Assessment of Serum Antibody Titers in Cows and Buffalos Post-Septicaemia Epizootica Outbreak in Aceh Singkil District, Indonesia

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Abstract. The control of Septicaemia epizootica (SE) through a vaccination program has been implemented, but fatal cases are still frequently reported in Indonesia. This study aims to measure the antibody titers of bovines in the area affected by SE outbreaks and assess the coverage of ongoing vaccinations. We collected 117 serum samples from bovines and buffalos in Gunung Meriah District (54 samples), Singkohor (9 samples), Singkil (9 samples), and Singkil Utara (45 samples) in the Aceh Singkil area, using a simple random sampling method to select the sub-districts. We examined serum samples using the Enzyme-Linked Immunosorbent Assay (ELISA) considered antibody titers against SE seropositive if they were above 77 ELISA Units (EU). The vaccination coverage of sampled cattle was only 22.22%, and the percentage of cattle/buffalo with seropositive SE titers (>77 EU) in the four sub-districts of Aceh Singkil was 29.06%.

1 Introduction

Septicaemia epizootica (SE) is a disease that affects cattle and swamp buffalo. It is caused by Pasteurella multocida serotype B: 2 (Asian type) and E:2 (African type) [1]. It is included in the list of 22 communicable animal diseases prioritized to control in Indonesia by the Minister of Agriculture under decree No. 4026/Kpts/OT.140/3/2013. The emergency of SE is detrimental to livestock infection is very detrimental to the livestock industry as it causes large-scale livestock deaths [2]. In recent years, SE outbreaks have been reported in several regions of Indonesia. For example, in 2015, there were 71 incidents of SE in South Sulawesi, according to BPS South Sulawesi data. In the North Central Timor District of NTT, SE cases in cattle resulted in the death of 45 cows in 2014 [3]. In West Aceh, SE affected 138, 28, 18, 335, and 637 buffaloes in 2006, 2007, 2008, 2009, and 2010, respectively, and contributed to fluctuations in the region's total population [4].

Control of SE is achieved through vaccination programs to increase individual immunity, good livestock management, periodic surveillance of antibody profiles, isolation and identification of P. multocida, and structuring of SE cases in the field [5]. ELISA tests

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are used to observe the incidence of infection and response to vaccination by assessing antibody titer descriptions. ELISA tests can quickly and easily test large numbers of samples and can be standardized. ELISA tests can produce specific and predictive results. Follow-up monitoring and evaluation of SE vaccination programs are critical [6]. Therefore, researchers conducted a study to evaluate antibody titers against SE in cattle and buffalo in the endemic areas of SE outbreaks in Aceh Singkil District.

2 Methodology

2.1 Blood Samples Collection

Blood samples were obtained via the jugular vein using a venoject tube without anticoagulant. Approximately 3-4 ml of blood was collected and incubated at room temperature for 30 minutes overnight. The collected blood was centrifuged, and the resulting serum was stored in separate Eppendorf tubes at -20°C until testing. A total of 117 serum samples were collected from four districts using simple random sampling based on proportional population sizes to increase sample accuracy [7]. Vaccination history data was asked via interviews with breeders.

2.2 Enzyme-Linked Immunosorbent Assay (ELISA) Testing

The procedure used to determine the antibody titers against *P. multocida* in serum samples is carried out using an ELISA test [8]. The antigens used in the test are crude extracts from *P. multocida* type B: 2 prepared beforehand. Then, 100 µl of serum samples diluted 1:100 in PBS was added to each well and incubated at 37°C for 1 hour. After incubation, the ELISA plate was rewashed using PBST solution to remove unbound serum proteins. Then, 100 µl of goat anti-bovine IgG horseradish peroxidase conjugate (HRP) diluted 1:3000 in PBS was added to each well and incubated at 37°C for 1 hour. After the second incubation, the ELISA plate was rewashed with PBST solution, and 100 µl of 3,3',5,5'-tetramethylbenzidine (TMB) substrate solution was added to each well. The ELISA plate was then incubated at room temperature for 15-30 minutes, and the reaction was stopped by adding 100 µl of 1 N HCl to each well. The absorbance was measured using a microplate reader at a wavelength of 450 nm. The antibody titer was determined as the reciprocal of the highest dilution of serum that gave an absorbance value greater than the mean absorbance of the negative control serum plus two standard deviations.

Positive and negative results for SE antibodies from the samples examined were interpreted according to the diagnostic standards of the Center for Veterinary Science Bogor, where the titer value < 77 EU (ELISA Unit) showed no antibodies (seronegative), the titer value of 77 indicates doubtful/dubious. The titer value >77 EU showed the presence of antibodies to *P. multocida* (seropositive).

2.3 Statistical Analysis

The location and vaccination status data were tabulated and analyzed using the Chi-Square test with the SPSS 26 statistical software to investigate the potential influence of vaccination status on Elisa’s test outcomes. A negative test result indicates that the animal may not have been vaccinated, while a positive test result indicates that the animal has received the vaccination.
3 Result and Discussion

Blood samples were collected from 117 animals in the Singkil district (Fig 1), including 3 buffaloes and 114 cattle. Out of these, 26 animals were reportedly vaccinated, while 84 were not vaccinated. However, the vaccination status of the remaining 7 animals was unclear, as it was impossible to differentiate between vaccinated and non-vaccinated animals that were subsequently expelled. These animals were excluded from further analysis. The data from Table 1 shows that the vaccination coverage of the sampled cattle was 22.22%, with 26 out of 117 animals vaccinated (Table 1).

![Fig. 1. Research Sampling Location](image)

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Amount of Sample</th>
<th>Vaccinated</th>
<th>Not Yet Vaccinated</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gunung Meriah</td>
<td>54</td>
<td>13</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>Singkohor</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>North Singkil</td>
<td>45</td>
<td>13</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>Singkil</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>26</td>
<td>84</td>
<td>7</td>
</tr>
</tbody>
</table>

There could be several reasons for the low vaccination coverage of the sampled cattle. One possible reason could be the lack of awareness among farmers regarding the importance of vaccination in preventing diseases. Farmers may not have access to information on the benefits of vaccination or have sufficient resources to vaccinate their animals. Additionally, there could be issues with the availability and accessibility of vaccines, beside the difficulties for vaccinators officer to go to farms because cows or
buffaloes usually released in forests or mountains. Those are some of contributing factors of low vaccination coverage. Other factors, such as the cost of vaccination, lack of government support, incentives for vaccination, could also play a role. It is important to identify the underlying reasons for low vaccination coverage to develop targeted interventions to increase coverage and prevent the spread of diseases.

The ELISA test on whole blood serum samples collected from Aceh Singkil district in 4 sub-districts revealed positive results for antibody titers against SE. Among the sub-districts, the Singkil sub-district showed the highest percentage of positive cases at 4/9 (44.44%), while the Mount Meriah sub-district showed the lowest percentage at 13/54 (24.07%) (Table 2). The positive results were obtained due to the presence of antibodies to *P. multocida* (seropositive) with an antibody titer value $\geq 77$ EU (ELISA Unit). Several factors contribute to the variation in the percentage of positive results for antibody titers against SE in different sub-districts. These factors could include differences in the level of exposure to the pathogen, differences in the management practices of livestock in each sub-district, differences in the effectiveness of vaccination programs, and differences in the genetic susceptibility of the animals. It is important to investigate these factors further to understand the epidemiology of the disease better and to develop effective control measures.

<table>
<thead>
<tr>
<th>Sampling Location</th>
<th>Amount of Sample</th>
<th>Positive</th>
<th>Negative</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gunung Meriah</td>
<td>54</td>
<td>13</td>
<td>41</td>
<td>24.07</td>
</tr>
<tr>
<td>Singkohor</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>33.33</td>
</tr>
<tr>
<td>North Singkil</td>
<td>45</td>
<td>14</td>
<td>31</td>
<td>31.11</td>
</tr>
<tr>
<td>Singkil</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>44.44</td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>34</td>
<td>83</td>
<td>29.06</td>
</tr>
</tbody>
</table>

The ELISA test has been a crucial serological tool in laboratories since its invention in 1971 due to its speed, sensitivity, specificity, and ability to quickly test large quantities of samples [9]. Its versatility has made it a staple in various diagnostic procedures [10].
Table 3 shows the results of the ELISA test on cattle vaccinated with SE were measured as seropositive as much as 21/26 (80.77%), and animals have been detected as seronegative as many as 5 tails 5/26 (19.23%). On the other hand, livestock that has not been vaccinated is detected with seropositive SE as much as 58/84 (69.04%). Cattle with positive antibody titers but no vaccination may have acquired the antibodies through natural infection, even if they do not exhibit any symptoms. The high seropositivity rate among unvaccinated animals is concerning because these animals, which may not show any signs of illness, can still transmit the infection to other animals in the area, potentially leading to an SE outbreak if the population is stressed. Additionally, vaccinated cattle may have temporary low or undetectable antibody titers, requiring booster vaccinations to maintain immunity. Low vaccination coverage and low antibody titers against SE should also be monitored [11,12].

Table 3. Vaccination Status and ELISA Test Results of Sample for SE Antibody in Aceh Singkil

<table>
<thead>
<tr>
<th>Status of Vaccination</th>
<th>ELISA Test Result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Not yet Vaccinated</td>
<td>58</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>31</td>
</tr>
</tbody>
</table>

Note: P > 0.05 (0.246), 7 animals had no clear vaccination status.

The sampled population has a relatively high seropositive rate for SE antibodies. The variation in the percentage of positive results in different sub-districts may be due to factors such as differences in pathogen exposure, livestock management practices, the effectiveness of vaccination programs, and the genetic susceptibility of the animals. It is important to investigate these factors further to understand the epidemiology of the disease better and to develop effective control measures. Furthermore, the low vaccination coverage and low antibody titers against SE in some animals are areas of concern. This highlights the need for effective vaccination programs and monitoring of vaccination coverage to prevent the spread of SE in the population [13].

However, the relationship between vaccination status and ELISA test results was insignificant, possibly due to the low vaccination coverage compared to the number of samples taken, thus not representing the wider population. Therefore, further studies with larger sample sizes must confirm the relationship between vaccination status and SE test results.

4 Conclusion

Based on the findings and statistical analysis, it can be concluded that the vaccination coverage of the sampled cattle was only 22.22%, with 26 out of 117 animals being vaccinated, and the number of seropositive cattle and buffaloes for SE (>77 EU) in the four sub-districts of Singkil district, Aceh Singkil district was 34 animals (29.06%) out of the total sample of 117.
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References

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