Seroepidemiology of Brucellosis on Dairy Cattle in Small-Holders Farms, in West Java

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ABSTRACT

Brucellosis in cattle causing abortion and infertility in adult animals and the main causative agent is Brucella abortus. The objective of the study was to determine the seroprevalence and several putative factors in the occurrence of brucellosis among herds of dairy cattle in small holder farms. The study was carried out in the districts with high risk of brucellosis in dairy cattle production centers in Bandung regency, West Java Province. The sampling strategy in this study was risk-based surveillance. A total of 260 blood samples were collected from cows owned by farmers who have had a history of cow abortion and were suspected of having a risk of brucellosis. Serological examination of bovine serum with RBT and CFT tests were performed. True infection reactions cannot be distinguished from serological reactions caused by the Brucella abortus vaccine strain S19. Currently, B. abortus vaccine strain RB51 is being applied in Java islands for preventing of brucellosis in dairy cattle. Thus, the positive result of serological reaction test shows brucellosis reactor. The overall seroprevalence of brucellosis was found to be 15.77%. The finding of this study suggested that more than 50% abortion occurred at gestational age more than 5 months. The result indicated that there was a relationship between brucellosis seropositive and the incidence of abortion, in which the risk of brucellosis was 3.35 times more in cows that had experienced abortion than in cows that has not experienced abortion. Vaccination coverage was relatively low (44.11%), and prevented 3.1% of dairy cows against brucellosis. This study is essential for disease control and prevention measures. Further studies need to be carried out on the factors that influence the low efficacy of the RB51 vaccine in dairy cows.

Key Words: Seroepidemiology, Seroprevalence, Brucellosis, Abortion, Dairy Cattle, Risk Factors

INTRODUCTION

Brucellosis is an infectious disease caused by Gram-negative bacteria from the Brucella genus that is pathogenic in various animals as well as humans (Sanogo et al. 2012). Bovine brucellosis is a livestock disease with worldwide distribution (Abernethy et al. 2011). The disease is a chronic infectious disease causing abortion, weak or stillbirth calves, infertility and decreased milk production (Enright 1990). Brucellosis infection can last for years. In cow, abortus is a major clinical symptom, generally occurs between 5-7 months of pregnancy; in bulls, brucellosis may cause infection of the testis (Young 1983). This disease has a socioeconomic impact, due to abortion in pregnant animals, loss in milk production and infertility in adult males, as well as on human health (OIE 2009a, b).

Brucellosis in Indonesia was first detected in 1925 by Kirschner from a dairy cow in Bandung (Sudibyo & Ronohardjo 1989), then the disease spread throughout the province except Bali where is historically free of brucellosis. Brucellosis in cows
remains endemic in Java, Sulawesi and East Nusa Tenggara with an economic loss of Rp138.5 billion per year (Dirjennak 2006). In Indonesia, control of brucellosis in cattle is carried out through a combination of vaccination and test-and-slaughter of positive animals, which has been able to reduce and eliminate brucellosis in cattle (Table 1).

According to Naipospos (2014), the overall eradication program of Brucellosis is running slowly, yet there are 14 provinces with very low prevalence rates, some of which have been declared free (Table 1). Epidemiological information, particularly the prevalence rate, is a key and essential element in the implementation of proper brucellosis eradication activities in the field. Prevalence rates can serve as program performance indicators, and is a tool for establishing actual disease status in an area or group of livestock. So far, many reports of brucellosis only include the number of positive cases (unknown to its denominator, the number of samples examined), so it can cause bias in assessing the actual disease status. The purpose of the study was to determine the seroprevalence and several putative factors in the occurrence of brucellosis among herds of dairy cattle in small holders farms in West Java.

**Table 1.** Provinces in Indonesia that has been declared free of Brucellosis

<table>
<thead>
<tr>
<th>Provinces</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bali, Lombok Island (NTB)</td>
<td>2002</td>
</tr>
<tr>
<td>Sumbawa Island (NTB)</td>
<td>2006</td>
</tr>
<tr>
<td>South Kalimantan, West Kalimantan, East Kalimantan, and Central Kalimantan</td>
<td>2009</td>
</tr>
<tr>
<td>West Sumatra, Riau, Jambi and Riau Islands</td>
<td>2009</td>
</tr>
<tr>
<td>South Sumatera, Lampung and Bangka Belitung Islands</td>
<td>2011</td>
</tr>
<tr>
<td>Madura, P. Sumba-NTT and North Sumatra</td>
<td>2015</td>
</tr>
</tbody>
</table>

**Sources:** Naipospos (2014); Direktorat Kesehatan Hewan (2018)

**MATERIAL AND METHODS**

**Study design and sample collection**

The sampling strategy in this research activity is risk-based surveillance. According to Stärk et al. (2006), the benefits of risk-based surveillance, one of which is to detect cases and estimate prevalence in the endemic conditions of a population. Samples are stratified, and strata are grouped according to the risks. Sampling is focused on the part of the population with the highest possible infection.

Location was chosen based on the districts with high risk of brucellosis in dairy cattle production centers in Bandung regency, West Java Province. This information was obtained after discussing with the head of the animal health sector and local livestock agency. A total of 260 blood samples were collected from cows owned by farmers who have had a history of cow abortion and are suspected of having a risk of brucellosis. Those samples were from 80 herds, *i.e.*: 25 dairy farms in district of Lembang, 29 farms in district of Pangalengan, and 26 dairy farms in district of Pasirjambu.
Semi-structured questionnaire were administered to each farm owner to collect the information of animal’s status included age, history of abortion, and vaccination. Most dairy cattle farmers did not practice vaccination regularly against brucellosis. Blood samples were collected from jugular vein or vein in the base of cow’s tail of each selected cow and these were transported to the laboratory on ice.

Serological examination of bovine serum with Rose Bengal Test (RBT) and The Complement Fixation Test (CFT) were performed following the procedure described by Alton (1988). True infection reactions cannot be distinguished from serological reactions caused by the Brucella abortus vaccine strain S19. Currently, B. abortus vaccine strain RB51 is applying in Java islands for preventing of brucellosis in dairy cattle. Thus, the positive result of serological reaction test shows brucellosis reactors

**Rose Bengal Test (RBT)**

Before conducting the RBT, serum samples and RBT antigen should be put in room temperature (22±4°C). Then, 25 µl of each serum sample was placed on a WHO plate and added 25 µl of RBT antigen to each serum, mixed thoroughly. The mixture was shaken for 4 minutes using a rotary agglutinator at ambient temperature, and then observed for agglutination under a white lamp box. Positive reaction should be confirmed by using CFT.

- Interpretation of RBT results:
  - Negative : No agglutination
  - Positive : Any visible agglutination

**Complement Fixation Test (CFT)**

The test uses the principle (indicator) of complement in the antigen and antibody bonds. The CFT is performed using standard 96-well microtitre plates with round (U) bottoms. The CFT procedure is as follows: volumes of 25 µl of diluted inactivated test serum are placed in the well of the first, second and third rows then add 25 µl of veronal buffer to the wells of the first row (anti-complementary controls). Volumes of 25 µl of veronal buffer are added to all other wells except those of the second row. Serial doubling dilutions are then made by transferring 25 µl volumes of serum from the third row onwards; 25 µl of the resulting mixture in the last row are discarded.

Volumes of 25 µl of antigen are added to each well except in the first row then add 25 µl of complement to each well. Control negative wells containing diluent only. The plates are incubated at 37°C for 30 minutes, and then 25 µl of sensitised sheep red blood cells (SRBC) is added to each well and the plate is re-incubated at 37°C for 30 minutes. The results are read after incubation at 4°C overnight. The degree of haemolysis is compared with standards corresponding to 0, 25, 50, 75 and 100% lysis.

**Data analysis**

Association measurements between the incidence of abortion and the results of the Brucellosis serology test were analysed by a Chi Square Test and computed using equation:
X2 = \[\sum \left| \text{Obs} - \text{Exp} \right| - 0.5\)^2/\text{Exp}\]

The efficacy of vaccines is the proportion of seropositive brucellosis that can be prevented by a vaccination program in a group of animals receiving vaccination. The proportion of seropositive brucellosis in vaccinated cows was calculated using Attributable fraction (AF). One of the AF applications is to calculate the efficacy of vaccines. The formula of AF is:

\[
\text{AF} = \frac{\text{AR}}{\text{Ie}}
\]

AR: Attributable risk; Ie: Incidence in exposed to a risk

Attributable risk (AR) or excess risk is the difference in rate of a condition between an exposed population and an unexposed population to a risk. The formula commonly used in epidemiology for attributable risk is \(\text{AR} = \text{Ie} - \text{Iu} \), where \(\text{Ie} \): Incidence in exposed to a risk, and \(\text{Iu} \): Incidence in unexposed to a risk (Budiharta & Suardana 2007). Therefore, AR is the difference between incidence brucellosis of vaccinated cow and unvaccinated cow.

**RESULTS AND DISCUSSION**

Serological test results of blood samples are presented in Table 2. Out of the 260 tested sera, 74 (28.46%) were positive by the RBT test, after being confirmed with the CFT test showed that 41 out of 74 (55.40%) samples were positive brucellosis reactors. The positive CFT results can be used as a reference, in which the overall seroprevalence of brucellosis in the study area were 41/260 (15.77%). The prevalence of brucellosis in this study was slightly higher than that found in India (14.81%) (Mahajan et al. 2012), but 2.4 times higher than that reported in Jordan (6.5%) (Al-Majali et al. 2009), 3 times higher than that in Malaysia and Myanmar (4-5%) (Zamri-Saad & Kamarudin 2016) and it was about similar that reported in Ethiopia (15%) (Molla & Delil 2015).

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of samples (n)</th>
<th>RBT (+)</th>
<th>CFT (+)</th>
<th>Seroprevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lembang</td>
<td>99</td>
<td>26</td>
<td>12</td>
<td>12/99 (12.1)</td>
</tr>
<tr>
<td>Pangalengan</td>
<td>106</td>
<td>47</td>
<td>28</td>
<td>28/106 (26.4)</td>
</tr>
<tr>
<td>Pasir Jambu</td>
<td>55</td>
<td>1</td>
<td>1</td>
<td>1/55 (1.8)</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>74 (28.46)</td>
<td>41 (55.40)</td>
<td>41/260 (15.77)</td>
</tr>
</tbody>
</table>

RBT: Rose Bengal Test; CFT: Complement Fixation Test

The serology test results showed positive RBT of 26/99 (26.3%) in Lembang and 47/106 (44.3%) in Pangalengan; after being tested with CFT brucellosis seropositivity were 12/26 (46.2%) and 28/47 (59.6%), respectively. The seroprevalence of Brucellosis in Lembang was lower than in Pangalengan. However, the prevalence of Brucellosis at this study sites was much higher compared to the prevalence of Brucellosis in East Java island i.e. 1.82% in 2009, 1.13% in 2010 and 0.51% in 2011 (Rahmahani et al. 2017).
In contrast, seroprevalence of Brucellosis in Pasirjambu district was 1.8%. In accordance with the provisions, areas with a prevalence of less than 2% will be applied a ‘Test and slaughtered’ policy. The results of this study have been reported to the Provincial Livestock Service and to the Livestock and Fisheries Office of Bandung Regency, so it can be followed up in line with applicable control procedures. According to Naipospos (2014) the policy of controlling and eradicating brucellosis is based on the level of prevalence in a region. ‘Test-and-slaughter’ method currently is used to control brucellosis in many ASEAN countries, as it is suitable for eradicating zoonotic livestock diseases when the prevalence of diseases is low (Zamri-Saad & Komarudin 2016). Related to implementation of brucellosis control, Indonesia central government institutionally plays a role in making guidelines, circular to regions and coordination with relevant agencies for support programs including public health (zoonosis) aspects (Bahri & Martindah 2006).

Information gathered from observation and questionnaire suggested that farm management practices and the hygienic conditions of the farms were relatively similar at each site. The cattle population was very dense and the cattle pens were close to each other, there was no special pen for partus or for the isolation of brucellosis reactors. In addition, the water sources used for the farms come from the same source. This is in line with Ai-Majali et al. (2009) who identified risk factors for the incidence of brucellosis.

Table 3. History of vaccination against brucellosis and the occurrence of abortion

<table>
<thead>
<tr>
<th>History of vaccination and abortion</th>
<th>Location</th>
<th>Total (n = 260)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lembang (n = 99)</td>
<td></td>
</tr>
<tr>
<td>Vaccinated (%)</td>
<td>61 (60.0%)</td>
<td>116 (44.11%)</td>
</tr>
<tr>
<td>Number of cow experienced abortion</td>
<td>32 (32.3%)</td>
<td>70 (26.62%)</td>
</tr>
<tr>
<td>Number of abortion cases</td>
<td>39 cases</td>
<td>93 cases</td>
</tr>
<tr>
<td>Abortion (&gt;1×)</td>
<td>6/32 (18.75%)</td>
<td>18/70 (25.71%)</td>
</tr>
<tr>
<td>Abortion at gestational age &gt;5 months (%)</td>
<td>28/39 cases (71.8%)</td>
<td>62/93 cases (64.58%)</td>
</tr>
</tbody>
</table>

Cases of abortion in Lembang (32.3%) was higher than in Pangalengan and Pasir Jambu around (Table 3). The funding of this study suggested that more than 50% abortion occurred at gestational age more than five months. The chi-square value counts at a significant level of 5% was greater than the chi-square table value, so the result suggested there was a significant relationship between Brucellosis seropositive and the incidence of abortion.

In general, the seroprevalence of brucellosis in the dairy cow population at the study site was 15.8 per 100 cows (Table 4). However, the incidences of Brucellosis seropositive in cows that have experienced and never had abortion were 32.8 and 9.8 per 100 cows, respectively (328 and 98 per 1000 cows, respectively). Seroprevalence
of dairy cattle brucellosis in the study area were higher compared to study conducted in Ethiopia (Ibrahim et al. 2010), in which sero-prevalence of bovine brucellosis was also positively associated with the occurrence of abortion (26.98 and 1.54% in those with and without previous history of abortion, respectively). Escamilla et al. (2007) suggested that result of serologic testing for certain infectious agents in an animal that had abortion must be interpreted with great care, as antibodies due to vaccination are difficult to be differentiate from those due to natural infection.

A relative risk (RR) test was performed to determine the association between the abortion and incidence of brucellosis, with a result of 3.35 (RR >1). This result showed that there was a strong association between abortion and brucellosis seropositivity at the study site. In other words, the risk of brucellosis was 3.35 times more in cows that had experienced abortion than in cows that has not experienced abortion. This finding was differ to study conducted by Mahajan et al. (2012) that brucellosis to be non-significantly associated with abortion, however, the risk of brucellosis was 2.059 times more in organized farms than in unorganized farms. The reason may be greater use of artificial insemination than natural services which provides a chance for magnified perpetuation of disease.

**Table 4.** Dichotomous data of the serology test of Brucellosis and the incidence of abortion in dairy cows at study sites

<table>
<thead>
<tr>
<th>Category</th>
<th>Brucellosis (+)</th>
<th>Brucellosis (-)</th>
<th>Total</th>
<th>Attack rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortions</td>
<td>22</td>
<td>45</td>
<td>67</td>
<td>32.8 per 100 animals</td>
</tr>
<tr>
<td>No experience abortion</td>
<td>19</td>
<td>174</td>
<td>193</td>
<td>9.8 per 100 animals</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>219</td>
<td>260</td>
<td>15.8 per 100 animals</td>
</tr>
<tr>
<td>Proportion of abortion</td>
<td>22/41 (0.54)</td>
<td>45/219 (0.20)</td>
<td>67/260 (0.26)</td>
<td></td>
</tr>
</tbody>
</table>

Vaccination against brucellosis using strain RB51 has been carried out at the study site, but the coverage is still very low. Historically coverage of vaccination against brucellosis at the study sites was 44.11%, ranged from 29 to 60% (see Table 2). Chi Square test was conducted to determine the effect of vaccination on the incidence of positive brucellosis. Dichotomous data between the results of the vaccination and results of serology test on brucellosis are presented in Table 5.

**Table 5.** Dichotomous data of the brucellosis serology test and vaccination at study site

<table>
<thead>
<tr>
<th>Variables</th>
<th>Brucellosis seropositive</th>
<th>Total</th>
<th>Attack rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (%) (+)</td>
<td>Negative (-)</td>
<td></td>
</tr>
<tr>
<td>Vaccination</td>
<td>18 (15.5%)</td>
<td>98</td>
<td>116</td>
</tr>
<tr>
<td>Unvaccination</td>
<td>23 (16.3%)</td>
<td>121</td>
<td>144</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>219</td>
<td>260</td>
</tr>
</tbody>
</table>
Chi Square statistical calculation value, at a significant level of 5% was smaller than the chi-square table value; therefore result suggested that the implementation of vaccination against Brucellosis has no significant effect on the incidence of brucellosis seropositivity. The incidence of brucellosis seropositivity in cows that have been vaccinated and unvaccinated was similar 15.5 and 16%, respectively (Table 4). This indicates that incidence of brucellosis seropositivity in vaccinated or unvaccinated cattle at the study sites had a similar chance. Although Brucella abortus vaccines strain 19 and RB51 were most commonly used to protect cattle against infection and abortion, however, they have side effect, such as interference with diagnostic tests, pathogenicity for humans, potential to cause abortion in pregnant animals (Dorneles et al. 2015).

The efficacy of vaccines, i.e. the proportion of seropositive brucellosis that can be prevented by a vaccination program in a group of animals receiving vaccination (data is in Table 4), is calculated as follows:

- Attributable risk (AR) = 0.160 - 0.155 = 0.005
- Attribute Fraction (AF) = 0.005/0.160 = 0.031 or 3.1%

Based on these results, vaccination at the study site only prevented 3.1% of dairy cows against brucellosis, in other words the efficacy of the vaccine was 3.1%. Therefore, it is recommended that further studies should be carried out on factors that influence the low efficacy of RB51 vaccine in dairy cattle. Several factors that can be studied further are RB51 vaccine protection level, RB51 vaccine quality, vaccination (cold chain) implementation and its coverage. To date the efficacy of the Brucella RB51 vaccine has not been evaluated yet due to limitations in serological detection where conventional serological tests (RBT, CFT, and Elisa) cannot detect antibodies in vaccinated cows.

CONCLUSION

Case of abortion and seroprevalence of brucellosis was fairly high on dairy cows in Bandung Region (Lembang and Pangalengan Districts). Vaccination coverage and the efficacy of brucellosis vaccine in dairy cattle was low, resulting in low protection on vaccinated and unvaccinated cows against brucella infection. This study is essential for disease control and prevention measures based on scientific finding. Further studies need to be carried out on the factors that influence the low efficacy of RB51 vaccine in dairy cows.

ACKNOWLEDGEMENT

This study was funded through the BBLitvet DIPA research budget. Technical assistance during the research was supported by the Bandung Regency Livestock and Fisheries Office, North Bandung Cattle Breeders Cooperative (KPSBU) Lembang, South Bandung Breeders Cooperative (KPBS) Pangalengan, and Bandung Regency Pasir Jambu Animal-Health Post. The gratitude is conveyed to technicians from the Bacteriology Laboratory, Sumirah (Ira) and Supartono, who have helped activities in the field and in the laboratory.
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