Molecular Screening of *Salmonella sp*. from fecal sample of Sparrows (Passer domesticus) in Yogyakarta, Indonesia

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Abstract. Wild birds is one of the reservoir agent of some of various zoonotic diseases. The study was aim to see the potential of sparrow as the reservoir agent of *Salmonella sp*. using polymerase chain reaction (PCR) method. We detected the *invA* gene of *Salmonella sp*. from faecal sample of sparrows (*Passer domesticus*) in local area of Yogyakarta, Indonesia. A total of 30 faecal dropping samples were collected from sparrows. DNA was extracted from the faecal samples, then amplified by PCR for the target genes. The amplicons were electrophorized to see the visualization of DNA on the agarose gel. The result showed the prevalence of the positive result of *Salmonella sp.* was 3,3% . The study indicated that sparrows can spread zoonotic pathogens and this necessitates monitoring for the epidemiologic status of these pathogens among birds, also applying the appropriate intervention measures to prevent the transmission of zoonotic diseases from birds to humans.

1 Introduction

The current pandemic issues in the world currently has made public attention to the various possibilities of wild animals as the important objects in the spread of zoonotic diseases. In addition to mamals, wild birds are also considered as an important reservoir of some pathogens zoonoses [1]. Several species of wild birds are known to act as reservoir for zoonotic agents such as *Chlamydophila psittaci* [2], *Avian Influenza* [3], α-, β-, γ-, and δ-coronaviruses [4] enteropathogenic *E.coli* (EPEC) and Shiga-toxin producing *E. coli* (STEC) [5], and Salmonella [6]. The transmission of the diseases occurs between the wild birds to other animals and humans through droplets of feces or nasal fluid [7, 8]. *Salmonella sp*. spreaded through direct contact with bacteria-contaminated feces [9, 10]. Type of wild birds included in the order Passeriformes, Psittaciformes, and Columbiformes are recorded as a reservoir of various zoonotic diseases caused by bacteria [11, 12].

Salmonella enterica is one of the important member of enterobacteriaceae family. *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) is responsible for acute gastroenteritis or typhoid fever in humans [13], acute enteritis in cattle and pigs, *S. enteric*a serovar Gallinarum (*S. Gallinarum*) and Pullorum (*S. Pullorum*), the cause of fowl typhoid

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and pullorum disease, respectively diseases in avian [14]. Diagnosis of *Salmonella* can be carried out by culture of faeces, blood, spleen, liver, and intestinal contents. In addition, very small numbers of viable organisms present in the feces may fail to grow in artificial laboratory media [15, 16]. Recently, the molecular techniques like polymerase chain reaction (PCR), DNA microarray-based detection, and DNA sequencing were developed and used to detect bacterial infection in different clinical materials [17, 18]. Molecular testing has been most successful in areas for which conventional microbiologic techniques do not exist, are too slow, or are too expensive [19].

2 Objective

This study was aim to detect *Salmonella sp*. from fecal sample of the sparrow birds, and to know the prevalence of Sparrows as vectors of *Salmonella sp.* in Yogyakarta area, Indonesia.

3 Method

3.1 Samples collection and DNA extraction

This study has the ethical requirements for research in experimental animals from the Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University with certificate number: 00033/04/LPPT/VI/2019. Thirty sparrow birds were obtained from the bird hunter in Yogyakarta. Fresh fecal samples were collected and Genomic DNA was extracted using Stool DNA Isolation Mini Kit (Favorgen, Biotech Corp).

3.2 Detection of *invA* gene of *Salmonella sp.*

Salmonella sp. detection was carried out using *invA* gene targeted primers forward (5'-CGGTGGTTTTAAGCGTACTCTT-3') and reverse (5'-CGAATATGCTCCACAAGGTT A-3') with the *invA* gene target (769 bp).

The PCR reaction for *invA* gene of *Salmonella sp*. was carried out under initial denaturation conditions at a temperature of 94 °C for 2 mins, followed by 38 cycles consisting of denaturation at a temperature of 94 °C for 20 s , annealing at 60 °C for 1 min, and extension at 72 °C for 1 minute. The PCR products were electrophorized to see the amplified DNA band. Electrophoresis was performed at 100V for 30 minutes. The gel then visualized under UV light to see the DNA band.

4 Results and Discussion

This study reports the prevalence of *Salmonella sp*. in sparrow birds from wild of Yogyakarta region, Indonesia from the fecal samples. Fecal swab samples performed PCR using *invA* genes showed one positive sample of *Salmonella sp* (3.3%) (Figure 1). *Salmonella sp*. detection was carried out using *invA* gene.

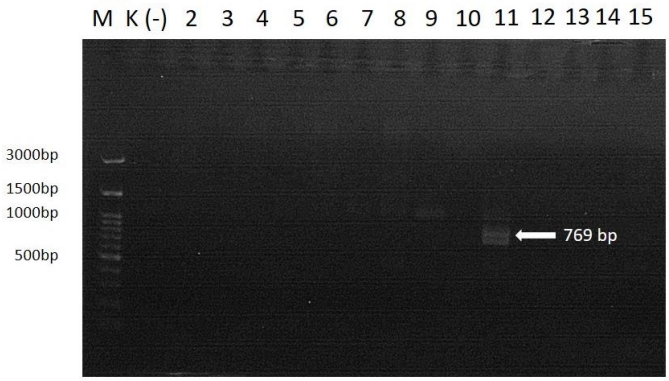

Fig. 1. Polymerase chain reaction product amplifying *invA* gene of *Salmonella sp.* (arrow) on agarose gel electrophoresis. M: marker, K (-): negative control, 2-15: samples

According to Malorny *et al*. [20] the *invA* gene became an international standard for detecting *Salmonella sp*. at the genus level using clinical samples such as faeces. The percentage of *Salmonella sp*. in sparrows in Yogyakarta is higher when compared to previous studies in various countries except UK, which has a percentage of *Salmonella sp* in wild birds of 1.6% [21]. Research conducted by Afema & Sisco [22] found that the percentage of *Salmonella sp* in birds was 4.1%, this is higher than the percentage shown in this study. The same result was revealed by Tizard [23], who found the prevalence of *Salmonella sp* in captive raptors was 7.36%, higher than the prevalence of Salmonella in sparrows in Yogyakarta. Sparrows as reservoir for *Salmonella sp* was expressed as carriage with no obvious disease manifestations. Some family of passerines are the carriers of Salmonella strains. In the study by Krawiec *et al*. [24], they discovered *Salmonella* serovar Typhimurium positive samples from Eurasian siskins and greenfinches. Salmonella can infect wild birds through direct contact with the vectors such as insects and rodents, or through food-producing animals [25].

Free-living birds as well as migratory and captivated birds, are potential as reservoirs for bacterial agents [26]. They also may act as vectors in the transmission of some pathogens [27]. Matias *et al.* [28] stated that there are relevance in illegal trading of wild birds with outbreaks potential of some zoonotic diseases in human. Environmental changes, forest loss, human demographic changes, industry expand, could lead direct contact between wild animals and humans. There is also the possibilities of the increase risk of pathogen spread from the wild animals to farm animals, thus resulting in foodborne diseases to humans. The interface between livestock and wildlife has been reported associated with rising incidences of pathogens [29]. Safety measure for humans at high risk of zoonotic infection from livestock and wild animals must be taken to prevent the occurrence of outbreak in humans.

5 Conclusion

This study showed the prevalence of *Salmonella sp*. from the faecal sample of wild sparrows in Yogyakarta was 3.3%. The sparrows can act as a carrier by spreading the bacteria through faeces without showing any clinical symptoms. Further research on the pathogens in wild birds, that are abundant in Indonesia, especially in Yogyakarta region is needed to determine variations in the types of zoonotic diseases that can be carried by wild birds. Evaluation and monitoring of the epidemiologic status of these pathogens among birds and humans is needed for applying the appropriate intervention measures to prevent the transmission of zoonotic diseases.

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