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Influence of Endotoxin in Poultry Farm Dust on the Increasing Number of Polymorphonuclear (PMN) Cells in *Nasal Lavage* Fluids Workers

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Abstract-Endotoxin in poultry farm dust can cause inflammation in the respiratory tract of workers, marked by an increasing number of PMN cells in the local tissue. This study aimed to analyze the relationship between the levels of personal dust with endotoxin in personal dust and influence endotoxin on the increasing number of PMN cells in nasal lavage fluids workers. Type of this study is observational analytic with prospective cohort study design. Sample consisted of seven workers. Measurement of PMN cell numbers in nasal lavage fluids using Papanicolaou staining method. Results showed there was no correlation between personal dust with the levels of endotoxin in personal dust ($p>0.05$). Linear regression showed that the endotoxin level have significant effect ($p<0.05$) on the increase number of PMN cells in nasal lavage fluids workers. Advised for the workers always use Personal Protective Equipment (PPE) during work to reduce the exposure of endotoxins in poultry farm dust.

Keyword-Poultry farm dust, endotoxin, nasal lavage, PMN

1. INTRODUCTION

Working in the farm industry has the potential exposure to airborne disease agents is quite large, including pollutants from chemicals, dust particles, allergens, endotoxins, microorganisms and organic gas. Where these pollutants have been reported as the cause of symptoms of acute respiratory disease and chronic occupational[1]. Acute effects of exposure to endotoxins such as fever with leukocytosis and flu ending within 18 hours after initial exposure such as Organic Dust Toxic Syndrome (ODTS)[2]. Long-term or chronic effects occur after repeated exposure to dust containing endotoxin such as chronic bronchitis and Chronic Obstructive Pulmonary Disease (COPD).

Endotoxin is a Lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria and a strong immunostimulan[3]. Some studies show that poultry farm is the place with the highest concentration of endotoxin compared to the pig farm, the animal feed industry, and wheat. All samples from all parts of the poultry farm showed a positive result to the presence of endotoxin[4].

Non-specific immune reaction or congenital triggered through Toll Like Receptors (TLR) that recognize different microbial products such as LPS, peptidoglycan, flagellin and CpG motifs in bacterial DNA. The introduction of TLR4 to endotoxin in a non-specific immune response or congenital will further stimulate macrophages to produce cytokines and Nitric Oxide (NO). Cytokine production will stimulate the formation of pro-inflammatory cytokines, namely Tumor Necrosis Factor alpha (TNFα), Interleukin 1 (IL-1) and Interleukin 6 (IL-6). The increase in pro-inflammatory cytokines subsequently will give a sign of local inflammation and picture an increase in systemic inflammatory cells. Local inflammatory response due to endotoxin exposure occurred in 2 to 6 hours after exposure include increased neutrophils and cytokines[5].

Neutrophils also referred to as Polymorphonuclear cells (PMN) is part of the white blood cells or leukocytes that have the highest number among the other leukocyte cell components. Increased PMN cells in the target tissue is to run the process because of its ability to phagocytes the pathogens and cell debris together with macrophages[6]. Several studies have been conducted to prove that exposure to endotoxin may increase the number of PMN cells[7] [8] [9].

One way to detect the onset of inflammation that occurs on workers is through nasal lavage fluids or liquid rinsing the nasal cavity. Nasal lavage can be performed as early diagnosis to determine the activity of an agent inhaled by the respiratory tract with the pathophysiological response humoral view that illustrates the possibility of the upper respiratory tract[10]. Changes in protein and levels of inflammation

or inflammation can be described by their cell influx in the nasal cavity, antioxidants, eicosanoid mediators, release of neuropeptides, nasal glands, increased vascular permeability, cytokines, and other products from cells such as eosinophils, neutrophils, and mast cells contained in nasal lavage fluids[11].

This study aimed to analyze the relationship between the levels of personal dust with endotoxin in personal dust and influence endotoxin on the increasing number of PMN cells in nasal lavage fluids workers.

2. METHOD

Type of this study is observational analytic with prospective cohort study design. Where research is conducted through measurement of exposure at ground level (baseline), and followed suit with the passage of time that has been determined is before and after work (cross shift) or for 8 hours, followed by measurement of the effects of acute exposure to workers. The study was located in poultry farm, Kiringan Village, Takeran District, Magetan. The sample size of 7 people.

The independent variables in this study is the personal dust levels and endotoxin level in the personal dust. PMN cell as the dependent variable, and the characteristics of workers (age, time work, gender, nutritional status, use of PPE, and packyears become confounding variables. Techniques and procedures for data collection: (1) measuring of personal dust level from poultry farms using personal dust sampler, (2) measurement of endotoxin using ELISA sandwich techniques with Limulus Amebocyte Lysat (LAL) method, (3) taking a liquid nasal lavage, (4) checking number of PMN cell from nasal lavage fluids using Papanicolaou staining method.

The data obtained will be analyzed using statistical data processing applications. The relationship between the levels of personal dust with endotoxin in the personal dust were analyzed using Pearson correlation if the normal distribution, and Spearman test if the data are not normally distributed. Influence of endotoxin to increase the number of PMN cells were analyzed using linear regression.

3. RESULT

Respondents in this study is a poultry farm workers who are willing to respondents, have not history of respiratory disease and never get job history that is estimated to have a high risk of causing respiratory diseases.

Table 1. Respondents characteristics

Characteristics	n	Percentage (%)
Age (years)		
21-25	1	14.3
26-30	3	42.9
31-35	1	14.3
36-40	2	28.6
Mean ± SD		31,00 ± 5,42
Work period (years)		
0-5	4	57.1
6-10	3	42.9
Mean ± SD		4,86 ± 2,03
Gender		
Male	7	100
Female	0	0
Nutritional Status		
Very Underweight	1	14.3
Underweight	0	0
Normal	5	71.4
Overweight	1	14.3
Obesity	0	0
Mean ± SD		22,06 ± 2,86
Using PPE		
Always	0	0
Sometimes	0	0
Never	7	100
Packyears		
0-10	3	42.9
11-20	3	42.9
21-30	1	14.3
Mean ± SD		12,03 ± 7,17

In Table 1 can be seen that the majority (42,9%) of respondents aged between 26-30 years old, with the mean age was 31 years. The mean work period was 4,86 years and all respondents (100%) are male, so it is not statistically tested because the data are homogeneous. The high proportion of nutritional status (71,4%) fall into the category of normal with the mean of 22,06. All respondents (100%) never use PPE during work so it is not statistically tested. Identification of packyears mean cumulative number of cigarettes smoked was 12,03 packyears.

Personal dust level are measured using personal dust sampler, then from the personal dust filter is used to measure endotoxin using a ELISA sandwich technique with LAL method. Results of measurement for personal dust and endotoxin are presented in Table 2.

Table 2. Measurement personal dust and endotoxin

Parameter	Mean ± SD	Min/ Max
Personal dust (mg/m^3)	0,287 ± 0,002	0,286/0,292
Endotoxin (EU/m^3)	118,57 ± 31,85	70/170

Table 2 shows that the mean level of personal dust is 0.287 mg/m^3 with the lowest score is 0.286 mg/m^3 and the highest is 0.292 mg/m^3. While the mean level of endotoxins is 118.57 EU/m^3, with the lowest value is 70 EU/m^3 and the highest is 170 EU/m^3.

Number of PMN are measured through nasal lavage fluids using microscopic examination with Papanicolaou staining method or Hansel. Here are the results of measurement for number of PMN cells.

Table 3. Distribution on the increasing number PMN cells in nasal lavage fluids workers after working

Number of PMN Cells (10 LP)	F	%
Increase	7	100
Permanent	0	0
Decrease	0	0
Total	7	100
Minimum	1	
Maximum	8	
Mean ± SD	3,57± 2,57	

In Table 3 shows that all workers (100%) have increased the number of PMN cells after working with a mean increase of 3,57 per 10 field of view.

Table 4. Relationship between personal dust level with endotoxin in personal dust

Variable	R	*p*-value
Personal dust	-0,396	0,380

Results from Pearson correlation test in Table 4 showed that no relationship between the levels of personal dust with endotoxin in the personal dust ($p>0,05$).

Table 5. Influence between the levels of personal dust, endotoxin and respondents characteristics on the increasing number of PMN cells workers

Variable	Results of Regression Test	
	β	*p-value*
Personal dust levels	0,042	0,913
Endotoxin	-0,925	0,009*
Age	-0,689	0,027*
Work period	0,059	0,819
Nutritional status	0,506	0,069
Packyears	-0,445	0,317

*=Significant ($p<0,05$)

Table 5 shows that endotoxin and age significantly affect an increasing number of PMN cells in nasal lavage fluids poultry farm workers.

4. DISCUSSION

Endotoxin is a structural component of the outer membrane of Gram-negative bacteria which become air contaminants that are found in the agricultural sector, including at the poultry farm. Inhalation of endotoxins can cause acute symptoms such as dry cough, shortness accompanied by a decrease in lung function, fever, and malaise. After a few hours, these symptoms will develop into bronchoconstriction, headache and joint pain. In some studies have been done on the effects of acute incurred after inhalation of endotoxin showed that in patients with asthma and people with inflammation of the nasal mucosa can cause bronchial obstruction accompanied by increased reactivity.

Results from Pearson correlation test analysis are presented in Table 4 shows that there is no relationship between the levels of personal dust with endotoxin. These results prove that high or low level of personal dust had no effect on levels of endotoxin although measurement of endotoxin derived from personal dust extraction.

This is caused by endotoxin in personal dust is one component of Gram-negative bacteria that is the outer membrane of the bacteria[3]. So the more the number of Gram-negative bacteria, the more well endotoxin level.

Thus the increase occurred in the number of PMN cells only influenced by the presence of endotoxin alone, while personal dust level not give any influence. In other words, no matter how much the number of dust contained in the personal working environment as long as no endotoxin in it then it will not give you a change to the PMN cells on workers. This study are consistent with previous studies that examined the relationship endotoxin and personal dust level in the rice mill where the results of these studies

indicate that there is no relationship between endotoxin and personal dust level[12] [13] [9].

In Table 5 shows that the increasing in the number of PMN cells in nasal lavage fluids workers is significantly affected by endotoxin level (p=0,009) and age (p=0,027). Endotoxin activates macrophages to release cytokines and several other inflammatory cells such as neutrophils, mast cells, basophils, eosinophils. Local inflammatory response for their endotoxin exposure occurred in 2 to 6 hours after exposure, marked an increase in neutrophils and cytokines[5]. In this research note that the endotoxin significantly influence the increase in PMN cells according to research that has been done before[7] [8] [9].

PMN cells are the main cells in the early inflammatory role in the process of phagocytosis, ie swallow pathogens and cell debris together with macrophages[6]. Increasing number of PMN cells associated with an inflammatory response to endotoxin. The occurrence of inflammatory reactions are governed by alveolar macrophages carrying the receptor binding endotoxin (LPS binding protein, CD14, MD2, TLR4), which plays an important role in the activation of PMN cells were then perform phagocytosis and followed by the inflammatory process[14]. Significant increase in the absolute number of lymphocytes and PMN cells occurred at 6 hours after inhalation, followed by an increase in viable cells, macrophages, PMN cells and lymphocytes in the 24 hours after inhalation of endotoxin[15].

5. CONCLUSION

The results showed there is no correlation between the levels of personal dust with endotoxin in the personal dust (p>0.05). Results of linear regression showed that endotoxin have significant effect (p<0.05) on the increasing number of PMN cells in nasal lavage fluids workers.

6. SUGGESTION

To reduce the exposure of endotoxins in poultry farm, advised for the workers to use PPE during work.

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