Poster Presentation (PF-11)

Isolation and Identification of Gram-Negative Bacterial Pathogens of Bat Guano from Liang Bukal and Liang Petang Cave on Sumbawa Island

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INTRODUCTION

Bats are important reservoirs of many bacteria. The occurrence of infectious diseases that caused by Gram-negative bacteria has increased the interest in bats as potential reservoir hosts of many bacteria. Several bacteria, including *Salmonella spp*, *Escherichia coli*, and *Bartonella spp* were isolated from wild bats in various country [1]. Despite the fact that little is known about the bats as a reservoir of gram-negative bacterial pathogens in Sumbawa, Indonesia.

Gram-negative bacteria can cause human disease was isolated from fresh bat guano of *Rousettus leschenaultii* at the Robber's Cave, Mahabaleshwar, Maharashtra, India, including *Escherichia coli, Yersinia, Enterobacter and Proteus* [2]. In Pakistan, Gram-negative bacteria like *Salmonella* and *Pseudomonas, Bartonella*, and *Klebsiella* was isolated from guano of Indian flying fox (*Pteropus giganteus*) [3].

Bat guano was used as fertilizer with a farmer in Sumbawa Island. So, Close contact between human and bat guano, it is possible the gram-negative bacteria from guano can infect the human. The present study reported that close contact with both domestic animals and humans, contaminating houses with guano and urine, additionally, humans occasionally encroach into bat habitats [4].

Sumbawa Island has many caves for breeding place of bats. Identification of gram-negative bacterial pathogens from bat caves which are a risk to human, animal and environment health on Sumbawa Island is needed as early detection of the presence of Gram-negative bacterial pathogens from bat guano.

MATERIALS AND METHODS

Study Site

Descriptive observational survey with purposive sampling method in two bat caves in Sumbawa Island from During April 2018 was used in this research. The Population Targets in this research are unknown population of the two Bat Caves in Sumbawa Island (Liang Bukal Cave and Liang Petang Cave). The Tanjung Liang Bukal Bat Cave is located at 8.680^0 S, 117.475^0E and the Liang Petang Bat Cave is located at 8.681^0 S, 117.475^0E.

Sampling strategy

The guano of bats were collected by spreading a polythene sheet of 1 m × 1 m (length × width) under the roosting sites of bats. 4 a polythene sheets were spread in bat cave, 2 a polythene sheets in Liang Bukal cave and 2 a polythene sheets in Liang. Selection of a polythene sheet site based on the highest population of the bat roasters. Each sheet remained spread for 10 h from 08.00 AM to 17.00 PM. The guano on the polythene sheets were collected with the tags indicating bat cave, plot number and date [5].

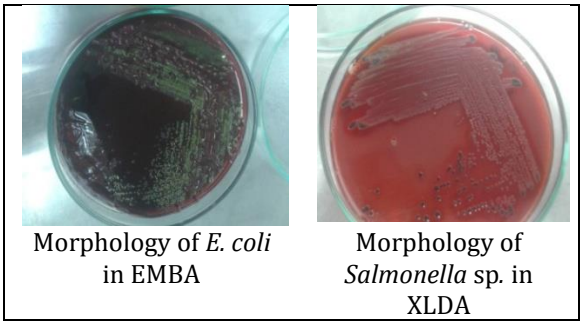
Bacterial analysis

One gram of the guano was placed into 9 ml Brain Heart Infusion (BHI) (Oxoid) and they were incubated over the night in an incubator (Memmrt). After 24 hours the samples were inoculated in Eosin Methylene Blue Agar (EMBA) and Xylose Lysine Desoxycholate Agar (XLDA) under aerobic conditions at Laboratory of Public Health and calibration, Province of Nusa Tenggara Barat. The colonies will be purified and characterized by standard Gram staining and biochemical methods. The reagent of Gram staining was used Crystal violet solution (Merck), Lugol's *solution* (Merck), Safranin solution (Merck), and Alcohol 95%. Gram-negative of bacteria isolated were determined by standard biochemical procedures using Bergey's Manual, book of Clinical Veterinary Microbiology, and Basic Laboratory Procedure of World Health Organization [6-8]. All activity of isolation and Identification of bacteria was used Biosafety Cabinet Class IIA.

RESULT AND DISCUSSION

Isolation and identification of Gram-negative bacterial pathogen from the 4 site of 2 bat cave were *Escherichia coli* and *Salmonella spp.* 2 Guano samples from Liang Petang bat cave were isolated *Escherichia coli* and 2 guano samples from

Liang Bukal bat cave were isolated *Salmonella spp*. The colony characteristics and morphology of *E. coli* and *Salmonella* spp are summarized in Figure 1 and Table 1.



Morphology of *E. coli* in EMBA | Morphology of *Salmonella* sp. in XLDA

Figure 1. The Morphology colonies of *E. coli* and *Salmonella* spp

Table 1. Colony characteristics and Morphology of *E. coli* and *Salmonella* spp

Location	No of Sample	Gram-Negative Bacteria	Colony characteristics and Morphology (staining Characters)
Liang Bukal	2	*Escherichia coli*	Green metallic colonies, Gram negative bacilli
Liang Petang	2	*Salmonella* spp	Red colonies, some with black centers, Gram negative bacilli

Biochemical test of *Escherichia coli* were isolated of bat guano from Liang Bukal bat cave are summarized in Table 2.

Table 2. Biochemical characteristics of *E. coli*

CHO fermentation and other biochemical tests	Result
Glucose fermentation	+
Lactose fermentation	+
Indol production	+
Urea utility	-
Simon Citrate Agar	-
Sulfide Indole Motilty Agar	+ H_2S
Triple Sugar Iron Agar	Alkaline/acid

Biochemical test of *Salmonella* spp were isolated of bat guano from Liang Petang bat cave are summarized in Table 3.

In this study were isolated *E. coli* and Salmonella sp agree with the previous research that *E. coli* and Salmonella sp were isolated from faeces of *Myotis myotis* in Italia [9]

In the present study the isolated *E. coli* organism fermented glucose, lactose with the production of both acid and gas, and the Indole test of were positive as reported by Buxton and Fraser 1977. In Gram's staining, the morphology of the E. coli were pink, small rod shape, Gram negative bacilli which was supported by several authors [10]. The results of biochemical tests of *E. coli* agree with Daniela *et al.* [11] for isolates of *E. coli* from the short-nosed fruit bat in Malaysia.

Table 3. Biochemical characteristics of *E. coli*

CHO fermentation and other biochemical tests	Result
Glucose fermentation	+
Lactose fermentation	-
Sucrose fermentation	-
Sorbitol fermentation	-
Indol production	-
Malonat	-
Urea utiliy	-
Simon Citrate Agar	-
Sulfide Indole Motilty	+ H_2S
Triple Sugar Iron Agar	Acid/Acid

The result of biochemical test of *Salmonella* spp. in this study were fermented glucose and they did not ferment lactose and it gave negative result for Indol test agree with Bergey's Manual for identification of *Salmonella* spp.

Besides thus fact, Cave-dwelling bats can transmit bacterial pathogens to human population and environment by dropping their feces in water, fruit and another way. Based on study reported that bats are widespread in urban areas and come in close contact with both domestic animals and humans, contaminating houses with guano [4]. This mean that *E. coli* and *Salmonella* sp. of bat guano from Liang Bukal and Liang Petang cave can infect the farmer in Sumbawa Island.

CONCLUSION

Escherichia coli were isolated of bat guano of from Liang Bukal cave and *Salmonella* spp. were isolated of bat guano from Liang Petang cave. They can infect the farmer that use the guano for fertilizer in Sumbawa Island.

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