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Greetings from Indonesia,

I have submitted article via bioscience research email on February 14, 2019,
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The Selective Isolation and Characterization of Pathogen Bacteria *Salmonella* sp. from Poultry Coop Soil

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Poultry commodities in Indonesia had high market prospects. The availability of poultry can decreased with the outbreak of disease that attack poultry. This occurrence can cause bacterial infections from the environment such as soil, water and air. This study purposed to detect the presence of *Salmonella* sp. derived from the poultry coop soil. Pathogenic bacteria was isolated from 3 different sample locations and was selected by using Salmonella Shigella Agar (SSA) selective media. The colonies that successfully isolated were carried out their characteristics. Macroscopic characters of colony were conducted by observing the morphology of bacterial colonies on SSA media, while microscopic characters of cell were carried out with Gram staining. Physicochemical characteristics of soil sample were evaluated by measuring pH, humidity, and soil texture. Based on the result, it was found that pathogenic bacteria from poultry coop soil phenotypically identified as *Salmonella* sp.

Keywords: Poultry, Soil, *Salmonella* sp.

INTRODUCTION

Efforts to accelerate development of the livestock sector in Indonesia, are the government current targets. Development of livestock sub-sector as one of the producers of animal food needs jointly supported. Fulfillment of meat needs in 2015 is mostly came from broilers 53.13%, chicken buras 10.25%, beef is only 17.11% of total production national meat (BPS, 2016).

Poultry breeding industry in Indonesia in the last few years is undergoing a slowdown. In 2009, there were a lot of dead poultry caused by *Salmonella* sp. (Sudaryanti and Santoso, 2003). This occurrence can cause bacterial infections from the environmental such as soil, water, and air. *Salmonella* is a family of Enterobacteriaceae which are facultative anaerobic, gram negative, rod shape (Yan, et al., 2003). *Salmonella* grow optimally at 35^0C to 37^0C, except *Salmonella gallinarum* and *Salmonella pullorum. Salmonella* that infected chickens is *Salmonella pullorum,* causing pulorum disease). Disease caused by *Salmonella* sp. was known as Salmonellosis (Shivaprasad, 2000).

Salmonellosis is one of the most frequently reported foodborne diseases worldwide (Panzenhagen et al., 2015). Foodborne diseases caused by non-typhoid *Salmonella* represent an important public health problem. Most human salmonellosis cases are associated with consumption of contaminated egg, poultry, pork, beef and milk products (Geimba, et al., 2004; Zaki, et al., 2009). Transmission of *Salmonella* to humans typically occurs when ingesting foods that are directly contaminated by animal feces or cross-contaminated by other sources (Modaressi

and Thong, 2010). Salmonellosis can manifest in a number of disease syndromes including gastroenteritis, bacteremia, typhoid fever and focal infections (Darwin and Miller, 1999). Study of pathogen such as *Salmonella* sp. bacteria from poultry has been done (Park, et al, 2014), but few data are available about this bacteria from poultry coop soil. The purpose of this study was to detect the presence of *Salmonella* sp. from the poultry coop soil.

MATERIALS AND METHODS

Sampling site

Soil sample was taken from poultry coop soil which located in Tajinan, Malang. The soil was taken from three different locations as deep as 10 cm from ground surface.

Soil Sample Characterization

Physicochemical characteristics of soil sample were evaluated by measuring pH, humidity, and soil texture. Measuring pH and humidity were done by using soil tester.

Isolation and Screening *Salmonella* sp.

Salmonella sp. was selected by using Salmonella Shigella Agar (SSA) selective media. Five-fold serial dilution of the sample was carried out and inoculated using the pour plate method: one mililiter (1ml) of serial dilution was inoculated on sterile Petri dish, after that media was poured aseptically on the inoculated plates. The plates were incubated at 37^0C for 24 hours. After incubation, morphologically colonies *Salmonella* sp. are shown with colorless colony with black center.

Salmonella sp. Characterization

Characterization of *Salmonella* sp. isolate including observation of macroscopic, microscopic and physiological characters. Macroscopic characters of colony were conducted by observing the morphology of bacterial colonies on SSA media, while microscopic characters of cell were carried out with Gram staining and physiological characters used *Microbact* TM GNB 12 A and 12 B *Identification Kits OXOID*.

RESULTS AND DISCUSSION

Soil sample was taken from three different locations in poultry coop soil. The three retrieval locations have different characteristic. The location of the first sample was a location with good intensity of light, the second sample was a location with less intensity of light and near to wellspring, and the last sample was the location that didn't expose with light. Physicochemical characteristics of soil sample test result were presented in Table 1 with three replications. The picture of the soil sample from three locations was presented in Figure 1.

Table 1. Physicochemical characteristics of soil samples

Samples	pH	Humidity	Soil textures
I	7	9	Brown and sandy soil
II	7.5	9	Black and sandy soil
III	8	10	Brown and sandy soil

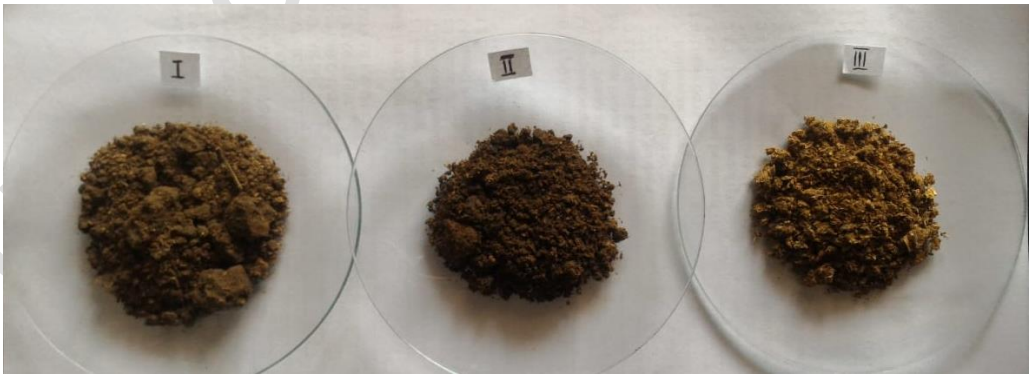

Figure 1. Soil sample from three locations in poultry coop soil.

The results of selective isolation of pathogenic bacteria from poultry coop soil, showed the presence of colonies. The colony has a colorless with black center, round, smooth and elevation shaped like a button. The morphology of the

colony is presented in Figure 2.

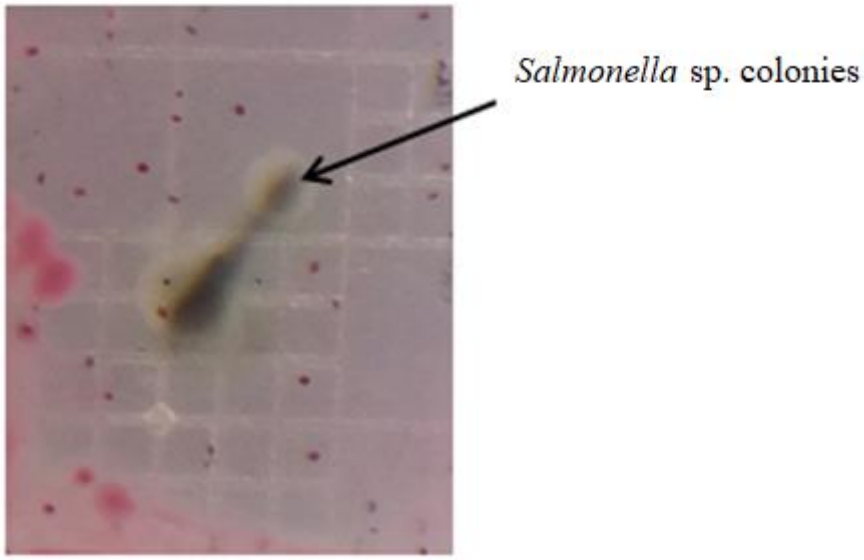

Figure 2. *Salmonella* sp. colonies in Salmonella and Shigella Agar

Microscopic *Salmonella* sp. was carried out with Gram staining. The result indicated that the colony belongs to Gram negative group and has a rod shaped that presented in Figure 3.

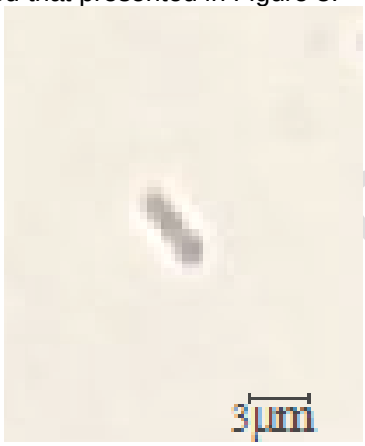

Figure 3. *Salmonella* sp. bacteria cell with 1000x magnification

The result of physiological test showed that the colony of bacteria has similarity with *Salmonella* sp. Physiological test results were presented in Table 2.

The result of physiology test showed that the colonies belongs to *Salmonella enterica* ssp *arizonae* with 77% similarity, based on Cowan and Steel's Manual for the Identification of Medical Bacteria.The result of pathogenic bacteria *Salmonella* sp. selective isolation from poultry coop soil, showing the presence of colonies *Salmonella* sp. This bacteria was found in poultry coop soil because *Salmonella* sp. could replicate in animal digestive. The habitat of *Salmonella* sp. was found in the digestive tract and then issued through feces, so these bacteria can contaminate feed and the environment such as water, soil, plants, and dust (Humprey, 2006).

Based on the characteristic of soil samples, pathogenic bacteria *Salmonella* sp. can be found in poultry coop soil because the condition of the soil has an optimal condition for its growth. The survival of *Salmonella* sp. in soil was determined by various factors such as: temperature, moisture, soil type, presence of plants, exposure to sun (UV) light, protozoan predation and the initial number of present organisms. *Salmonella* sp. has been reported to survive from a few days up to 332 days in manure-amended soils (Islam, et al., 2004). Temperature was also an important factor in the growth of *Salmonella* sp., High temperatures will cause death in *Salmonella* sp. (Jacobsen & Bech, 2012). Growth of *Salmonella* sp. both at neutral pH and relatively resistant to acidic conditions (Finn, et al., 2013). It was in accordance with the results of the soil characteristic that indicate a neutral pH and had a fairly good humidity.

Isolation of *Salmonella* sp. using Salmonella Shigella Agar (SSA) selective media will show colorless with black center colonies of *Salmonella*.

Table 2. Physiological characteristics of *Salmonella* sp. using Microbact Identification Kits 12 A and 12 B

GNB	Characters	Results
12A	Oxidase	-
	Motility	+
	Nitrate	+
	Lysine	-
	Ornithine	-
	H_2S	+
	Glucose	+
	Mannitol	+
	Xylose	+
	ONPG	+
	Indole	-
	Urease	+
	V-P	+
	Citrate	+
	TDA	-
12B	Gelatin	-
	Malonate	+
	Inositol	-
	Sorbitol	+
	Rhamnose	+
	Sucrose	-
	Lactose	+
	Arabinose	+
	Adonitol	-
	Raffinose	-
	Salicin	+
	Arginine	+

The pure colonies displayed typical *Salmonella* morphological characteristics on Salmonella-Shigella agar (SSA), which were clearly with a black spot in the center due to H_2S gas production (Gunasegaran, et al., 2011).

Diseases caused by *Salmonella* sp. was a disease in poultry that transmitted through feces, especially in young birds with high number of mortality, while adult birds act as carriers (career) (Shivaprasad, 1997). It had severe bad impact on the economy which will cause huge losses because of production decreased and high embryo mortality. Poultry disease was caused by *Salmonella* sp. known as salmonellosis, and it was the most commonly reported foodborne disease in the world (Schlundt, et al., 2004).

The *Salmonella* group that found in this research was determined as *Salmonella enterica* ssp *arizonae*. This spesies is Gram negative, bacil shape, family of enterobacteriaceae, and is a rare species. *Salmonella* sp. is cause disease in human, turkeys, chickens, and some others animal. (Nuhu, et al., 2017), it's part of the normal reptile intestinal flora but can cause disease in monotremes, turkeys, chickens, goats, and humans (Aiken, et al., 2010). S. *enterica* ssp. *arizonae* enteritis or systemic infections have been well described in patient resident in the southern states of the USA (Casner, et al., 1990). Arizonosis is an important disease of turkeys and less important of poultry caused by *Salmonella enterica* ssp *arizonae*. Mode of infection and symptomps are very similar to those of avian paratyphoid. Adult birds do not show any symptomps but young chicks show diarrheas, pasting around vent and paralytic signs due to dehydration (Chauhan & Roy, 2003).

Salmonella sp. found in this explorating research had 77% similarity with *Salmonella enterica* ssp *arizonae*. The percentage of similarity was relatively far away and was not included in the species. This was supported by the literature from the book entitled Cowan and steel's manual for the identification of medical bacteria which states that with a percentage of 85-100% are positive strains which is mostly similar, 16-84% are positive strains which is only a few in common, and 0-15% is equivalent with one, few, some, or even none at all positive strain. The results of *Salmonella* sp. exploration research in

the animal husbandry environment are become important because with knowing the existence of the spread of *Salmonella* in the farm environment then it can prevented by the addition of probiotics in food cattle so as to prevent *Salmonella* infection in poultry.

CONCLUSION

The condition of the poultry coop is a factor which greatly influences the poultry's health. Exploration of *Salmonella* sp. is an effort to understand the condition of the poultry coop and preserve the health of the poultry. Based on the result of this research, *Salmonella* sp. pathogens can be found in poultry coop, spesifically in the coop soil.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

RDF, NAB, GI, VW, RKA, WNP, FAT design and perform the experiment and also write this manuscript. NMZ review, correct and edit the manuscript. All authors read and approve the final version..

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