommendation

In areas with a history of brucellosis vaccination, it is necessary to carry out the c-ELISA test because the CFT test as a confirmatory test is not effective in determining the diagnosis of brucellosis. To support brucellosis-free status, it is necessary to have seriousness and high commitment from the regional government of Metro City and Lampung Province to cooperate in eradicating brucellosis.

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Conflict Of Interest

We certify no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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