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Research Report

RELATIONSHIP BETWEEN CLINICAL MANIFESTATIONS AND ANTIBODY SERUM IN OUTBREAKS ANTHRAX

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ABSTRACT

Introduction: *Anthrax is a zoonotic disease that often affects the grass-eating animals, which occurs due to the entry of spores into the bodies of animals and can be transmitted to humans. This disease often appear in certain seasons and occurs in endemic areas, including Indonesia. Cutaneous anthrax is the clinical manifestations that often arise on outstanding events in the area. This study aims to determine how the relationship between the clinical manifestations of the serum antibodies in people who are exposed to anthrax.* ***Material and methods:*** *This study is an observational cross sectional analytic approach, in people exposed to anthrax to assess the clinical manifestations and antibody serum Anthrax.* ***Results:*** *Obtained in this study respondents were 101 people with a history of contact with animals suffering from anthrax. The number of respondents with the highest age distribution was 31 to 40 years by 42%, and most were female gender, which is 57.7%, the highest level of education is 74% finished elementary school. Forty-four percent of working as a housewife. Risk factors are the most direct contact with and consume the flesh of animals as much as 34.6%. Results of Ig G antibody serum showed 50% negative, 15.4 borderline and 34.6% positive. Clinical manifestations that occur in the skin as much as 13.5%, that is the eschar on all respondents and 92.8% showed positive Ig G. While 86.5% did not show any clinical signs of anthrax, of that number 25.5% with Ig G positive, 16.6% and 57.7% showed borderline negative with p 0.02.* ***Conclusion:*** *There was a significant association between the clinical manifestation with antibody serum anthrax. But also found a positive Ig G without the appearance of clinical signs in the skin.*

Key words: *clinical, manifestation, anthrax, serum antibody ELISA, eschar*

ABSTRAK

Pendahuluan. *Antraks adalah salah satu penyakit zoonosis yang sering menyerang pada hewan pemakan rumput, yang terjadi karena masuknya spora ke dalam tubuh hewan dan dapat ditularkan ke manusia. Penyakit ini sering muncul pada musim tertentu dan terjadi di daerah endemi, termasuk Indonesia. Cutaneous Anthrax merupakan manifestasi klinis yang sering timbul pada kejadian luar biasa di suatu daerah. Penelitian ini bertujuan untuk mengetahui bagaimana hubungan antara manifestasi klinis terhadap serum antibodi pada orang yang terpapar antraks.* ***Bahan dan Metode.*** *Penelitian ini merupakan observasional analitik dengan pendekatan Cross Sectional, pada orang yang terpapar antraks dengan menilai manifestasi klinis dan serum antibodi Antraks.* ***Hasil.*** *Pada penelitian ini didapatkan responden sebanyak 101 orang dengan riwayat kontak dengan hewan yang menderita antraks. Jumlah responden dengan sebaran umur tertinggi adalah pada 31 sampai 40 tahun sebanyak 42 %, dan jenis kelamin terbanyak adalah perempuan, yaitu 57,7 %, tingkat pendidikan terbanyak adalah lulus SD 74 %. Empat puluh empat persen bekerja sebagai ibu rumah tangga. Faktor risiko terbanyak adalah kontak langsung dan mengkomsumsi daging hewan sebanyak 34,6%. Hasil pemeriksaan Ig G antibodi serum menunjukkan 50% negatif, 15,4 borderline dan 34,6% positif. Manifestasi klinis yang terjadi pada kulit sebanyak 13,5 % , yaitu adanya eschar pada semua responden dan 92,8% menunjukkan Ig G positif. Sedangkan 86,5% tidak menunjukkan adanya tanda klinis antraks, dari jumlah tersebut 25,5% dengan Ig G positif, 16,6% menunjukkan borderline dan 57,7% negatif dengan p 0,02.* ***Simpulan.*** *Ada*

hubungan yang bermakna antara manifestasi klinis dengan hasil serum antibodi Antraks. Namun juga didapatkan adanya antibodi Ig G positif tanpa disertai munculnya tanda klinis di kulit. Sehingga perlu dilakukan deteksi dini pada orang yang terpapar antraks.

Kata kunci: *manifestasi, klinis, antraks, antibodi serum ELISA, eschar*

INTRODUCTION

Anthrax is one of the types of zoonotic diseases, which can be transmitted to humans, animals suffering from anthrax. The disease is caused by bacillus anthracis. Anthrax commonly often attacked livestock such as cattle, sheep, goats and camels. Transmission to humans occurs when there is direct contact of animals or animal products that suffer from anthrax, can be skin, blood and flesh.[1,2]

Anthrax incident in Indonesia in the last ten years has occurred five times the plague that is 1996 to 2000 in West Java.[3,4]

Since the outbreak of anthrax 15 years ago in Indonesia, the patient 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 was able to detect PA (≥ 1 ng / ml PA) in the serum of patients with suspected anthrax.[4,5]

This Problem is how is anthrax protective antigen serum antibodies (PA) Ig G ELISA in people who are exposed to anthrax and its relationship with clinical manifestations in outbreak area ?

Diagnosis approach

Anthrax Diagnosis is made through history, clinical examination, laboratory and serology :

1. History: Early diagnosis of anthrax is difficult to know because it does not show the typical signs and symptoms, usually preceded by the appearance of reddish nodule with pain and swelling. It needs to be asked is whether previously had contact with an animal that died of anthrax, either direct contact or eating meat or contact with animals or their products (skin, bone), how the employment status (farmers fields, ranchers, RPH, tanners) and whether residence in endemic areas of anthrax.[2,6]
2. Clinical manifestations: There are 3 clinical manifestations that may arise in people is cutaneous anthrax, gastrointestinal and inhalation:
   a. Cutaneous anthrax
      Most cases (95%) anthrax is happening in the world is cutaneous anthrax. Patients usually have a history of contact with animals or their products, the anthrax bacteria or spores enter through the skin through a lesion on the skin, for example, when doing the slaughtering process (cutting, skinning or divide meat) cattle infected with anthrax. Then came a low germination rate at the location where the entry of spores and cause lesions on the skin that itch, then papuler lesions arise and develop into vesicles accompanied by edema and pain. These lesions became necrotic eschar formation and accompanied local soft tissue edema. Germination occurs within 1-3 hours after inoculation, but germination can not cause infection of the skin intact. Endospores will undergo phagocytosis by macrophages and then be taken to the regional lymph nodes, causing lymphadenopathy and lymphangitis. Hematogenous dissemination can occur, but with the provision of adequate spread of systemic antibiotics is quite rare. Several case reports of infections caused by insect bites suspected of being infected (eating carcasses containing anthrax bacillus).[4,5]
      Common location is on the face, extremities or neck. Endospores enter through skin abrasions or wounds. One to seven days after entry endospores, formed the primary skin lesions that are not painful and itchy papules. Twenty-four to 36 hours later lesions forming vesicles containing clear fluid or serosanguineus, and contains many Gram-positive bacteria. Vesicles then undergo central necrosis, dry out and cause eschar (necrotic ulcers) blackish typical vesicles surrounded by edema and purple. Edema usually occurs more severe on the head or neck than the body or limbs. Lymphangitis and lymphadenopathy that pain can be found following systemic symptoms occur. Although cutaneous anthrax can heal itself, but still need to be given antibiotics (to reduce systemic symptoms). In 80-90% of cases the lesions healed completely without complications or scarring. Malignant edema are rare, characterized by severe edema, induration, multiple bullae, and shock. Malignant edema can occur in the neck and chest area that causes difficulty breathing, requiring corticosteroids or intubation.[5,6]
   b. Gastrointestinal Anthrax
      Gastrointestinal anthrax, although it can be fatal, has not been reported in the US. Symptoms usually appear 2-5 days after eating raw or undercooked meat that is contaminated with germs. Some cases may occur in the home. On pathological examination under a microscope can be found in

the mucosa and submucosa basil lymphoid tissue and mesenteric lymphadenitis. Ulceration almost always be found. A large number of Gram-positive bacteria can be found in the peritoneal fluid. Mediastinal widening can also occur.

Clinical symptoms can include fever, diffuse abdominal pain, constipation or diarrhea. If there is ulceration of the bowel becomes blackish. Ascites can occur with clear liquids until purulent.[1,6]

c. Anthrax inhalation

Inhalation anthrax spores began with the entry into the alveolar cavity, then macrophages will fagocyt spores and some of the spores will lysis and broken. Spores that survive will spread to the lymph nodes and mediastinal nodes. The process of change in vegetative forms occur approximately 60 days later. The slow process of change in shape is not known with certainty, but well-documented cases of anthrax in Sverdlovsk that inhalation occurs between day 2 to day 43 after exposure. Once germination has occurred, the disease will arise quickly and replication of bacteria causing bleeding, edema and necrosis. The term anthrax pneumonia is not used because it turned out after pathological examination abnormalities were obtained mainly in the form of thoracic lymphadenitis and mediastinitis hemorhagis without typical bronchopneumonia. However, in the event of inhalation anthrax in Sverdlovsk, 25% of fatal cases was found bleeding focal necrosis and pulmonary lesions.[1,2]

Anthrax meningitis

A complication of cutaneous anthrax, inhalation and gastroitestinal, but is most common in inhalation anthrax (> 50%). Often addressing bleeding and meningoencepalitis. Anthrax death rate is over 90%.[6,8]

3. Investigations : In the diagnosis of anthrax needed routine blood tests, culture swab the wound or blood (on the skin), sputum (on inhalation) chest X-ray (on inhalation), electrolyte (gastrointestinal) and serology using ELISA (Enzyme linked immunosorbent Assay) and PCR (Polymerasi Chain Reaction). Samples were taken for laboratory examination of the above is the blood serum, rub the injured area, sputum and land near the cage or a dead animal.[9,10]

Criteria of Diagnosis

The criteria used in the diagnosis of anthrax consists of three types, namely suspected (suspect), Probable (possibility) and Confirmed (Confirmation).[10,11]

Suspected, is clinically shown one form of anthrax and there is epidemiological evidence that exposure to anthrax environment, but there is no definitive laboratory evidence. Probable, is in clinical symptoms of anthrax but do not meet the definition of confirmation, but shows one of the following : (1) In Epidemiology, there are environmental exposures. (2) B. anthracis DNA evidence collected from the lesion, usually sterile (such as blood or CSF) or lesion of other affected tissue (skin, lung or digestive) (3) Positive serology IgG ELISA Anthrax Lethal Factor (LF) in the examination of the positive Spectrometry. Confirmed, is in clinical symptoms of anthrax with one of the following : (1) B. anthracis culture positive (2) Demonstrate B. anthracis antigens of the network by immunohistochemical staining using the cell wall and capsule monoclonal antibody B. anthracis (3) Proven 4x increase in antibody titer during the acute period and fixes the quantitative examination of anti-PA IgG ELISA testing (4) The presence of environmental exposure to anthrax and PCR test positive.[10,11]

METHOD

This study is an observational analytic with cross sectional approach, by screening immunoassay based on people exposed to anthrax outbreak in the area in 2011. The location of sampling is an area of outbreak anthrax, Boyolali and Sragen, Central Java. All the people who are exposed to dead animals suffering anthrax, a blood sample for examination IgG antibodies in serum by ELISA.[3,4] Intepretation ELISA will get three categories: positive, borderline and negative, with inclusion criteria such as direct contact with infected animals anthrax, do not suffer from a disease that causes a decrease in the body's immunity, there are currently using Immune suppressant drugs, there are pregnant or breastfeeding and not in hormone therapy. There were exclusion citeria such as not willing to follow research and medium serious illness, such as sepsis.

Operational definitions of variables

Dependent variable: clinical manifestations Is the result of the interview and physical examination in people who are exposed to anthrax animals.

The independent variables: levels of Ig G Anthrax Serology examination to assess the titer of anthrax protective antigen (PA) Ig G ELISA in order to confirm the presence of infection with Bacillus antracis in human blood. Interpretation of test results :

<0.9 : No detectable IgG antibodies against PA protein in ELISA.

0.9 - 1.1 : Borderline

> 1.1 : Detected the presence of IgG antibodies to the protein PA, indicated patients were infected or infected with a never Bacillus anthracis.

Scale: nominal

RESULT

In this study, 101 people with a history of contact with animals that died of anthrax. Respondents in the youngest age is 6 years old (1%) and the oldest 80 years (1%). The distribution of the highest age at 21 to 40 years as much as 39.6%, and most are women sex, ie 57.4%. The education level of respondents at most 74.3% graduated from elementary school. Subsistence farmers as much as 21.8%. The basic characteristics of the study subjects are shown in Table 1.

Table 1. Baseline Characteristics of research subjects (n = 101)

Variable	N	%
Gender		
• Male	43	42,6
• Female	58	57,4
Age		
• 0 - 20 year	2	1,9
• 21 – 40 year	40	39,6
• 41 – 60 year	37	36,6
• 61 – 80 year	22	21,7
Pendidikan		
• Elementary	75	74,3
• Junior high school	15	14,9
• Senior high school	5	5
• University	6	6
Profession		
• No work	48	47,6
• Farmer	22	21,8
• Civil	5	5,0
• Private	26	38,8

Of the total sample, showed serum Ig G antibodies showed negative 50.5%, borderline 15.8% and 33.7% positive. ELISA serology results can be seen in Table 2.

Table 2. Results of ELISA

Variable	N	%
• Positive	34	33,7
• Borderline	16	15,8
• Negative	51	50,5

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 results 20.8%. Serology results Elisa Risks associated with the contact can be seen in Table 3.

Table 3. Results of serology Elisa Risks associated with contact

Risk Factors	Elisa		
	Positif	Border line	Negatif
• Wash the meat	1	0	1
• Eating	10	5	16
• Wash and eat	3	2	0
• Cooking and eating	11	6	19
• Slaughtering and eating	9	3	13
• Located near the cage	0	0	0

Overall there are 11.9% of respondents 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. The skin manifestations can be seen in Table 4.

Table 4. Distribution of clinical manifestation

Clinical manifestation	N	%
• Eschar	12	11,9
• No Eschar	89	88,1

At respondents with 10.9% positive serology results indicate a skin manifestation of the emergence of eschar, while only 1.0% with borderline serology showing the skin manifestations. There are as many as 22.8% with positive ELISA results, but does not cause any skin manifestation in the form of eschar or other clinical signs (fever, myalgia, stone, spasms, nausea and vomiting). Result of serology associated with skin manifestations such as eschar can be seen in Table 5.

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

Eschar	ELISA		
	Positive	Borderline	Negative
• Yes	11 (11%)	1 (1,0 %)	0 (0,0 %)
• No	23 (23%)	15 (14,8 %)	51 (51%)

P 0.02

Contact risk factor for the emergence of manifestations in the skin, especially on the respondents were slaughtered at once ate beef (6.0%), followed by the washing and eating meat, which is 3.0%, whereas only 1.0% wash and meating. The relationship between contact with the manifestation of the emergence of the eschar can be seen in Table 6.

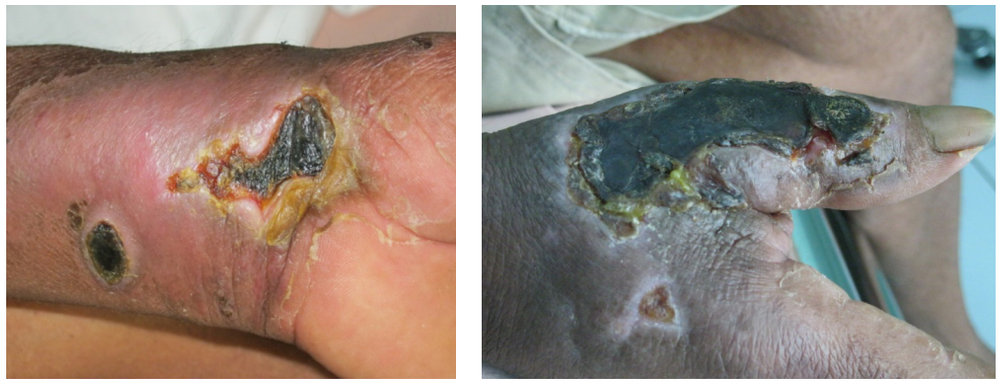

Figure 1. Anthrax manifestations in the skin with the advent of eschar

Table 6. Relationship between contact with the manifestation of eschar

Variable	Eschar	
	Yes	No
• Wash the meat	1 (1,0 %)	1 (1,0 %)
• Eating	2 (2,0 %)	29 (28,7 %)
• Wash and eat	3 (3,0 %)	2 (2,0 %)
• Cooking and eating	0 (0,0 %)	36 (35,6 %)
• Slaughtering and eating	6 (6,0 %)	19 (18,9 %)
• Located near the cage	0 (0,0 %)	2 (2,0 %)

DISCUSSION

During the 2011 outbreak of anthrax in Central Java. Initially 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 examined in laboratory of Central Java Province and tested positive for anthrax. Seven days later, seven people were complaining there are small bumps and itching, swelling and lesions accompanied wet in the area under the eyes, hands, legs or feet, then taken to the health center and declared suspected anthrax. Then in May 2011 in Sragen also occur the same thing and be some people who show symptoms of anthrax skin contact.

Clinical manifestations in the form of eschar present in 11.9% with cutaneous anthrax. Respondents were taken from both locations, obtained 101 samples were then examined serological Anthrax serum Ig G antibodies. Of these 50.5% negative and 33.7% positive, while 15.8% borderline. Clinical manifestations in the form of a skin disorder that begins their edema or injury which led to edema and ends with eschar present in 10.9% of the respondents who Ig G antibody positive and 1.0% of respondents with Ig G borderline results. This is due to the emergence of antibodies against anthrax bacteria on respondents who had clinical manifestations in the skin, but that can not be explained is the result obtained antibodies also borderline clinical manifestations (see Figure 1).

Twenty-two percent of respondents with a positive serum Ig G 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 can not be explained further, because of the endurance factor all pretty much the same condition, which probably is due to the virulence of the bacteria and germs that enter the number.

The risk of direct contact, ie cooking and eating meat of infected animals showed 30.5% Ig G positive results, but does not cause clinical manifestations with the advent of eschar (0%). This may be due to immune factors of patients as well as virulence B antrhacis that enters the body. Risk factors eating only 32% of respondents showed positive results. While the risk factors slaughter and eat 24% manifested by the appearance of skin eschar.

CONCLUSIONS

The conclusion of this study is the increase in serum antibody titer Ig G anthrax does not occur in all of the respondents were exposed to the anthrax outbreak area, all respondents were obtained eschar followed by an increase in Ig G antibody titers.

Researchers suggest screening anthrax using anthrax Ig G antibodies can be done in areas that are outbreaks of anthrax and can proceed with dealing with how the eschar and its effect on Ig G antibody titer anthrax.

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