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Potency of Cockroaches (*Periplaneta americana* and *Blattella germanica*) on the ship as Vector of Salmonellosis in Baubau Port

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Abstract

Cockroaches are insects that act as mechanical vectors of pathogenic agents to humans such as Salmonella sp. The aim of this study was to identify the presence of Salmonella sp. in cockroach bodies on cargo and passenger ships. This research was conducted at Baubau Port from October to December 2017. A total of 24 ships were examined consisting of 12 passenger ships and 12 cargo ships. Cockroaches were collected from rooms inside each ship namelygalley, bridge, deck andbathroom. A total of 3196 cockroaches caught consisting of Periplaneta americana (69.50%), Blattella germanica (29.60%), Periplaneta brunnea (0.66%), Pseudophorapsis sp. (0.03%), Pycnoscelus surinamensis (0.03%), Periplaneta australasiae (0.03%), and Neuphoeta cinerea (0.03%). The presence of Salmonella sp. was only examined in species with a large populatuon, namely Periplaneta americana and Blattella germanica. A total of 95.3% of the 42 cockroaches examined in the laboratory contained Salmonella sp.. Based on this research, it can be concluded that the cockroaches collected from the ships leaning at Baubau Port can serve as vectors of salmonellosis for both ship crew and passengers.
Keywords:*Cockroaches, Ships, Salmonella sp, Salmonellosis*

Introduction

Marine transportation consists of passenger and cargo ships is one of transportation that becomes the daily necessity for the Indonesian people to move from one place to another. High transportation needs can induce health problems, including vector infestations. The entry of the vector is usually through freight goods that are the vector breeding grounds. Vectors that are often found in ships are cockroaches.

Cockroaches are vectors that can be associated with various pathogens, such as bacteria, protozoa and viruses. One of the pathogenic species that can cause disease is *Salmonella* sp. The bacteria inhabit the digestive tract of humans and animals. Transmission of *Salmonella* sp. usually occurs through contaminated food and drink. Disease transmitted through the food is well known as food-borne disease. Transmission of disease, especially food-borne diseaseremained as public health problem. Rooney *et al.* (2004) said that contamination of food and beverage can cause 50 cases of diseases such as salmonellosis, shigellosis, typhoid fever, gastroenteritis, trichinosis, and cryptosporidiosis. Cox (2000) stated that *Salmonella* sp. is an etiological agent of food-borne disease named salmonellosis in humans *Salmonella* sp..

Gastroenteritis can arise from food contaminated by the bacteria. CDC (2017) reported that of 239 cruise ships in the United States Harbour have gastrointestinal outbreaks characterized by vomiting and diarrhoea in ship crew and passenger due to *Salmonella* sp.. Food contaminated by *Salmonella* sp. can cause abdominal pain, typhoid fever, dizziness, and itchy skin, even loss of consciousness (Srigede 2015).

Baubau Port is a transport port which connects islands in eastern and western Indonesia. The port is located inBaubau City, Southeast Sulawesi Province. Based on our observations, Baubau port has an inadequate sanitation which allowing insects to breed. This study aimed to identify the presence of *Salmonella* sp. in cockroach bodies on cargo passenger ships leaning on Baubau Port.

Material and method

Collection and Identification of Cockroaches

Catching of cockroaches was conducted at Baubau Port in November to December 2017 on freight boats and 24 passenger ships. The collection was done at the night on ship rooms such as deck, steering room, kitchen and bathroom with 4 enumerators involved. The caught cockroaches were put into a bag and then labelled by ship type. The prevalence of cockroach infestation was measured by calculating total percentage of captured cockroach. Identification of cockroaches to species stage conducted at Laboratory of Medical Entomology, Division of Parasitology and Medical Entomology, Faculty of Veterinary Medicine, Bogor Agricultural University using stereo microscope and identification key by Hadi and Soviana (2013).

Detection and Isolation of Salmonella sp.

The sample of cockroaches for detection and isolation of *Salmonella* sp. came from 2 types of cockroaches with the largest population (*Periplaneta americana* and *Blattella germanica*). Samples were collected from 21 ships consist of these two species of cockroaches (n=42). The cockroach sample was made of 10% w/v suspension (ratio of 1 part of cockroach: 9 parts of 0.9% sterile NaCl. Cottonwas crushed until smooth and sterile NaCl added until reach the appropriate ratio and then centrifuged at 1000 G for 10 minutes. The suspension leaved to be grown on a bacterial culture medium. Then, the suspensionwas cultured on an enrichment medium (Tetrathionate medium) and incubated for 2 x 24 hours at 35 ± 1ºC. Cultures of bacteria in the Tetrathionate medium transferred to be re-grown in *Salmonella Shigella Agar* (SSA) medium and then incubated for 2 x 24 hours. Colonies that give the characteristics of *Salmonella* is round-shaped like a fish eye will be collected. Typical colonies of *Salmonella* sp. transferred into subcultures in Trypticase soy agar (TSA) medium and incubated for 24 hours at 35 ± 1ºC. The growing colony was observed using by morphological examination with Gram staining and biochemical test. The biochemical tests carried out are tests on Triple Sugar Iron Agar (TSIA), urease, indole test, citrate and catalase test, oxidation test, motility test, glucose, lactose, and sucrose tests.

Interpretation of biochemical test results of *Salmonella* sp. based on the National Standards Agency (2008) and Cappuccino & Sherman (1987) presented as follows: 1) Positive in *Triple Sugar Iron Agar* (TSIA) test, which a black color in the bottom of the media and red or yellow color in above of the media are formed. 2) Negative in urease test, which no color alteration in the media. 3) Negative in indol test, which no red ring-formed appear in the surface of the media. 4) Negative in katalase test, which no bubble is formed. 5) Negative in oksidase test, which no color alteration in the media. 6) Positive in motility test, which root-liked is formed in media. 7) Positive in glukosa test, which a red medium changed to yellow color. 8)

Negative in laktosa test. which no color alteration. 9) Negative in sukrosa test, which no color alteration.

Data Analysis

The types of cockroaches and their distribution in ships and laboratory test results of Salmonella sp. analyzed descriptively by showing the result of isolation and identification of *Salmonella* sp. in pictures and tables.

Result and discussion

Total of 24 ships sampled in this research including cargo and passenger ships. The results showed that infection rate of cockroaches was higher in passenger ships (91.6%) than in cargo ships (83.3%) (Table 1). This is because the passenger vessels have higher human mobility with a variety of luggage compared to cargo ship, such as food boxes that make a comfortable place for cockroaches to breed.

Table 1. Number of ships infested by cockroaches in Baubau Port in November to December 2017

No	Type of ship	Number of ship sampled (N)	Number of ship infested by cockroaches (N)	Percentage (%)
1	Cargo ship	12	10	83.3
2	Passenger ship	12	11	91.6
Total		24	21	87.5

Distribution of Cockroaches on the Ships

A total of 3196 cockroaches were caught with several species such as *Periplaneta americana* (2223), *Blattella germanica* (942), *Periplaneta brunea* (21), *Pseudophoraspis* sp. (1), *Pycnoscelus surinamensis* (1), *P. australasiae* (1) and *Nauphoeta cin*erea (1), as described in Table 2. The cockroaches are scattered in galley, bridge, deck andbathroom. The presence of cockroaches in each room due to the high population of cockroaches in the ship so the cockroach can spread in the room. The highest cockroach population is found in galley and bathroom. This is because there are many sources of food favored by cockroaches in the galley, while the high population of cockroaches in the bathroom due to the location of the bathroom near to the galley.

Detection and Isolation of Salmonella sp

A total of 42 cockroaches from two species (*Periplaneta americana* and *Blatella germanica*) were taken for microbiological testing. Culture results showed that 40 cockroaches were positive of *Salmonella* sp. with the characteristics of colonies and morphology as shown in Table 3. The macroscopic observation on SSA (Fig. 1) shows colourless colonies, due to *Salmonella* sp. does not ferment the lactose. The bacteria that ferment lactose will form colourful colonies and turn the medium into pink (Tilleand Scott's 2013).

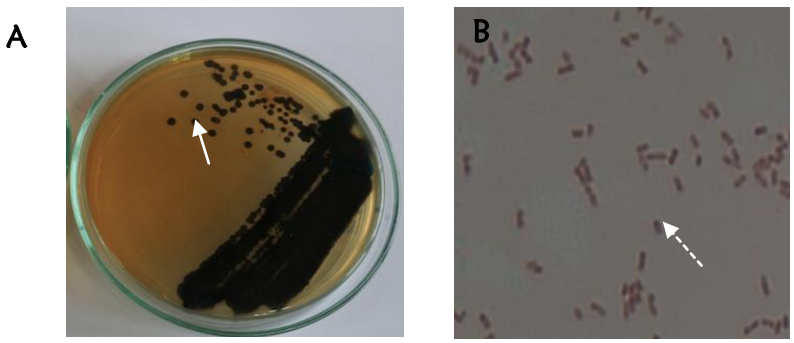
Table 2.Species of cockroach caught from cargo and passenger ships in Baubau Port in November-December 2017

No.	Species of cockroach	Number of cockroach (N)	Percentage (%)
1	*Periplaneta americana*	2223	69.5
2	*Blattella germanica*	948	29.6
3	*Periplaneta brunnea*	21	0.66
4	*Pseudophoraspis sp.*	1	0.03
5	*Pycnoscelus surinamensis*	1	0.03
6	*P. australasiae*	1	0.03
7	*Nauphoeta cinerea*	1	0.03
	Total	3196	100

Table 3. The result of Colony morphology and cell characteristic of *Salmonella* sp.

Colony Form	Whole rounded
Margin	Rounded like afish eye
Elevation	Convex
Colour	Black in the middle, clear on the margin
Surface Texture	Smooth
Cell Form	Rod
Gram	Negative (-)

Figure 1A shows the shape of *Salmonella* sp. colony with round-shaped like a fish eye. The colony has inflated elevation and black colour because it contained H_2S deposits as well as smooth surface texture. Figures 1B shows *Salmonella* sp. morphology which bacillus form and red color in gram staining (White *et al.* 2000).



Figures 1. An arrow shows bacteria coloni form, fish-eye shaped in *SalmonellaShigellaAgar* (SSA) which inaculation of *Salmonella* sp.(A) and dash dot arrow shows *Salmonella* sp. morphology with Gram staining under microscope-1000x(B)

The result of biochemistry test also confirmed that from 42 samples, 40 isolates are positive *Salmonella* sp. as presented in Table 4. The biochemical tests of TSIA medium showed that of 95.3% of cockroaches contain *Salmonella*sp. Positive result of the presence of *Salmonella* sp. was indicated by the change of colour of TSIA medium from red to pink on slant, yellow on butt and the formation of H_2S gas characterized by black colour on butt. The presence of H_2S indicates that the bacteria ferment methionine and cysteine (amino acids having H_2S structure). On Simmons citrate medium, the result showed positive results which marked by colour change from green to blue. The change colour means that bacteria use citrate as the only carbon source (Tilleand Scott's 2013). The other bacterium found on the bodies of

cockroaches is *Proteus* sp. Fathpour*et al.* (2003) reported that 70% of cockroaches caught in hospital in Isfahan, Iran have been infected by *Salmonella* sp. Other study by Chaicanawongsaroj *et al.* (2004) in Bangkok also reported other species of bacteria in the cockroach bodies (*Enterobacter cloacae* and *Escherichia coli*). Contrast result reported by Menasria*et al.* (2014) and Kassiri*et al.* (2014) which did not find any *Salmonella* sp. from *Blatella germanica* and *Periplaneta americana* caught from hospitals.

Table 4. Biochemical result of *Salmonella* sp. from cockroach samples

Test	Result of test	Explanation
TSIA	Pink on slant, yellow on but, and formation of H_2S gas characterized by yellow colour on butt	H_2S +
Indole	No red rings are formed on the surface of the culture	-
Urease	Red	-
Citrate	Blue	+
Catalase	No gas is formed	-
Oxidation	There is no change of colour to violet on the scratch marks	-
Motility	Yellow	+
Glucose	Yellow	+
Lactose	Red	-
Sucrose	Red	-

(+): Positive test for *Salmonella* sp.;(-): Negative test for *Salmonella* sp.

Indole test results showed negative results, because on the surface does not form a ring pink layer, meaning that the bacteria do not form indole from tryptopan as a carbon source, which is known when added Kovac's solution. Similar result reported by Rahmi*et al.* (2014) that indole test showed a negative result on examination of *Salmonella* sp. from orangutans at Orangutan Reintroduction Center. In the urease test, the colour of medium did not change into pink. Negative results show that bacteria do not break down urea to form ammonia.

The catalase test is a test used to determine the nature of bacteria against oxygen demand. This type of enzyme can catalyze the decomposition of hydrogen peroxide (Lay 1994). The catalase test showed a negative result because the glass object does not form bubbles when contact catalase reagent. Oxidation test showed a negative result because the reagent of oxidation does not change the colour into violet on the scratch marks. Motility test results indicate a negative result. This can be occurred because the culture medium does not show any movement of *Salmonella*sp. Glucose test signifies a positive result. It can be seen in the test tube that the colour of culture medium was changed from red to yellow. Lactose and sucrose tests showed negative results, due to the absence of colour change in culture in each test tube.

Twenty one of 24 (87.5%) examined ships are infected by cockroaches. Species of cockroaches found varied, namely *Periplaneta americana*, *Blattellage rmanica*, *Periplaneta brunnea*, *Pseudophoraspis* sp., *Pycnoscelus surinamensis*, *Periplaneta australasiae* and *Neuphoeta cinerea*. *Periplaneta americana* and *Blattella germanica* were the most abundant species with 2223 individuals and 948 individuals while others were few (Table 2). The presence of cockroaches in the ships is closely related to ship construction, room cleanliness, room conditions (lighting, temperature and humidity), as well as vector control. Mandagie

(2011) reported that the discovery of cockroaches in the motor boat "Queen Maria" in Manado-Talaud due to dirty room conditions and poor lighting.

The kitchen was a location with the highest risk of insect Pests infestation, especially cockroaches. The cockroaches usually come to places where the food was left over. The presence of food waste in the kitchen on cargo and passenger ships allows the occurrence of cockroach infestations in the port of Baubau. the result of observations that made on cargo and passenger ships lean in Baubau port show the low sanitation systems, especially in hygiene water supply.

Transmission of the disease through food (*foodborne disease*) is a very serious health problem. this can be caused by food that contaminated by cockroaches which contain bacteria. Foods contaminated with Salmonella sp. can cause abdominal pain, typhoid fever, dizziness, and itchy skin and even loss of consciousness (Srigede 2015). Cockroaches have regurgitation digestion system which regurgitates food from their stomachs so that pathogens in the cockroach can contaminate food (Fathpour *et al.* 2003; Hadi and Soviana 2006).

Poor sanitation can cause transmission of Salmonellosis. This is because food and water that consumed have been contaminated by *Salmonella* sp. (Irianto *et al.* 2007; Candra 2013). the cockroaches will transmit bacteria that are inside the body or surface of their body so that it can cause diseases in the crew and passengers of the ship. Salmonellosis can cause a rapid outbreak of gastroenteritis, which is characterized by symptoms of nausea, diarrhea, abdominal cramps, fever, chills, headaches, and vomiting.

Personal hygiene needs to be improved to avoid Salmonellosis. Good behavior such as washing hands routinely before holding a food and after leaving the toilet, washing fruits and vegetables with flowing water and cleaning food equipment before use is very important to avoid *Salmonella* sp. contamination.

Conclusion and suggestion

Conclusion

There high number of cockroaches that found in the ships leaning at Baubau Port. The cockroaches could potentially cause the Salmonellosis to ship crew and passengers in the ship.

Suggestion

The ship crew need to be aware about the potency of transmission of salmonellosis from cockroaches. Cockroaches control program need to be considered as one of program by the providers of ship cargo and passenger transportation services.

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