A comparison of the immune response between early exposed and 1 year post exposure to *Bacillus anthracis* in Indonesia

D Redhono¹, A Kusumawardani² and P Dirgahayu³

¹Sub Division of Tropical Medicine & Infectious Disease, Faculty of Medicine, Sebelas Maret University, Moewardi Hospital, Surakarta, Indonesia
²Departament of Dermatology Venerology, Faculty of Medicine, Sebelas Maret University, Moewardi Hospital, Surakarta, Indonesia
³Departement of Biomedicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia
⁴Corresponding author: dhaniredhono@gmail.com

**Abstract.** Anthrax is one of the zoonotic diseases that usually affects animals and can be transmitted to humans. Immune response of the body during an infection is the presence of antibodies as an effort to defend the body and it will survive for some time in the blood. The aim study is to find out how the initial response to the formation of antibodies and how these antibodies survive after one year. This study is cohort to people exposed to anthrax and found 130 people exposed to anthrax. The most risk factor was direct contact and consumed infected animal meat, which was 34.6%. Clinical manifestations of the skin were 12.3% and all respondents showed positive IgG. While 87.7% did not show any anthrax symptoms. IgG serum examination after 1 year of exposure to anthrax obtained 3.8% still detected antibodies in the body. The relationship between IgG titer with clinical manifestations of anthrax at one year post-outbreak is highly significant p 0.028. In conclusion a significant association between the clinical manifestation with antibody serum anthrax and it still detected after one-year post outbreaks of anthrax.

**1. Introduction**

Anthrax is one of the types of zoonotic diseases, which can be transmitted to humans from animals suffering from anthrax. The disease is caused by *Bacillus anthracis*. Anthrax commonly attacks livestock such as cattle, sheep, goats and camels. Transmission to human occur when there is direct contact of animal products or animal that suffer from anthrax. It can be skin, blood or flesh. The cutaneous form of the disease is the majority reported cases[1,2]. Anthrax in West Java, Indonesia for the last ten years were reported to be occurred five times than that was in 1996 to 2000[3,4,5].

Since the outbreak of anthrax 15 years ago in Indonesia, the patient’s sample should be sent abroad (USA) for the diagnosis of anthrax investigation. Based on these events, the Moewardi hospital cooperate with Integrated Biomedical Laboratory of the Faculty of Medicine, University Sebelas Maret has been trying to develop anthrax test-based immunoassay using Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of proteins PA by using the Anthrax Protective Antigen Calbiotech (PA) IgG ELISA Kit, as catcher agents that are sensitive to the ELISA which is able to detect PA (≥1 ng/ml) in the serum of patients with suspected anthrax[3,5,6,7].
This study aims to determine early antibody response and one year post exposed in *Bacillus anthracis*.

2. Methods
This study is a cohort study which method is by immunoassay based on people who exposed to *Bacillus anthracis*. The location of sampling is an area of outbreak anthrax. Anyone who is exposed to the anthrax germs will be dialed for a blood sample for examination IgG antibodies in serum by ELISA and immediately checked for its antibody and one year after exposure. ELISA Interpretation will be put in three categories; positive, negative and borderline\[^{8,9,10}\].

Population and samples in this study are those who has been exposed to anthrax from a dead animal which meet the inclusion criteria. The inclusion criteria are those who do not suffer from a disease that causes a decrease in the body’s immunity, there are currently using immunosuppressant drugs, there are pregnant or breastfeeding, not in hormone therapy. The exclusion criteria are who are not willing to follow research and attempt with serious illness such as sepsis.

2.1. Mechanical Sampling
Sample size is determined based on the criteria with minimum samples of 40.

2.2. Serological Test
The protective antigen (PA) is a component of the anthrax toxins. This is an essential virulence factor of *Bacillus anthracis* and is the major protective immunogenicity. Serological test to assess the titer of anthrax protective antigen (PA) IgG ELISA were used to assess the immune response in populations exposed Anthrax either direct contact or by consuming meat\[^{11,12}\].

2.3. Identification of Research Variables
2.3.1. Dependent variable: the titer of anthrax protective antigen (PA) IgG. The independent variables are characteristics (age, sex, education, and occupation), contact exposure and clinical manifestations. External variables that can be controlled are immunocompromise disease and severe infections. External variables that cannot be controlled are immunity, socio-economic conditions of cultural and taking antibiotics.

2.4. Operational definitions of variables
Dependent variable is the titer of anthrax protective antigen (PA) IgG. Definition is titer of anthrax protective antigen (PA) IgG is the titer in serology examination to confirm the presence of infection with *Bacillus anthracis* in human blood and evaluate patient’s immune status or exposure to anthrax. The following is intended as a guide to interpretation of PA IgG antibody index (Ab Index) test results\[^{12,13,14}\]:

- \(<0.9\) : No detectable IgG antibodies against PA protein in ELISA.
- \(0.9 - 1.1\) : Borderline positive. Recommended for re-examination.
- \(>1.1\) : Detected the presence of IgG antibodies to the protein PA, indicated patients were infected or infected with a never *Bacillus anthracis*.

3. Result
3.1. Individual Characteristic
In this study, 130 people with histories of contact with animals suffered from anthrax. The highest distribution are in the age of 21 to 40 years which is as much as 40% and most of them are female. The education level of respondents at most are 73.8% graduated from elementary school. The basic characteristics of the study subjects are shown in Table 1.
Table 1. Demographic characteristics of research subjects.

| Characteristic          | N  | %   |
|-------------------------|----|-----|
| Gender                  |    |     |
| • Male                  | 58 | 44.6|
| • Female                | 72 | 55.4|
| Age                     |    |     |
| • 0 – 20 old year       | 4  | 3.0 |
| • 21 – 40 old year      | 52 | 40.0|
| • 41 – 60 old year      | 46 | 35.4|
| • 61 – 80 old year      | 28 | 21.6|
| Education               |    |     |
| • Elementary            | 96 | 73.8|
| • Junior high school    | 23 | 17.7|
| • Senior high school    | 7  | 5.5 |
| • University            | 4  | 3.0 |
| Profession              |    |     |
| • No work               | 54 | 41.5|
| • Farmer                | 33 | 25.4|
| • Civil employment      | 7  | 5.4 |
| • Private               | 36 | 27.7|

3.2. ELISA examination
The serum IgG antibodies showed 50.5% negative, 14.6% borderline and 32.3% positive (Table 2).

Table 2. Results of ELISA.

| Variable    | N  | %   |
|-------------|----|-----|
| Positive    | 42 | 32.3|
| Borderline  | 19 | 14.6|
| Negative    | 69 | 50.5|

3.3. Analysis of ELISA and risk contact
In cross-table analysis results between risk factors contact with ELISA serology results obtained at the same respondents who cook and eat the highest risk on positive serologic (Table 3).

Table 3. Results of serology Elisa associated with risk contact.

| Risk Factors             | Antibody PA |
|--------------------------|-------------|
|                          | Positive    | Borderline | Negative |
| Wash the meat            | 5           | 1          | 4         |
| Eating                   | 10          | 4          | 19        |
| Wash and eat             | 3           | 5          | 3         |
| Cooking and eating       | 10          | 4          | 23        |
| Slaughtering and eating  | 9           | 3          | 15        |
| Located near the cage    | 5           | 2          | 5         |

3.4. Clinical manifestations
Respondent with eschar are found in 12.3% of people exposed to anthrax who showed clinical signs of the appearance of the skin in the form of vesicles, accompanied by fever and ulcers which ended with eschar formation (Table 4).
Table 4. Distribution of clinical manifestation.

| Clinical manifestation | N  | %   |
|------------------------|----|-----|
| • Eschar               | 16 | 12.3|
| • No Eschar            | 114| 87.7|

3.5. Analysis eschar with ELISA
All people with eschar, showed positive serology results. People without eschar 20% positive IgG. Result of serology associated with skin manifestations such as eschar can be seen in Table 5.

Table 5. Results serology of ELISA associated with skin manifestations in the form of eschar.

| Eschar | Antibody ELISA |
|--------|----------------|
|        | Positive | Borderline | Negative |
| • Yes  | 16       | 0          | 0        |
| • No   | 26       | 19         | 69       |

3.6. Analysis risk contact with the manifestation of eschar
Contact risk factor for the emergence of manifestations in the skin, especially on the respondents were slaughter a cow and eat it (6.0%), followed by the washing and eating meat, which is 3.0%, whereas only 1.0% wash the meat. The relationship between contact with the manifestation of the emergence of the eschar can be seen in Table 6.

Table 6. Relationship between contact with the manifestation of eschar.

| Variable                  | Eschar | No |
|---------------------------|--------|----|
| • Wash the meat           | 1      | 4  |
| • Eating                  | 2      | 34 |
| • Wash and eat            | 3      | 6  |
| • Cooking and eating      | 2      | 45 |
| • Slaughter and eating    | 6      | 23 |
| • Located near the cage   | 2      | 2  |

3.7. ELISA after one year
IgG serum examination after one year of exposure to anthrax obtained 3.8% still detected antibodies in the body and 88.5% was negative. The relationship between IgG titers with clinical manifestations of anthrax at one year post-outbreak is highly significant p0.028.

Table 7. Results of ELISA after one year.

| Variable  | N  | %   |
|-----------|----|-----|
| • Positive| 5  | 3.83|
| • Borderline| 10 | 7.67|
| • Negative| 115| 88.5|

4. Discussion
During outbreak of anthrax obtained a cow belonging to one of the people who had collapsed and accompanied by seizures. The owner decided to slaughter cattle meat and sold to residents of 40 packs. Beef meat and blood samples are examined in laboratory of Central Java Province and tested positive for anthrax. Seven days later, seven people were complaining small bumps and itching, swelling and lesions accompanied wet in the area under the eyes, hands, legs or feet, then were taken to the health center and declared suspected anthrax.}[3,5,7]
Clinical manifestations in the form of eschar present in 11.9% with cutaneous anthrax. Respondents were taken from both locations, obtained 130 samples were then examined serological Anthrax serum IgG antibodies that showed negative 50.5%, borderline 14.6% and 32.3% positive. Clinical manifestations in the form of a skin disorder that begins their edema or injury which lead to edema and end with eschar present in 10.9% of the respondents whose IgG antibody positive and 1.0% of respondents with IgG borderline results. This is due to the emergency of antibodies against anthrax bacteria in respondents who had clinical manifestations in the skin, but that cannot explain why in the result of obtained antibodies which appeared to be borderline clinical manifestations (see Figure 1).\textsuperscript{3,5}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{eschar.jpg}
\caption{Anthrax manifestations in the skin with the advent of eschar.}
\end{figure}

Twenty percent of respondents with a positive serum IgG antibody, did not lead to clinical manifestations. It might be due to the durability of the respondents, bacterial virulence factors and the amount of exposure that occurs may not be too much. But this cannot be explained further, because of the endurance factor all pretty much the same condition, which probably is due to the virulence number of the bacteria and germs that enter.

The risk of direct contact, i.e. cooking and eating meat of infected animals showed 30.5% IgG positive results, but does not cause clinical manifestations with the advent of eschar. This may be due to immune factors of patients as well as virulence \textit{B. anthracis} that enters the body. Risk factors eating only 32% of respondents showed positive results. While the risk factors of slaughter and eating will be 24% manifested in the appearance of skin eschar.

IgG serum examination after one year of exposure to anthrax obtained 3.8% still detected antibodies in the body, 88.5% was negative and relationship between IgG titers with clinical manifestations of anthrax at one year post-outbreak is highly significant \textit{p} 0.028. This may be due to direct contact with animals or components of an infected animal. So, the risk of transmission and eschar occurrence is higher in people who are in direct contact than those who do not.

5. Conclusions
The conclusion of this study is the increase in serum antibody titer IgG anthrax does not occur in all of the respondents who were exposed to the anthrax outbreak area. All respondents were obtained eschar followed by an increase in IgG antibody titers. Anthrax Protective Antigen (PA) ELISA IgG was detected after one year post outbreaks of anthrax and meaningful relationship to clinical manifestations.

References
[1] Jeremy F, Peter J H, Thomas J, Gagandeep K, David L and Nicholas J W 2014 Anthrax \textit{Manson's tropical diseases} vol 31 pp 395-8
[2] Fred F F 2015 Anthrax \textit{Ferri's clinical advisor} p 115
[3] Redhono and Paramasari 2011 Anthrax outbreaks in Indonesia proceeding in APSIC 2011-the 5th international congress of the asia pacific society of infection control Melbourne 152
[4] Mehmet D, Karen E B, Arya A, Stanley G, Darrell R G, Alfred J, Alan S C and Leslie W B 2011 The early humoral immune response to Bacillus anthracis toxins in patients infected with cutaneous anthrax FEMS Immunol. Med. Microbiol. 62(2) 164–72
[5] Redhono D, Sumandjar T and Hermawan G 2011 Pemetaan antraks di jawa tengah Antraks Sebelas Maret press 11-7
[6] Baillie L W 2009 Is new better than old? The development of human vaccines for anthrax Human Vaccines 5 806–16
[7] Dirgahayu P 2011 Pemeriksaan laboratorium deteksi antraks berbasis immunoassay Antraks Sebelas Maret press 18-26
[8] Dixon T C, Meselson B S M, Guillemin J and Hanna P C 2005 Anthrax N. Engl. J. Med. 341 815-26
[9] Pile J C, Malone J D, Eitzen E M and Friedlander A M 2005 Anthrax as a potential biological warfare agent Arch. Intern. Med. 158 429-34
[10] Shafazand S, Doyle R, Ruoss S, Weinacker A and Raffin T A 2005 Inhalation anthrax, epidemiology, diagnosis and management Chest 116 1369-76
[11] John E B, Raphael D and Martin J B 2015 Anthrax Mandell, douglas, and bennett's principles and practice of infectious diseases second edition (Saunders) 209 pp 2391-409
[12] Inglesby T V, Henderson D A and Barlett J G 2005 Anthrax as a biological weapon medical and public health management JAMA 281 1735-45
[13] Holmes R K 2009 Diphtheria, other corynebacterial infection and anthrax Harrison’s principles of internal medicine 16th edition ed A S Fauci, E Braunwald, et al. (New York: McGraw-Hill) pp 892-9
[14] Quinn C P, Semenova V A, Elie C M, et al. 2002 Specific, sensitive, and quantitative enzyme-linked immunosorbert assay for human immunoglobulin G antibodies to anthrax toxin protective antigen Emerg. Infect. Dis. 8 1103–10