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Seroprevalence, Accuracy and Precision Value of Brucellosis Surveillence at The Region Area of Balai Karantina Pertanian Kelas I Balikpapan

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ABSTRACT

The Technical Implementation Unit Balai Karantina Pertanian Kelas I Balikpapan plays a role in efforts to prevent the entry, spread, and release of HPHK. Direct observation was performed by surveying the target brucellosis in the region of Balai Karantina Pertanian Kelas I Balikpapan. This surveillance aimed to determine the seroprevalence of brucellosis and to support the maintenance of brucellosis status in East Kalimantan. Sampling in areas with reported clinical symptoms and brucellosis reactors. The sampling areas were based on the region of Balai Karantina Pertanian Kelas I Balikpapan in Balikpapan City, North Penajam Paser Regency, Paser Regency, and Kutai Kartanegara Regency. The method for calculating the number of samples to detect the disease uses the Rose Bengal Test (RBT) and Complement Fixation Test (CFT). The test results showed a seroprevalence of 0.29%, positive and negative predictive values of 50% and 99,7%, respectively, an accuracy value of 99.11%, and a precision of 50%. The test performance based on the accuracy value was excellent because it had a value of 99.11%, which means that the ability of the CFT to detect all samples tested correctly was 99.11%. The test carried out using the CFT test on this surveillance had a precision or test consistency of 50%, and the sensitivity and specificity were 50% and 99.7%, respectively.

Keyword: Accuracy, Brucellosis, Complement Fixation Test, Balikpapan Agricultural Quarantine, Seroprevalence

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INTRODUCTION

The Agricultural Quarantine Agency through the Technical Implementation Unit Balai Karantina Pertanian Kelas I Balikpapan plays an active role in preventing the entry and spread of Pests and Diseases Quarantine Animals. Chapter 11 (2) of the Regulations Government Number 82 Year 2000 on quarantine animals states that apart from being carried out at the point of entry while the carrier media is being sequestered to observe the appearance of HPHK symptoms, observation also means observing the situation of quarantine animal pests in a country, area, or place. Observation of the HPHK situation can be done in two ways: observing directly or indirectly.

Direct observations were made by taking samples directly from the Balai Karantina Pertanian Kelas I Balikpapan Technical Implementation Unit Balai Karantina Pertanian Kelas I Balikpapan by implementing surveillance of HPHK distribution areas based on Regulation Minister Agriculture Number 22/ Permentan /OT.140/4/2008 concerning the Organization and Work Procedure of the Agricultural Quarantine Technical Implementation Unit. The surveillance function was then carried out by observing the status and situation of HPHK, especially against brucellosis disease caused by *Brucella abortus* in the distribution area of Balikpapan, namely Balikpapan, Kutai Kartanergara, Penajam Paser Utara, and dan Paser.

Brucellosis is a disease of the reproductive organs that causes a decrease in livestock productivity. Serological surveillance of *B. abortus* in cattle is expected to be useful in detecting the possibility of brucellosis cases occurring in the cattle population in an area earlier; thus, efforts to increase the livestock population can avoid the potential for reproductive disorders in cattle in the region of Balai Karantina Pertanian Kelas I Balikpapan.

Kalimantan Island, especially East Kalimantan Province, is declared free of brucellosis based on the Decree of the Minister of Agriculture Number 2540/Kpts/Pd.610/6/2009 Concerning the Declaration of Kalimantan Island Free from brucellosis in cattle and buffalo so that surveillance activities are carried out. This will provide benefits in the form of an overview of the status and situation of brucellosis in an area that is spreading for animals imported through the Balikpapan seaport.

The results of seroprevalence and hypothesis testing can be used as a policy basis to prevent the spread of HPHK within the Republic of Indonesia, especially in Balikpapan. This hypothesis test was conducted to determine whether the Balai Karantina Pertanian Kelas I Balikpapan work area had a seroprevalence of less than 0.2%. An area is said to be free of brucellosis if its prevalence is less than 0.2% (Nasution

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2020). In addition, in the brucellosis test, the accuracy, precision, predictive, and negative values of the test will be calculated to determine whether the test has good accuracy and precision values and the predictive and negative values of the test results. This illustrates the effectiveness of the tests on surveillance brucellosis in the Balai Karantina Pertanian Kelas I Balikpapan areas.

MATERIAL AND METHOD

Sampling Area

Areas sampled for surveillance included areas where abortions were reported or clinical symptoms were observed, where brucellosis reactors had occurred, and areas with dense livestock populations. This area is based on the work area of Balai Karantina Pertanian Kelas I Balikpapan, namely Balikpapan, Penajam Paser Utara, Paser, and Kutai Kartanegara.

Sample Counting

The number of samples was calculated using the detection disease method because Kalimantan is declared free of brucellosis in cattle and buffalo, based on the Decree of the Minister of Agriculture No.2540/Kpts/PD.610/6/2009. The sample size required for laboratory testing in surveillance activities was calculated using the following formula.

$$n = [1 - (1 - a)^{\frac{1}{D}}][N - \frac{(D - 1)}{2}]$$

Description :
n = Samples
a = Confidence level
N = Amount Population
D = Approx animal Sick in the population

This equation calculates the sample size needed for laboratory testing using an epidemiological application, namely Epitools (Ausvet, 2021). This site was developed and maintained by Ausvet and is used by epidemiologists and researchers involved in estimating disease prevalence or demonstrating disease-free status through structured survey.

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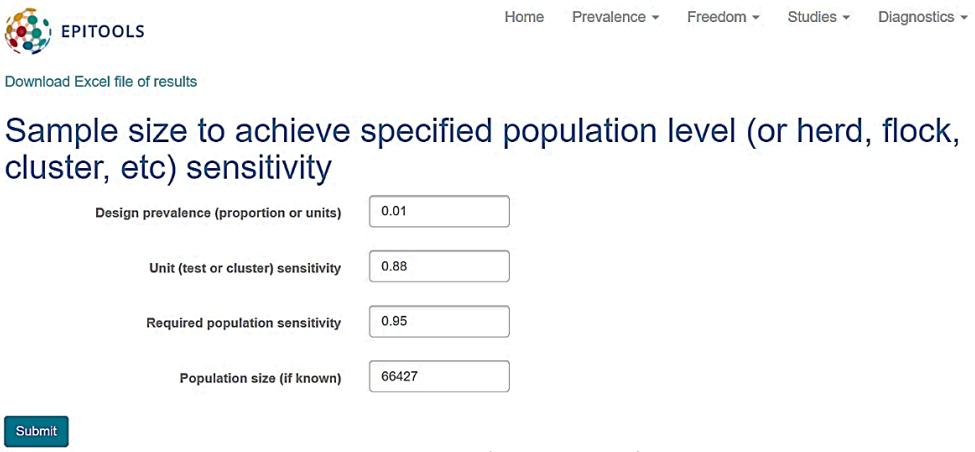

Figure 1. Sample size to achieve

Sampling and Testing

Sampling for surveillance to maintain areas that have been declared free has been mutually agreed upon based on the results of coordination with Balai Veteriner Banjarbaru. Sampling during surveillance activities was carried out by quarantine officers and accompanied by officers from the local service from April to August 2021. The brucellosis test sample was in the form of blood serum from beef cattle in the Balai Karantina Pertanian Kelas I Balikpapan-work area. Sample testing in the form of RBT and CFT tests was performed at the Balai Karantina Pertanian Kelas I Balikpapan Laboratory.

Figure 2. Surveillance sampling

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Data Analysis

The data obtained were calculated by calculating the seroprevalence of the total sample and testing the hypothesis to determine whether the seroprevalence of brucellosis in the Work Area of Balai Karantina Pertanian Kelas I Balikpapan is <0.2%. In addition, the accuracy, precision, and positive and negative predictive values of the tests were calculated.

RESULTS

Counting Sample Surveillance

Based on the results of sample calculations using the Epitools application (Ausvet, 2021), 340 samples were obtained based on the sensitivity of the RBT test is 88% (Muslimin *et al*., 2017) with a total population in Balikpapan, Penajam Paser Utara, Paser, and Kutai Kartanegara of 666,427 head of cattle. Subsequently, 340 samples were proportioned in each of the four areas.

The proportion of samples per district was calculated based on the cattle population in each district/city in Balai Karantina Pertanian Kelas I Balikpapan. The cattle populations from the Dinas Peternakan dan Kesehatan Hewan Provinsi Kalimantan Timur were as follows: Balikpapan 1,308, Penajam Paser Utara 16,463, Kutai Kartanegara 18,626, and Paser 30,03. The calculation is as follows:

$$\frac{\text{Cattle Population}}{\text{Total Population}} \times 340 = \frac{1308}{66427} \times 340$$

$$= 7 \text{ samples}$$

Sampling and Testing

The sampling technique was based on visible clinical symptoms and the presence of a brucellosis reactor. The targets and results of sampling are presented in Tables 1 and 2.

Table1. Brucellosis surveillance sampling targets and their test results

NO	DISTRICT	MP HPHK	TEST TYPE	SAMPLE NUMBER	INTERPRETATION	
					POSITIVE	NEGATIVE
1	Balikpapan	Cattle	RBT	7	-	7
2	Paser	Cattle	RBT	84	-	84
3	Penajam Paser Utara	Cattle	RBT	95	1	94
4	Kutai Kartanegara	Cattle	RBT	154	1	153
	TOTAL			**340**	**2**	**338**

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Table 2. CFT test sesults on Brucellosis surveillance

NO	DISTRICT	MP HPHK	TEST TYPE	RESULTS
1	Penajam Paser Utara	Cow	CFT	1 Seronegative
2	Kutai Kartanegara	Cow	CFT	1 Seropositive

Based on clinical symptoms in the field, namely, the presence of abortion or miscarriage, which is a clinical symptom of brucellosis (Ditjen PKH, 2014), based on RBT and a history of cases, CFT was carried out, one positive sample was obtained, and one negative sample.

Figure 3. RBT testing of surveillance sample

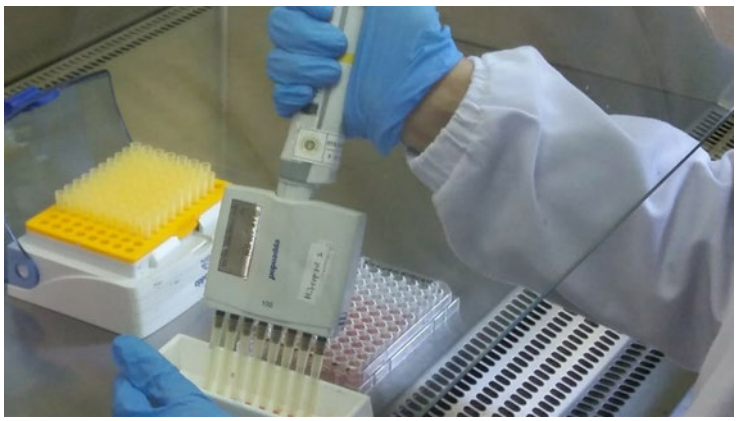

Figure 4. CFT testing of surveillance sample

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Seroprevalence

The calculation of seroprevalence in the surveillance of brucellosis carried out by Balai Karantina Pertanian Kelas I Balikpapan was as follows (Tenny, 2023).

$$\text{Seroprevalence} = \text{Total Seropositive} / \text{Total Sample} \times 100$$
$$= (1/340) \times 100$$
$$= 0.29\ \%$$

Data analysis was carried out to determine whether brucellosis seroprevalence in the work area of Balai Karantina Pertanian Kelas I Balikpapan was below 0.2%, which is required for a brucellosis-free area.

H 0: P ≥ 0.2 (brucellosis prevalence in the Balikpapan work area is ≥ 0.2%).
H 1: P < 0.2 (brucellosis prevalence in the Balikpapan work area less than 0.2%)

$$z = \frac{\hat{p} - p}{\sqrt{\frac{p(1-p)}{n}}}$$
$$= \frac{0{,}29 - 0{,}2}{\sqrt{\frac{0{,}2 \times 0{,}8}{340}}}$$

$$= 0.09/0.021$$
$$= 4.28 \quad \text{(Kiernan, 2021)}$$

P(z<4.28) ≈ 1, P-Value > 0.05, indicates that there is not enough evidence to show that the Balai Karantina Pertanian Kelas I Balikpapan work area has a seroprevalence of less than 0.2%. The Balai Karantina Pertanian Kelas I Balikpapan work area, which consists of Balikpapan, Penajam Paser Utara, Paser, and Kutai Kartenegara, has a seroprevalence of > 0.2%.

Predictive Value Test (Akobeng, 2007)

The predictive value of the test on surveillance of brucellosis carried out by Balai Karantina Pertanian Kelas I Balikpapan was calculated as follows:

$$\text{Positive P test } (D+ \mid T+) = TP/(TP + FP)$$
$$= 1/\ (1+1)$$
$$= 0.5 \times 100\%$$
$$= 50\ \%$$

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$$\text{Negative (D - } | \text{ T-)} = TN/(FN+TN)$$
$$= 337/338$$
$$= 1 \times 100\%$$
$$= 99.7\ \%$$

Accuracy and Precision

The calculation of the accuracy and precision of the test on the surveillance of brucellosis carried out by the Balai Karantina Pertanian Kelas I Balikpapan is as follows (Noerjanto, 2014).

$$\text{Accuracy} = (TP+TN/TP+FP+FN+TN) \times 100\ \%$$
$$= (1+337)/(1+1+1+337) \times 100\%$$
$$= (337/340) \times 100\ \%$$
$$= 0.9978 \times 100\%$$
$$= 99.11\%$$

$$\text{Precision} = (TP/FP+TP) \times 100\%$$
$$= (1/1+1) \times 100\%$$
$$= (1/2) \times 100\ \%$$
$$= 0.5 \times 100\ \%$$
$$= 50\%$$

The 95% confidence interval for accuracy and precision was calculated by simulating 100 random samplings using the R Studio software. The calculation for 154 samples in Kutai Kartanegara is as follows:

```
1=TP, 2=TN, 3=FP, 4=FN
> city3= c( data Kukar )
Kukar = sample(kota3,154)
```

The results of 100 runs yielded an accuracy of 99.41%. After calculating the average and standard deviation for the Balikpapan, PPU, Paser, and Kutai Kartanegara regions, an average accuracy of 99.85 was obtained with a standard deviation of 0.29. These results were entered into the 95% confidence interval formula: $\bar{x} \pm z\ \alpha/2\ SD = 99.85\% \pm 0.56$.

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Sensitivity and Specificity

Calculation of sensitivity and specificity values on surveillance of brucellosis carried out by the Balai Karantina Pertanian Kelas I Balikpapan as follows (OIE, 2018):

$$\begin{aligned}\text{Sensitivity} &= TP/(TP + FN) \times 100\% \\ &= 1/(1+1) \times 100\% \\ &= (1/2) \times 100\% \\ &= 50\%\end{aligned}$$

$$\begin{aligned}\text{Specificity} &= TN/(FP+TN) \times 100\% \\ &= 337/(1+337) \times 100\% \\ &= (337/338) \times 100\% \\ &= 99.7\ \%\end{aligned}$$

DISCUSSION

From the results of testing the samples with the RBT test and CFT, it was found that one sample was positive. One positive sample described a seroprevalence of 0.29 % %in the Balikpapan, Penajam Paser Utara, Paser, and Kutai Kartanegara areas. Based on these results, there are still cases of brucellosis. Based on the 2020 brucellosis-free status of the Directorate of Animal Health, East Kalimantan remains brucellosis-free.

The results of testing the hypothesis that H0 is not rejected indicate that the Balai Karantina Pertanian Kelas I Balikpapan work area has a seroprevalence of less than 0.2%. This condition can be used as information by relevant agencies that the brucellosis seroprevalence carried out through surveillance by Balai Karantina Pertanian Kelas I Balikpapan is more than 0.2%. These results can be used as material for further study in considering brucellosis-free status in East Kalimantan, where the tolerance limit for brucellosis-free status is (prevalence <0.2%) (Nasution, 2019). In addition, the existence of seropositive findings from surveillance results and the results of this hypothesis test conducted by Balai Karantina Pertanian Kelas I Balikpapan can be a joint study with relevant agencies to determine the risk factors for the presence of brucellosis cases and strategic steps to keep East Kalimantan free from brucellosis.

Predictive validity was assessed using positive and negative predictive values (Safitri, 2022). The predictive value is one of the criteria used to determine the reliability of a diagnostic test. This value can be used if the diagnostic test characteristics are

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considered when making decisions. The positive predictive value is the proportion of positives that are positive among the positive results detected by the test (Pisestyani *et al*., 2023). The positive predictive value obtained from the results of the surveillance of brucellosis in the Balai Karantina Pertanian Kelas I Balikpapan work area was 50%. These results illustrate that tests carried out with the CFT test could detect positive results in 50% of sick animals. The CFT test is recommended as a complementary test and confirmatory test (Rosmiati and Kurniawan 2022).

The positive predictive value of test results in surveillance is thought to be influenced by the presence of one false-positive result. One of the causes of false positives in tests for brucellosis is that the structure of the polysaccharide O chain of *Brucella abortus* is similar to that of other bacteria (Khan and Zahoor, 2018). The O chain of the lipopolysaccharide of *Brucella abortus* is similar to that of *Y. enterocolitica* O:9 and *E. coli* O:157. This factor can cause false positives in brucellosis tests for brucellosis (Kusuma *et al*., 2021).

The negative predictive value refers to the proportion of patients who are negative (healthy) among all patients who show negative test results (Safitri, 2021). In this case, the cows tested negative after the diagnostic test. The negative predictive value of the test using CFT in the surveillance was 99.7%, which means that the CFT test in surveillance brucellosis in the Balai Karantina Pertanian Kelas I Balikpapan working area was able to detect a negative result from cows that were not sick by 99.7%. This result was allegedly because no false negatives were detected at the time of testing. In a surveillance brucellosis study conducted by Balai Karantina Pertanian Kelas I Balikpapan, it was found that the negative predictive value was higher than the positive predictive value, which indicates that there are positive cases from the results of the screening test with RBT. However, many cattle do not have brucellosis or test negative results.

Diagnostic test performance can be seen in two dependent measures: accuracy and precision. The term accuracy is a synonym for validity. Accuracy is the closeness between the test results and the true value. Precision refers to the closeness between repeated measurements of the same sample under specified conditions (Greiner, 2000). In calculating the accuracy, the result was 99.11% with a confidence interval of 99.85% ± 0.56. This value indicates that the ability of the CFT test to detect all tested samples correctly was predicted to be 99.11% (Noerjanto, 2014). Tests carried out using the CFT test as test confirmation can be said to be accurate and good at detecting brucellosis in cattle in the Balai Karantina Pertanian Kelas I Balikpapan work area because the accuracy value based on testing is more than 50% (Widiyawati, 2018).

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Test accuracy shows the ability of a test to provide true results. The ability of the CFT to provide true results for this surveillance was very high (99.11%). The high accuracy value of the complement fixation technique is used as a confirmatory test for *B. abortus, B. melitensis,* and *B. ovis* infections, and it is the reference test recommended by the OIE for international animal traffic requirements (OIE, 2009). The precision was calculated to be 50%. The precision value indicates the consistency of the test results obtained using a certain method, that is, CFT. This precision result indicates that the CFT test performed on brucellosis under surveillance conducted by Balai Karantina Pertanian Kelas I Balikpapan has a test result consistency of 50%.

The sensitivity value obtained from the calculation results was 50%. Sensitivity is the ability of a diagnostic tool to identify patients with a disease with positive results (Gordis, 2014). A diagnostic test equipment with high sensitivity is required to detect the disease. Several factors can influence high sensitivity, including a high true positive value, which is a sick condition, and positive test results. In addition, it is influenced by the low value of false negatives (Noerjanto, 2014).

Meanwhile, low-sensitivity results increase false negatives. In the calculation results, the specificity is 99.7%. Test specificity indicates the ability of a test to show negative results in samples that do not suffer from the disease (Gordis, 2014). The specificity results for surveillance conducted by Balai Karantina Pertanian Kelas I Balikpapan are high, which means that the CFT test in this surveillance is predicted to be able to detect negative results from cattle that do not suffer from brucellosis (99.7%). A high specificity is needed to strengthen the suspicion of the disease. From the results obtained, it is suspected that there were many true negative results for surveillance brucellosis.

CONCLUSION

The results of the tests conducted showed that the brucellosis seroprevalence in the Balai Karantina Pertanian Kelas I Balikpapan work area was 0.29%. The positive predictive value of the surveillance results against brucellosis in the Balai Karantina Pertanian Kelas I Balikpapan work area was 50%, which illustrates that the test performed with CFT can detect a positive result in sick animals by 50%. This could be due to 1 a positive result. The negative predictive value of the test using the CFT was 99.78%. The CFT test is very good at surveillance brucellosis in the Balai Karantina Pertanian Kelas I Balikpapan work area because it can detect negative results from healthy livestock (99.78%). The test performance based on the accuracy value is very good, with a value of 99.11%, which means that the ability of the CFT to

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detect all samples tested correctly was 99.11%. Tests carried out with the CFT on this surveillance have a precision or test consistency of 50%. The sensitivity and specificity values of the tests performed for brucellosis surveillance were 50% and 99.7%, respectively.

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