



(RESEARCH ARTICLE)



## Indirect contact increases antibody titers in people exposed to Anthrax

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### Abstract

**Introduction:** Anthrax is a zoonotic infection that often becomes epidemic in endemic areas. It can be transmitted to humans through contact with infected animals. The most common manifestation is the appearance of *eschar* on the skin. Early detection as a screening for anthrax exposure can use serum anti PA Ig G antibody tests. This study aims to analyze the contact history and appearance of *eschar* on the skin against serum antibodies of people exposed to anthrax.

**Material and Methods:** This analytical observational study used a cross-sectional design. It assessed the relationship of contact history and *eschar* to Ig G anti PA serum antibodies. To determine the significance of the association between the factors studied, an analysis using SPSS version 22.0 for windows was used, with a 95% confidence level.

**Result and Discussion:** A total of 35 respondents who were exposed to anthrax in Gunungkidul District, Yogyakarta. Most of the respondents were women aged between 40 - 60 years and worked as farmers. It showed that Anti-PA Ig G antibodies are 40% positive, with 17% having a history of direct contact showing positive serology and 20% having *eschar* on skin with positive antibodies. It indicates a significant association between contact history and antibodies with  $p < 0.05$ , but there is no significant difference in the appearance of *eschar* on the skin.

**Conclusion:** There is a relationship between the results of serology with a history of contact with infected animals, so it's very important information in efforts to prevent and screen anthrax in endemic areas.

**Keywords:** Contact; Eschar; Anthrax; Antibody

### 1. Introduction

Anthrax is a zoonotic infection that often causes outbreaks in Indonesia. This infection can be transmitted to humans through contact with infected animals and is one of the priority programs of 14 zoonotic diseases in Indonesia.<sup>1-4</sup> This anthrax outbreak is closely related to a history of contact with infected animals.<sup>5</sup> This is evident in the anthrax outbreak that occurred in 2016 in China.<sup>6</sup> Pieracci et al., (2016), reported that there were 5,197 cases of anthrax in humans from 2009 to 2013, with a mortality rate of 1.7%, which occurred in Ethiopia.<sup>7</sup> Anthrax outbreaks in Indonesia have increased,<sup>3</sup> which is marked by anthrax cases from 2019 to 2020 in Yogyakarta, Central Java and East Java.

Based on the entry of spores into the body, including skin anthrax, inhalation, and gastrointestinal.<sup>2,7</sup> Almost 95% of anthrax cases that occur in the world manifest on the skin because of direct contact between the skin and *Bacillus anthracis* spores through the skin.<sup>5,6,8</sup>

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Early detection in the diagnosis of anthrax is the examination of IgG anti-Protective Antigen (PA) using the Enzyme Linked Immunosorbent Assay (ELISA).<sup>5,9,10</sup> However, tests based on this can cause false positive results.<sup>10</sup>

Therefore, this study aimed to analyze the correlation between contact history and *eschar* on the skin against serum IgG anti-PA antibodies during anthrax outbreaks in Indonesia.

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## 2. Methods

This research is an analytic observational study with a cross-sectional approach. The research location is in Gunungkidul Regency, Yogyakarta, Indonesia. This research took place from December 2019 to March 2020.

### 2.1. Sample

The population in this study were people exposed to *Bacillus anthracis* spores, namely 35 respondents who were then tested for anti-PA IgG serology at the Laboratory of the Indonesian Veterinary Research Center, Bogor.

Data was collected from residents who had been exposed to *Bacillus anthracis* spores from infected animals, including demographic information such as age, gender, occupation, contact history, the presence of *eschar* on the skin, and serological testing for IgG anti-PA serum.<sup>9,10</sup>

### 2.2. Contact History

One of the risks of transmission to humans is contact with infected animals. Direct contact occurs when there is direct exposure to *Bacillus anthracis* spores from anthrax-infected animals. They can enter through the skin or respiratory tract, when slaughtering animals or washing animal meat.<sup>5,11,12</sup> Indirect contact occurs when there is no direct contact with *Bacillus anthracis* spores from infected animals, for example, consuming animal meat or processing skin or bone products that have been cleaned.<sup>6,7,11</sup>

### 2.3. Eschar on the skin

Anthrax manifestations that often occur in anthrax outbreaks are lesions on the skin that end with the appearance of *eschar*.<sup>5-7</sup> This occurs approximately 2 weeks after exposure. 11 *Bacillus anthracis* spores enter through non-intact skin, for example during the slaughter of animals infected with anthrax.<sup>12,13</sup> The site of entry of the spores will cause lesions on the skin, beginning with itching, followed by the release of lethal toxin. Then papular lesions will develop into vesicles, accompanied by regional edema and pain.<sup>6,8,14</sup> These lesions then develop into vesicles. This causes localized necrosis, which is characterized by the appearance of *eschar* and surrounding soft tissue edema.<sup>7,11</sup> Germination occurs within 1-3 hours after inoculation, but this germination cannot cause infection in intact skin.<sup>6,9</sup>

### 2.4. Antibody Serology Test

In establishing the diagnosis of anthrax, it can be assessed by measuring the serum IgG anti-PA antibody titer using the ELISA method. The tool used is an ELISA reader, as well as materials such as Microwells coated with PA recombinant antigen, calibrator, positive and negative control, enzyme conjugate, and Tetramethylbenzidine (TMB).<sup>10</sup>

The serological test used was the Calbiotech Protective Antigen IgG ELISA kit®, Inc. USA. First, the 2.5 L test sample and control were diluted 1:41, then added to pre-coated plates, followed by incubation at room temperature for 30 minutes. Plates were washed three times with 300 L of wash buffer 1x, then conjugate solution, 100 L, was added to each well and incubated at room temperature for 30 min. After that, the plates were washed and 100 L of TMB substrate solution was added to each well for a 10-minute incubation at room temperature. Then each plate was read at 450 nm using an ELx800 absorbance microplate reader (BioTek, VT). The calibrator OD is multiplied by the calibrator factor in each bottle to determine the cut-off value. Each sample and control OD were divided by the cut off value to determine the antibody index. Positive if the antibody index is greater than 1.1, borderline between 0.9 and 1.1, and negative if it is less than 0.9.<sup>10,15</sup>

A positive result is a sign of an increase in the IgG antibody titer against exposure to *Bacillus anthracis* spores, while a negative result indicates a low antibody titer.

### 2.5. Data Analysis

To determine the correlation between variables, the data was analyzed using SPSS version 22.0 for Windows and the chi-square correlation test with a significant value less than 0.05.

### 3. Results

The basic characteristics of this study indicate that the number of respondents could be as many as 35 people, with 57% being female, 60% aged between 41-60 years, and 71% working as farmers (Table 1).

The results of the serum antibody examination showed that 40% of respondents were positive and 34.3% of respondents were declared negative. History of contact with animals infected with anthrax indirectly 71.4%, and 28.6% directly. Manifestations in the form of *eschar* on the skin occurred in 60% of respondents (Table 2).

Respondents with a history of direct contact with infected animals showed positive results of as much as 17%, while respondents who had indirect contact with positive antibody results were 23%. Contact history and serum IgG anti-PA antibodies showed a significant relationship ( $p = 0.045$ ) (Table 3).

The appearance of *eschar* was followed by an increase in antibody titer in 20% of respondents and a negative result of 20%. The appearance of *eschar* in the skin and serum IgG anti-PA antibodies showed no significant relationship ( $p = 0.418$ ). Skin *eschar* that occurred on the finger of a patient with cutaneous anthrax during the outbreak in Yogyakarta can be seen in Figure 1.

**Table 1** Characteristics of Population Exposed to Anthrax

Variable	N	%
Gender		
Male	15	43
Female	20	57
Age		
< 20 years	0	0
21 – 40 tahun	10	29
41 – 60 tahun	21	60
> 61 tahun	4	11
Job		
Farmers	25	71
Non-farmer	10	29

**Table 2** Distribution of Anti-PA Anthrax IgG titer, contact history and *eschar* (n=35)

Variable	N	%
Anti-PA Anthrax IgG Titer		
Positive	14	40
Borderline	9	25.7
Negative	12	34.3
Contact History		
Direct	10	28.6
Indirect	25	71.4
Skin Manifestation		
<i>Eschar</i>	21	60
No <i>Eschar</i>	14	40

**Table 3** Cross table of contact history and *eschar* against anthrax antibodies

Variable	Anti-PA IgG Serum			P
	Positive	Borderline	Negative	
Contact History				0.045
Direct	6 (17%)	1 (3%)	3 (8%)	
Undirect	8 (23%)	8 (23%)	9 (26%)	
Skin manifestation				0.418
<i>Eschar</i>	7 (20%)	7 (20%)	7 (20%)	
No <i>eschar</i>	7 (20%)	2 (5%)	5 (15%)	

**Figure 1** *Eschar* on cutaneous anthrax

#### 4. Discussion

Anthrax outbreaks are a problem that often occurs in endemic areas, especially in tropical countries, including Indonesia.<sup>3,5</sup> Clinical manifestations that often appear are skin anthrax, characterized by the presence of *eschar* on the skin, especially on the face, upper and lower extremities.<sup>5,6,13,16</sup> Respondents who were exposed to anthrax spores in this study were mostly female. This is in accordance with Redhono et al., (2016), which may be due to direct contact when washing and sharing infected animal meat, which is often done by women.<sup>5</sup> According to Ombala et al. (2016), the most common age range is between 15 and 29 years.<sup>17</sup> This is different from the research conducted by Mwakapeje et al., (2018), where 70% of the respondents were male, with a mean age of 32 years, while the results of this study showed that the most common age range was 41-60 years.<sup>18</sup> This is in accordance with Doganay et al., (2010), which stated that the median age in anthrax cases was 44 years, with gender in girls.<sup>7</sup>

Transmission from animals to humans begins with contact with infected animals.<sup>5,9</sup> This is one of the important factors in assessing the possibility of the entry of spores into the human body.<sup>19</sup> In this study, 17% of respondents who had direct contact showed Ig antibody results. Positive serum anti-PA G. This is in accordance with the research of Redhono et al, (2015), which states that most people who are directly exposed will show positive antibody results.<sup>11</sup> What is interesting about the results of this study is that 23% of respondents without a history of direct contact showed positive results for positive antibodies. There is a history of indirect contact in the form of consuming cooked meat from anthrax-infected animals, but there is still a risk of transmission to humans. The existence of a significant relationship strengthens the reason that contact history is one of the important information that must be known by people exposed to anthrax.<sup>11,20,21</sup>

In this study, most of the people exposed to anthrax had *eschar* on their skin, especially on their fingers and toes, which showed positive serum IgG anti-PA antibodies in 20% of the respondents, while negative serological results were also obtained in 20% of the respondents. There is an interesting thing in this study: 20% of respondents who appeared *eschar* but had negative serological results. This may be due to the relatively small amount of spore exposure or the good immune system of the individual. So far, there is no definite relationship between anti-PA IgG levels and clinical manifestations of anthrax patients. This is consistent with Mweemba et al., (2012), workers in the wool processing industry showed a positive anti-PA IgG titer, which supports a history of exposure to anthrax spores during work.<sup>22</sup>

An increase in serum IgG anti-PA antibody titers occurs in most people who have direct or indirect contact. This is in accordance with the study of Redhono et al., (2015), which showed positive anti-PA IgG, but no clinical manifestations were found with 22% of respondents with positive anti-PA IgG titers showing no clinical manifestations, but it was concluded that an increase in IgG titers was often accompanied by the appearance of *eschar*.<sup>11</sup> Borderline results occur, possibly due to exposure to small amounts of anthrax spores or the presence of a good immune system in a person, so that the antibody titer does not rise high.<sup>23</sup> Currently, there is no literature that states the relationship between anti-PA IgG titers and clinical manifestations of anthrax. Other factors that may play a role are host conditions, the virulence of the spores and the environment.<sup>24</sup>

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## 5. Conclusion

There is a relationship between the results of serology with a history of contact with infected animals, so it's very important information in efforts to prevent and screen anthrax in endemic areas.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

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