Seroprevalence of Brucellosis reactor among goats and sheep on an agribusiness farm in a peri-urban of Bogor, Indonesia

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Abstract. Brucellosis in small ruminants is a challenge in agriculture, particularly livestock development. In Indonesia, the determinants of the epidemiology of the disease are not widely known or reported. This study aimed to investigate the brucellosis reactor in goats and sheep to provide evidence for controlling the disease. A total of 216 sera samples (102 sheep and 114 goats) from an agribusiness MTF farm in a peri-urban of Bogor were collected and analyzed with RBT and CFT. The results showed that the seroprevalence was high at 21.30% (95% CI; 15.8-26.8). Brucellosis seroprevalence did not differ significantly between goats and sheep with 22.81% and 19.61% respectively, testing positive for the disease. Brucellosis reactor seroprevalence was significantly higher in older animals (over 2 years) than in younger animals (0-2 years), at 30.38% (95% CI, 20.2-40.5) and 16.06% (95% CI, 9.9-22.2), respectively. Increasing age was significantly associated with seropositivity (OR=2.28). These findings indicate a high prevalence of brucellosis reactor among small ruminants in the peri-urban of Bogor, Indonesia, and require appropriate precautions. Given the high density of people in the study site, this could endanger public health and lead to significant production losses.

1 Introduction

Goats and sheep, which are small ruminants, serve many different social and cultural roles, that vary across cultures, socioeconomic systems, agroecology, and tropical regions [1]. These animals' milk and meat are essential to rural populations' protein diets, so breeding them has a significant nutritional impact [2]. Goats and sheep agribusiness in Indonesia is very promising because of the high demand during the feast of sacrifice, for 'aqiqah', and culinary dishes.

Brucellosis is caused by bacteria of the genus Brucella. The disease occurs in many different types of livestock, such as cattle, sheep, goats, and pigs, causing production loss; it

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is zoonotic imposing significant impacts on social, economic, and public health worldwide [3, 4]. *Brucella melitensis* and *Brucella ovis* are the two primary causes of brucellosis in small domestic ruminants. Both agents are now present worldwide. Currently, *B. ovis* is prevalent in most sheep-raising areas whereas *B. melitensis* is still endemic and linked to a significant decrease in flock productivity in specific regions [5]. *Brucella militensis* is one of the causes of brucellosis in humans, which causes the most severe symptoms with sheep and goats as intermediate hosts [6], as well as being a potential reservoir for other animals.

In China, brucellosis in humans was reported to be quite high, reaching 162,329 people during 2004-2010, which increased in winter and spring when exposure to sunlight was minimal [7]. In Penang, Malaysia, it was reported that 79 people suffered from brucellosis after consuming milk purchased from farms with cases of brucellosis [8]. It was reported that sheep farm owners and sheep keepers suffered from fever, headache, and pain with a positive serological diagnosis of brucellosis. India has an endemic case of brucellosis, with annual median losses to the economy estimated at US $ 3.4 billion for livestock and US $ 6.39 for humans [9]. Meanwhile, in Indonesia, brucellosis in humans is a neglected disease [10]. The factors influencing the epidemiology of the disease in small ruminants in Indonesia are poorly understood and the data are sometimes sporadic and incomplete. The objective of the study is to investigate the brucellosis reactor in sheep and goats in a peri-urban area and provide evidence of the need to control the disease.

2 Material and Methods

2.1 Ethical approval

The study has been approved by the Research Ethics Committee, The National Research and Innovation Agency: Experimental Animal Research, Approval No.: 075/KE.02/SK/10/2022.

2.2 Research Design and Sample Collection

The research design was cross-sectional, with a purposive sampling approach. The study was carried out from July to December 2022, in the Ciampea district, a peri-urban area of Bogor. The location was chosen based on the relatively high goat and sheep population, easy to reach, and the livestock owner or person in charge of the farm is cooperative for blood sampling. A number of 216 blood samples, consisting of 102 samples from sheep and 114 from goats, in an agribusiness farm (MTF farm) were collected. Blood samples were taken via the jugular vein aseptically.

As no single serological test is proper in all epidemiological circumstances, a combination of RBT and the Complement Fixation Test (CFT) is the most popular used serial testing scheme [11, 12]. Samples were tested serologically to determine the presence of antibodies to brucella which was carried out in parallel with the Rose Bengal Test (RBT) and Complement fixation test (CFT); if one of the test results is positive it will be declared positive and if both are negative then the result is negative [12]. The RBT and CFT are tests commonly used in diagnosing brucellosis in goats and sheep [13] which are recognized for the benefit of international trade [14] although other serological tests, such as iElisa, cElisa, FPA, Coombs test can be used. The use of two serological test methods in parallel is necessary for diagnosing *B. melitensis* in goats and sheep to obtain higher sensitivity besides the use of standard serum controls and antigens that are suitable and more sensitive [15]. In order to obtain information on the occurrence and transmission of brucellosis on the farm, discussions with livestock keepers, paramedics, and field managers were conducted.

2.3 Data analysis

Laboratory results and information obtained from farmers' interviews were tabulated using Microsoft Excel (Microsoft Corporation) and analyzed descriptively. Individual animal prevalence is calculated by dividing the number of positive animals by the total number of animals tested, and group prevalence is computed by dividing positive groups by the total number of groups. The significance level of brucellosis seroprevalence was analyzed using Chi-square [16], and odds ratio, with 95% confidence level. Comparisons between species, location, age and sex group were statistically evaluated with equation :

$$\textit{Chi-square test } X^2 = \chi 2 = \Sigma\, [(\lvert Obs\text{-}Exp \rvert - 0.5)^2/Ex] \quad (1)$$

Odds ratios are used to assess the risk of brucellosis infection (a measure of the strength of the association). The odds ratio (OR) corresponds to the ratio of the odds of having a condition for those exposed to a certain treatment, versus the odds of having a condition for those not exposed to the treatment. Consider the following 2x2 crosstabulation :

	Reactor (+)	Reactor (-)
Exposed	a	b
Not Exposed	c	d

The strength of association between the putative risk factors and seroprevalence was calculated using the odds ratio (OR). The following formula is used to calculate the odds ratio of having the condition for those in the exposed group compared to the non-exposed group:

$$\textit{Odd Ratio (OR)} = (axd)/(bxc) \quad (2)$$

Results of interviews and observations at the farm were analyzed descriptively and used as supporting data for laboratory examination results.

3 Result and Discussion

Table 1. Seroprevalence of brucellosis in sheep and goats according to animal origin

Origin of animals	**Total samples (216)**	**Seroprevalence brucellosis**		**Seroprevalence positive % (CI 95%)**
		Negative (169)	**Positive (46)**	
West Java (Bandung)	22	14	8	36.4^a (16.3 – 56.5)
East Java (Kediri)	60	56	4	6.7^b (0.1 – 12.9)
West Java (Sukabumi)	8	6	2	25^a (0.1 – 0.6)
West Java (Bogor)	31	24	7	22.6^a (7.9 – 37.3)
Farm Bred	94	69	25	26.6^a (17.7 – 35.5)

Of 216 sera samples, collected from 114 goats, and 102 sheep at an organized farm, located in the sub-urban of the Bogor area, West Java, Indonesia. Goats and sheep reared at the MTF farm, come from various regions such as Bandung, Sukabumi, Bogor (West Java Province), and Kediri (East Java Province). When animal origin was considered, Table 1 shows goats and sheep from West Java had a much higher prevalence rate of brucellosis than those from East Java. The lowest prevalence of brucellosis recorded in this study was from East Java (Kediri) was 6% (95% CI, 0.1-12.9) which was significantly different from West Java ($p < 0.01$) with a range of 22.6% (95% CI, 7.9 – 37.3) and 36.4 (16.3 – 56.5) respectively from Bogor and Bandung. The difference may depend on the geographical area and the animal rearing practices in the origin of the livestock. This is in line with [17] that transmission of infection may be associated with environmental related factors.

Seroprevalence of brucellosis reactor (+) in small ruminants from 216 sera samples is presented in Table 2. It can be seen that 19 were positive with the RBT test, 26 were positive with the CFT test, and 1 (one) sample was positive both with the RBT and CFT tests. So, the brucellosis seroprevalence reactor (+) in this study was 21.30% (95% CI, 15,8 – 26,8). This result was much higher than the seroprevalence *Brucella melitensis* in West Sulawesi was 4,23% (10/236) and in South Sulawesi was 4,45% (23/516) [18]. Moreover, brucellosis seroprevalence in small ruminants in this study was much higher compared to the previous study in Mali, at 4.1% (2.8-5.6, 95% CI) [19], in Southern-Ethiopia was 3.2% (95% CI: 2.1–4.6) [11], and in Gujarat India was 13.60% [20]. The findings indicate alarmingly very high seroprevalence of brucellosis reactor among small ruminants in this study, so it required appropriate measures.

Table 2. Seroprevalence of brucellosis reactor (+) in small ruminants based on species, age, and gender in the sub-urban Bogor area

Parameter	Sample (N)	RBT (+)	CFT (+)	RBT & CFT (+)	% Reaktor (brucellosis)	Prevalence % (95% CI)
Farm "MTF"	216	19	26	1	46	21.30 (15.8 – 26.8)
1. Species						
Goats	114	17	8	1	26	22.81^{a} (15.1 – 30.5)
Sheep	102	2	18	0	20	19.61^{a} (11.9 - 27.3)
2. Age*						
0 – 2 year	137	17	5	0	22	16.06^{a} (9.9 – 22.2)
>2 year	79	2	21	1	24	30.38^{b} (20.2 - 4.5)
OR = 2.28						
3. Gender (Sex)						
Male	51	2	10	0	12	23.5^{a} (11.9 – 35.2)
Female	165	17	16	1	34	20.6^{a} (14.4 – 26.8)

* the calculated X^2 value (6.03) is greater than the X^2 table value of 5% (3.84)
CI: Confidence Interval

With regard to species, the seroprevalence of small ruminant brucellosis was higher in goats than in sheep (Table 2), however, there was no significant difference, of 22,81% (95% CI, 15.1 – 30.5) and 19,61% (95% CI, 11.9 – 27.3), respectively. This result was in

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line with seroprevalence in organized and non-organized rearing systems, in which was not a significant difference between the two species [4]; even though the brucellosis seroprevalence in sheep was 8.29% (95% CI 6.7-10.1) higher than in goats at 5.82% (95% CI 4.0-8.2). While [21] reported that the seroprevalence by rose bengal plate test (RBPT) in organized farms, with a history of repeat breeding, retention of placenta, and abortion was 8.57% in sheep and 2.73% in goats. Additionally, there was a significant correlation between risk factors for Brucella seropositivity and district, age group, flock size, and history of abortion (P<0.05) [11].

Brucellosis reactor seroprevalence was significantly higher in older animals (over 2 years) than in younger animals (0-2 years), at 30.38% (95% CI, 20.2-40.5) and 16.06% (95% CI, 9.9-22.2), respectively. The Chi-square analysis revealed a substantial difference in seroprevalence between age groups (p≤0.05). The result suggested there was a significant relationship between Brucellosis seropositive and the age of animals. Increasing age was significantly associated with seropositivity (OR=2.28), indicating that brucellosis reactor risk in mature animals > 2 years was 2.28 times more likely than in young goats and sheep. This study was similar to one conducted in a peri-urban region of Tajikistan [22], which found that seropositivity was substantially correlated with age (OR=1.4). However, it was relatively lower than previous studies [11], which reported young sheep and goats had a seropositive rate for Brucella infection that was 4.8 times lower than that of older animals (> 3 years) (OR = 4.8). In Central and Northeast Ethiopia, it was found that sheep 3 years of age and older had the highest sensitivity to brucellosis [3]. Furthermore, sheep and goats older than 8 months were shown to have significantly higher levels of *Brucella* seropositivity (OR 2.49, 95% CI 0.8-7.2), from the Southern Province of India [4].There was no significant difference in the seroprevalence of brucellosis reactors (+) between male and female animals based on gender (P>0.05), and it was relatively high in males at 23,5 % (95% CI 11,9-35,2) than females at 20,6 % (95% CI 14,4-26,8). In contrast, in breeding ranches of Central and North East Ethiopia revealed there was no significant difference between gender (P > 0.05) and that seroprevalence was generally greater in females 1.93% (95% CI: 1.01-4.63) compared to male sheep 0.95%, (95%CI: 0.42- 3.83%) [3].

The current study found sheep and goats with serological proof of brucellosis at a farm selected in sub urban Bogor area, West Java, Indonesia. The results demonstrated that the disease prevalence of brucellosis is persistent in the small ruminant population**.** Any animal that is infected, sick, or appears to be in good health could be a brucella source [2].

Information gathered from observation and interviews with animal keepers suggested that farm management practices and the hygienic conditions of the farms were relatively good. The sheep and goats population was very dense and the pens were close to each other of the two species. There was no special pen for partus or for the isolation of brucellosis reactors. This is in line with the study [23], which identified risk factors for the incidence of brucellosis. Potential risk factors for brucellosis seroprevalence in small ruminants include mixed flock and agropastoral production systems. A potential source of Brucella infection is any animal, whether it is healthy, sick, or infected. In this present study, we observed brucellosis seroprevalence could be associated with the frequent introduction of purchased animals into the flock; and the absence of segregation and mixing of different species. As a result, each affected animal should be regarded as a potential brucellosis source. Consequently, any control measures must consider both the presence of the sick as well as the carriers of the germs, as suggested by [2].

4 Conclusion

The study revealed that antibodies to brucella species are detected in sheep and goats on the farm in a peri-urban area of Bogor. The findings indicate a high prevalence of brucellosis reactor among small ruminants and require appropriate precautions. The Chi-square analysis revealed a significant difference in seroprevalence between the origin of animals ($p \leq 0.01$). However, between the two species, there was no significant difference ($p > 0.05$). Recurrent livestock movement will probably continue to worsen the disease endemicity in the area. Given the high density of people in the study site, this could endanger public health and lead to significant production losses.

It is advised that further epidemiological studies be conducted to isolate and characterize the Brucella species circulating among animals to determine the transmission of the disease. Increasing public awareness about the rearing livestock practices that have the potential to lead to Brucella infection exposure and strategies for preventing them is a necessity.

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