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Human Brucellosis among the farmworkers in Cilawu and Boyolali, Indonesia

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Abstract

Brucellosis is a neglected infectious disease caused by animals and is becoming a public health problem in developing countries. There are limited case reports of human brucellosis in Indonesia because the symptoms are not specific. This study investigated the association between serological tests for brucellosis among workers and their knowledge, attitude, and behavior. The study was conducted on farmworkers in a dairy cattle milking center in Boyolali and Cilawu. A total of 149 respondents, according to the inclusion criteria, were included. Around 3 ml of blood was collected from the respondents, followed by structured interviews to determine the level of knowledge, attitudes, and behavior towards brucellosis in humans. The sera were tested with the rose bengal test (RBT) and complement fixation test (CFT). Results showed that 3.3% of respondents were positive for RBT in Boyolali and 7.01% in Cilawu. However, CFT showed that 5.3% of respondents were positive in Cilawu, but 0% in Boyolali. The level of knowledge and attitudes of respondents regarding brucellosis in humans was still lacking, and the behavior of respondents on the farm was mostly good, in the sense of understanding the procedures for maintaining healthy dairy cattle correctly, but maintaining personal health was still lacking. This study underscores the need for immediate action to improve the situation. Public health professionals, researchers, and policymakers have a crucial role in implementing regular health education programs for farmworkers and improving the availability of personal protective equipment.

Keywords

Complement Fixation test, Dairy farmers, Neglected zoonotic diseases, Rose Bengal Test, Brucellosis

Introduction

Food and Agriculture Organization (FAO), World Health Organization (WHO), and World Organization for Animal Health (WOAH) stated that Brucellosis is one of the infectious zoonotic diseases that rapidly and widely spread in farm animals and humans (Corbel 2006).WHO noted that brucellosis is a neglected zoonotic disease that extends to a developing country. Brucellosis significantly impacts the economy due to reduced productivity in affected animals and humans, stemming from the high treatment costs and the lengthy duration of the treatment. Brucellosis has a public health impact worldwide (Jama'ayah *et al.* 2011).

Brucellosis was first reported in 1915 in Indonesia from cattle in Java. Until 2014, only 9 provinces were reported free from brucellosis out of 34 provinces. Thus, most areas in Indonesia still have animal brucellosis cases, which generates a high risk of disease transmission to humans. There is a limited report on brucellosis cases in humans.In 1995, Sudibyo reported the seropositivity of brucellosis among the abattoir workers of cattle and pigs in Jakarta was around 13.6% and 22.6%, respectively (Noor *et al.* 2006, Muflinah *et al.* 2013, Sudibyo 1995).

The concept of one health must be upheld to achieve brucellosis-free Indonesia. This requires optimal cross-sectoral coordination between the Ministry of Health and Agriculture. To start this cross-sectoral collaboration, primary data on cases of brucellosis in humans are needed, especially in dairy farm workers. This is the main objective of this

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research. Primary data on human brucellosis cases, like CFT examination results, is crucial for policymakers to develop strategies to achieve optimal public health, including eradicating zoonotic diseases in livestock communities.

Materials and Methods

Ethical approval

All respondents were enrolled under informed consent procedures, which were reviewed and approved by the National Institute of Health Research Development, Ministry of Health of Republic Indonesia Ethics Committee (LB.02.01/5.2/KE.343/2016) and gave written informed consent under the declaration of Helsinki, before the sample collection.

Samples Collection and Site

The study was conducted at small dairy cattle farms in Cilawu West Java Province and Boyolali in Central Java Province (Figure 1). The respondents were dairy cattle farmers living in rural Cilawu and Boyolali.

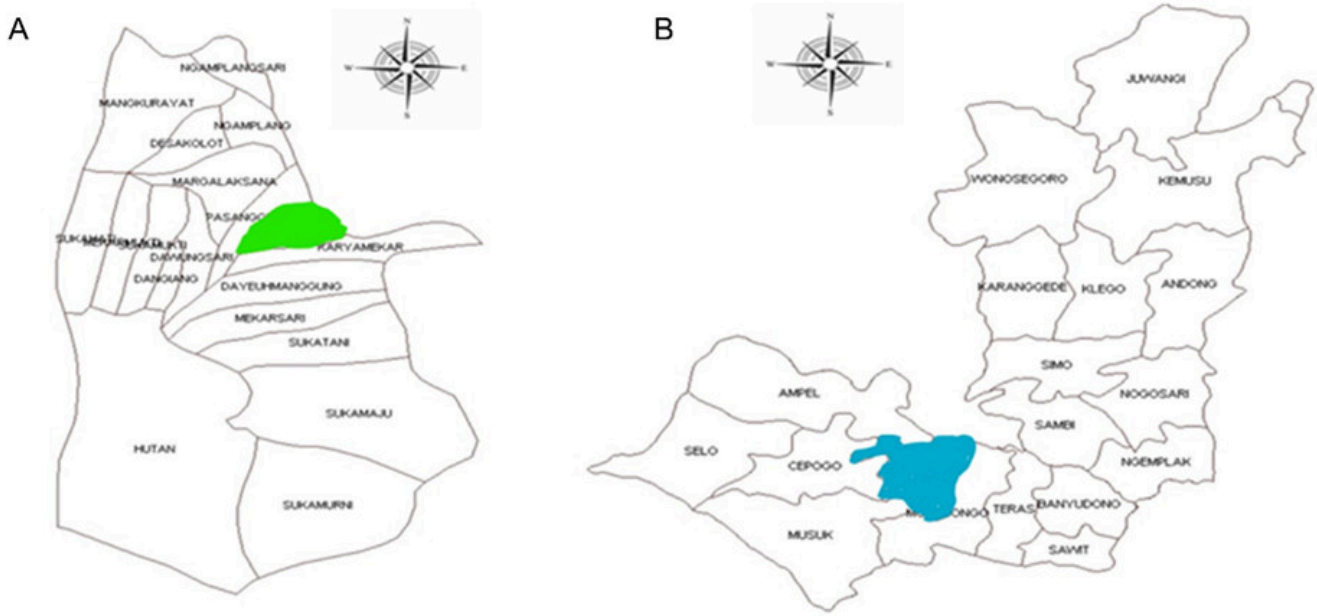

Figure 1. The sampling locations were in Cilawu and Boyolali. (A) Cilawu in West Java (green area). (B) Boyolali in Central Java (blue area). This picture was an image created by Healthmapper (https://healthmapper.informer.com).

The number of respondents collected was based on the prevalence of brucellosis cases in humans in Malaysia, which was 14.9% in 2014 (Jama'ayah 2011). The sample 83 size formula was used based on the proportion estimation test (NCSS 2022).

$$n = \frac{[Z21 - \alpha/2\ p\ (1 - p) \times Deff}{d^2}$$

Information :

p = the proportion of Brucellosis cases in dairy cattle milking workers (0.14)

d = Absolute deviation (5%)

$Z^2_{1-\alpha/2}$ = Z standard deviation value at a certain degree of gap

Deff = 2

Respondents with inclusion criteria were 149, consisting of 92 people from Boyolali and 57 from Cilawu.

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Sample Collection Method and Processing

Medical professionals collected 3 mL of venous blood. The blood was further processed for the Rose Bengal Test (RBT) at the National Institute of Health Research Development, Ministry of Health of the Republic of Indonesia, and the Complement Fixation Test (CFT) at the Bacteriology Laboratory of the Indonesian Research Center for Veterinary Science following standard procedure. Briefly, for RBT, 30 µL of serum was dispensed on a slide plate and mixed with 30 µL of RBT antigen (ID Vet) and processed according to the manufacturer's instructions.

All positive serum samples with RBT were tested for CFT for confirmation following manufactures recommendations.

Knowledge, Attitude, and Practices on Human Brucellosis

Respondents' knowledge, attitudes, and behavior on brucellosis in humans were measured using a set of questionnaires developed to measure human brucellosis knowledge, attitude, and practice (KAP). Knowledge of human brucellosis measures the transmission of the disease, the symptoms of human brucellosis, and the treatment and preventive ways for human brucellosis. Attitude about human brucellosis assessed respondents' opinions about the biosafety practices in the cage to avoid human brucellosis, such as cleaning the cage, showering the cattle, providing a clean environment before milking the cows and drinking raw milk. A set of questions identified practices to identify the behavior of the respondents in the cages, for example, cleaning the cages, the cows, the water, processing the feces, and visiting health care facilities whenever they feel unwell. A respondent was considered to have "good knowledge" if two or more questions were answered correctly for human brucellosis pathways and "poor understanding" if only one answer was answered correctly.

Statistical analysis

RBT and CFT test data were analyzed using SPSS version 11.5 (IBM, Armonk, NY, USA) to determine the prevalence of brucellosis in dairy farmers in Cilawu and Boyolali. A univariate analysis of the respondents' characteristics was performed for the data from the questionnaire. Chi-Square statistical test was used to evaluate the significant influence between observed variables; respondents' knowledge, attitudes, and behavior where $p \leq 0.05$ were considered significant.

Results

RBT and CFT results

The RBT test data result in Boyolali showed that 3.3% of people were positive (3 out of 92 people), while in Cilawu, 7.01% of people were positive (4 out of 57 people) (Table I).

According to the CFT results in Table I, there were no positive cases in Boyolali (0 out of 92 individuals). In Cilawu, only 5.3% of the population tested positive (3 out of 57 individuals), as summarized in Table I. The majority of individuals who tested positive for brucellosis using the CFT test did not exhibit clinical symptoms.

Number of respondents	RBT		CFT	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)
92 (Boyolali)	3 (3.3%)	89 (96.7%)	0 (0%)	92 (100%)
57 (Cilawu)	4 (7.02%)	53 (92.98%)	3 (5.3%)	54 (94.7%)

Table I. RBT and CFT results of respondents in Boyolali and Cilawu.

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Respondents Characteristics

In this study, the characteristics of the respondents were identified based on gender, age, occupation, length of work on the farm, cattle ownership, education level, and income.

Category	Percentage (%)	
	Cilawu	Boyolali
Gender		
Male	50	71.7
Female	50	28.3
Age		
<30 years old	19.6	7.6
30-39 years old	19.6	18.5
40-49 years old	37.5	28.3
>50 years old	23.2	45.7
Main job		
Veterinarian	0	6.6
Dairy farmer/Milkmaid	76.8	41.8
Farm cleaning service	8.9	7.7
Paramedic	3.6	7.7
Milk processor	5.4	6.6
Others	5.4	29.7
Level education		
No formal education	0	1.1
Primary (not pass)	21.05	4.3
Primary	59.65	33.7
Junior high school	14.04	15.2
Senior high school	3.51	29.3
University	1.75	14.2
Other	0	2.2
Ownership status		
Owner	0	72.7
Owned by someone else	78.6	25.0
Do not have cattle	21.4	2.3
Length of work on the farm		
< 1 Year	0	2.6
< 5 years	10.9	15.6
6-10 years	30.9	24.7
>10 years	58.2	57.1
Income Per-Month*		
<IDR 1,000,000	17.9	21.8
IDR 1,000,000-IDR 2,000,000	55.3	31.5
IDR 2,000,000-IDR 3,000,000	16.1	30.4
>IDR 3,000,000	10.7	16.3
Is there a history of abortion or premature birth in your family?		
Yes	7.1	16.3
No	92.9	83.7
Have you had an intermittent fever that has gone up and down in the last 3 months?		
Yes	7.1	11.0
No	92.9	74.7
Sometimes	0	14.3

*1$ = Rp 16.000

IDR: Indonesian Rupiahs. Rp: Rupiahs

Table II. Distribution by characteristics of respondents in Cilawu and Boyolali.

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In general, the characteristics of the respondents in Boyolali and Cilawu are similar: the majority are male, aged 40-49 years, graduated from elementary school, the main job is a milkmaid, owns dairy cattle, has worked for more than 10 years, has an income of Rp 1,000,000 – Rp 2,000,000, has no history of abortion in the family, and never had an undulant fever in the last 3 months (Table II). The respondents who were CFT positive also did not have an abortion history in the family and had an undulant fever in the last 3 months.

The occupation with a high risk of infection observed in this study was milkmaids, as 3 milkmaid respondents were infected (Table II). Family members of these patients also have a high risk of brucellosis infection, suggesting that the impact of brucellosis infection is not only on the infected people but also on the family.

The average education of respondents in Cilawu was elementary school, which makes them vulnerable to being infected by brucellosis. Respondents with education above high school have a lower risk of brucellosis infection. Low education causes low knowledge, and it is challenging to provide health education.

In general, there were similarities in the characteristics of dairy cattle in Boyolali and Cilawu; most heads did not have a history of abortion, and the time the cattle were kept was more than 6 months. The noticeable difference was that cattle in Cilawu were mostly not vaccinated against *Brucella* (88.9%), while in Boyolali, 32.5% had not been vaccinated (Table III). Since the number of dairy cattle that were not vaccinated in Cilawu was higher than that in Boyolali, this could lead to a higher risk of brucellosis in Cilawu and may be reflected in the higher abortion rates in this area (14.8%), compared to Boyolali (5.2%). The average milk production was the same in Cilawu and Boyolali, with more than 10 liters daily.

We also observed that many cattle were housed near the kitchen or in the front or backyards of houses in both Boyolali and Cilawu. The distance between the animal housing and the habitations was under 12 meters, which was less than the recommendations by the Indonesian Ministry of Agriculture. Cattle owners who own less than 10 cattle tend to place the cattle in an empty area around the house.

Characteristics of Dairy Cattle	Percentage (%)	
	Cilawu	Boyolali
Abortion history		
Yes	14.8	5.2
No	85.2	94.8
***Brucella* vaccinated**		
Yes	11.1	67.5
No.	88.9	32.5
Time of kept		
<3 months	0	3.8
3-6 months	1.9	12.7
>6 months	98.1	83.5
Cattle origin		
Local market	87	88.2
Legacy	1.9	9.2
Government	3.7	2.6
Different province	7.4	0
Number of cattle owned		
<5	48.1	42.4
5-10	46.3	44.7
>10	5.6	12.9
Cattle age		
<1 year	1.9	79.2
1-5 years	72.2	19.5
>5 years	25.9	1.3
Milk production		
1-5 liter	1.9	22.4
6-10 liter	1.9	38.8
>10 liter	96.2	38.8

Table III. Characteristics of dairy cattle in Cilawu and Boyolali.

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Respondent's knowledge, attitude, and behavior

Respondents' knowledge, attitudes, and behavior toward brucellosis in humans were measured using a questionnaire. Dairy farmers in Boyolali had good knowledge, but only a few had heard about human brucellosis compared to those in Cilawu (Table IV).

Variable/Category	Percentage (%)	
	Boyolali	Cilawu
Have you heard about brucellosis in cattle?		
Yes	100	100
No	0	0
Do you think humans could be infected by brucellosis?		
Yes	37.5	17.54
No	0	29.83
Do not know	62.5	52.63
In your opinion, what causes someone to be infected with brucellosis?		
a. Drinking unpasteurized milk		b. Eating meat from brucellosis animals
c. Close contact with brucellosis animals		d. I do not know
If Answer > 2 true	27.33	40.33
If Answer < 2 true	72.67	59.67
Are fever, Chills, Head pain, sweat, and abortus clinical signs of human brucellosis in women?		
Yes	24.8	22.8
Do not know	75.2	77.2
What is the treatment for human brucellosis?		
a. Antibiotics		b. Antipireptics
c. Good management practices in farm animals		d. I do not know
If Answer ≥ 2 true	66	75
If Answer < 2 true	34	25
What is the preventive way for human brucellosis?		
a. Drinking pasteurized milk		b. Disinfect cages and equipment
c. Good management practices in farm animals		d. I do not know
If Answer ≥ 2 true	77.66	86.33
If Answer < 2 true	22.34	13.67

Table IV. Descriptive analyses of the respondent's knowledge of human Brucellosis in Boyolali and Cilawu.

Most farmers understood that personal hygiene and drinking milk were noteworthy for their health. Attitudes were considered good if the respondents agreed about the importance of cleaning cages, washing hands after contact with the dairy cows and before milking the cows, and drinking milk (Table V).

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Good Attitudes	Percentage (%)	
	Boyolali	Cilawu
Keeping the cage clean is important	13	32
Washing hands with soap after contact with cattle is very important	97	100
Cleaning the cattle udder with disinfectant after milking	36	36
Drinking milk is very important to maintain a healthy body, especially for children	98	95
Processing cattle dung	36	52

Table V. Attitudes of the respondents regarding human brucellosis.

Practices were considered good if the farmers used biosafety practices such as cleaning the cages, washing the cow every day, cleaning the milking equipment before and after milking, processing cow's urine and feces, and visiting health care center whenever they felt unwell (Table VI).

According to the respondents' behavior section in the questionnaire, most of the respondents in Boyolali did not wash the cattle before milking them. As much as 68.25% of respondents in Cilawu washed their cows, while in Boyolali, only 43.25% of respondents washed their cows (Table VI). This might be due to the limited water sources in Boyolali, which caused cattle to be cleaned only twice a month. Water shortage due to geographical location has caused water to be prioritized by humans. In contrast, cattle were washed at least every 3 days in Cilawu, following good farming practices. Not many farmers in Boyolali and Cilawu cleaned the cow's teat and udder before and after milking, with 21.25% and 27.75%, respectively. They also did not immediately separate the aborted fetus from the herd, which can be seen in the low percentage of 34.25% and 21.5% in Boyolali and Cilawu, respectively (Table VI). Most farmers conducted biosafety practices such as cleaning the cage and milking equipment in the two sites.

Good practices	Percentage	
	Boyolali	Cilawu
Biosafety practices in cages (cleaning the cage and its equipment)	72.55	75.55
Showering the dairy cows	43.25	68.25
Use a disinfectant every time you clean after milking the cattle	21.25	27.75
Wash hands after contact with cattle	48	51.75
Aborted cattle are separated from the herd	34.25	21.50

Table VI. Practices of respondents regarding human brucellosis.

There was no significant difference between knowledge and attitudes, knowledge with behavior, and attitudes with respondents' behavior in both locations towards humans brucellosis ($p<0.05$) (Table VII).

Variable	p-Value	
	Boyolali	Cilawu
Knowledge VS Attitude	0.156	0.901
Knowledge VS Behaviour	0.1	0.320
Attitude VS Behaviour	0.757	0.724

Table VII. The p-value of knowledge, attitude, and behavior variables.

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Discussion

A previous study showed that brucellosis risk factors in humans are gender, age, and type of work. More brucellosis cases were reported in men aged between 39-50 years and working on farms (Rahman *et al*. 2014). This aligns with this study in which respondents who tested positive in the CFT test were male, aged 40-49 years, and worked as a milkmaid.

As a zoonotic disease, the incidence of human brucellosis is highly dependent on animal prevalence. Currently, Indonesia is still not free from brucellosis in animals. Thus, people working with infected cattle who consume unpasteurized milk have a high risk of *Brucella* infection. We conducted sero-surveillance and, for the first time, conducted a knowledge, attitude, and practice (KAP) study of brucellosis among farmworkers in two areas with high dairy cattle populations in Indonesia.

Transmission of *Brucella* from animals to humans can occur through raw milk, direct contact with infected animals through wounds on the skin and mucous membranes, and the respiratory route (Araj *et al*. 2005). This agrees with a previous study that showed that the infective dose of brucellosis in humans is low, likely due to exposure over a long period and causing asymptomatic clinical signs (Njeru *et al*. 2016). In our study, most infected respondents have worked closely with cattle for over 10 years. So, it is likely that the respondents who have been working for less than 10 years are safer from *Brucella* infection. This study also found asymptomatic cases, with most CFT-positive respondents not showing clinical symptoms related to *Brucella*.

The RBT is the best screening tool for animal brucellosis, as well as for humans. Both of our respondents had high RBT results, but compared with CFT, only respondents from Cilawu had positive CFT results. It indicated that RBT had a high sensitivity level compared to the CFT. This result correlates with a previous study, which showed positive RBT but negative CFT. Ekiri et al., 2020 found that during acute infection (infected for <6 months), sub-acute infection (infected for 6-12 months), and chronic infection (infected for >1 year), patients had positive RBT results of 71.4%, 94.2%, and 54.5% respectively. Therefore, positive RBT most likely shows acute infection, while negative RBT in chronic infection might be false negative (Ekiri AB *et al.* 2020).

Brucella from animals to humans can be transmitted through drinking raw milk, direct contact with infected animals through skin and mucous membrane wounds, and the respiratory route (Maurin 2005). Respondents may be contracting brucellosis through direct contact with infected animals and inhalation, considering the average length of work on the farm, which was more than 10 years, with unvaccinated cattle. The respondents working less than 10 years are less likely to have *Brucella* infection. It is like a previous study that showed the infective dose of brucellosis in humans is low, likely due to exposure over a long period and causing asymptomatic clinical signs (Glanville W *et al.* 2017). This study also identified asymptomatic individuals among most respondents who tested positive for CFT. Only a few respondents reported having undulant fever, but no data on the history of abortion cases and premature death in their family were available.

The low level of education of the respondents in the Cilawu puts them at risk of contracting brucellosis. The level of formal education is associated with the knowledge of human brucellosis and the practices of this disease (Lindahl et al. 2005; Arief S et al. 2017; Ekiri AB *et al*. 2020). Respondents with low formal education had poor knowledge, but respondents with high education had good knowledge about human brucellosis.

Data on the prevalence of brucellosis in humans, risk factors, hosts, and potential intermediaries of brucellosis are essential to developing and implementing suitable prevention efforts and control strategies (Dean *et al*. 2012). In this study, CFT-positive respondents had similar conditions, such as having unvaccinated cattle, being male, working in dairy cattle for more than 10 years, and CFT-positive animals.

The important point of our study was that brucellosis cases in cattle correlated with human brucellosis. Both of our study sites were not free from brucellosis, and this could be one of the risk factors for humans, besides poor biosafety practices on the farm. In our study, all respondents did not drink raw milk from their cattle. Still, *Brucella* could infect them due to their poor biosafety practices, such as not using gloves while in contact with possible infected cattle and poor hygiene of the cage and the cattle. Both in Cilawu and Boyolali, they rarely showered their cattle because of problems with water supplies.

Limitations of the study

This study remained an initial screening, with brucellosis detection in humans relying solely on RBT and CFT, without further confirmation using PCR, ELISA, or bacterial culture.

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