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Early Warning for Humans Regarding Contamination with Salmonella bacteria Originating from Duck

Alerta temprana para humanos por la contaminación con la bacteria Salmonella originada en el pato

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SUMMARY

Introduction: *Pathogenic bacteria of the genus Salmonella are one of the leading causes of foodborne disease. Salmonella sp. are pathogenic bacteria excreted through feces from the digestive tract of animals and humans. These bacteria can contaminate food of animal origin, both from animals to humans and humans to animals. This study aimed to determine the presence of Salmonella in Surabaya duck farms.*
Methods: *The research samples were taken from 24 duck eggs and 24 cloacal swabs. The criteria for the egg samples taken were newly hatched eggs. The sample were placed in a sterile plastic bag and labeled to prevent bacterial contamination from other eggs. Cloacal swab samples were taken from newly laid ducks using a sterile cotton swab and put into Tetrathionate broth as an enrichment medium. Samples were isolated on Salmonella Shigella Agar (SSA) medium and incubated at 37^{o}C for 24 hours and identified by microscopic examination with gram staining and biochemical tests on Triple Sugar Iron Agar (TSIA), Sulphide Indole Motility (SIM), Simon's Citrate Agar (SCA), urease.*
Results: *The results showed three positive samples of Salmonella enterica in cloacal swabs, and no Salmonella was detected in the egg white samples.*
Conclusion: *The detection of Salmonella enterica from cloacal swabs of ducks is undoubtedly an early warning so that the community is more aware of the dangers of Salmonella disease.*

Keywords: *Salmonella, albumin, cloacal swab.*

RESUMEN

Introducción: *Las bacterias patógenas del género Salmonella son una de las principales causas de enfermedades transmitidas por alimentos. Salmonella sp. son bacterias patógenas excretadas con heces del tracto digestivo de animales y humanos. Estas bacterias pueden contaminar alimentos de origen animal, tanto de animales a humanos como de humanos a animales. Este estudio tiene como objetivo determinar la presencia de Salmonella en las granjas de patos de Surabaya.*
Métodos: *Las muestras de investigación se tomaron de 24 huevos de pato y 24 hisopos cloacales. Los*

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criterios para las muestras de huevos tomadas fueron huevos recién puestos. La muestra se colocó en una bolsa de plástico estéril y se etiquetó para evitar la contaminación bacteriana de otros huevos. Se tomaron muestras de hisopos cloacales de patos recién puestos utilizando un hisopo de algodón estéril y se sembraron en cultivo de tetrationato como medio de enriquecimiento. Las muestras se aislaron en medio Agar de Salmonella Shigella (SSA) y se incubaron a 37ºC durante 24 horas y se identificaron mediante examen microscópico con tinción de Gram y pruebas bioquímicas en Triple Sugar Iron Agar (TSIA), Sulphide Indole Motility (SIM), Simon's Citrate Agar (SCA), ureasa.

Resultados: *Los resultados mostraron que hubo tres muestras positivas de Salmonella entérica en hisopos cloacales y no se detectó Salmonella en las muestras de clara de huevo.*

Conclusión: *La detección de Salmonella entérica a partir de hisopos cloacales de patos es sin duda una alerta temprana para que la comunidad sea más consciente de los peligros de la enfermedad de Salmonella.*

Palabras clave: *Salmonella, albúmina, hisopado cloacal*

INTRODUCTION

Salmonella has been considered an important pathogen that threatens global public health (1,2). These bacteria are the source of foodborne diseases that cause infection and food poisoning in humans (3). In China, 70 %-80 % of foodborne pathogens are caused by Salmonella (2). Ducks proved to be an essential reservoir for Salmonella species. Salmonellosis in ducks can cause acute, subclinical forms, while chronic non-lethal or carrier status usually develops in adult ducks (4,5). Poultry products contaminated with Salmonella can seriously impact life, health, and food safety. The spread of salmonellosis from ducks to humans may be higher than from chickens, and the main factor is the presence of infection without recognizable clinical signs and poor hygiene conditions (6). Salmonella bacterial contamination in ducks in Yangzhou, Jiangsu province, and Gaomi, Shandong province, China, shows a severe condition with a Salmonella isolation rate of 35.7 % observed at the hatching stage, indicating a potential threat to the downstream duck production chain (7).

Although ducks are resistant to systemic infections caused by Salmonella, ducks are a potential reservoir of these microorganisms and can release them in feces and contaminate the environment (8). Outbreaks of human salmonellosis caused by contact with ducks have been reported in several countries, such as Australia, the United States, America, and Denmark. In addition, consuming contaminated duck eggs has been reported as a cause of salmonellosis outbreaks in Italy, Thailand, and the United States (9,10). Eggs are one of the essential products that can transmit Salmonella infection to humans (11). Eggs and egg products have been identified as the most common food vehicle for salmonellosis in humans in Europe, mainly caused by *Salmonella enterica* serovar Enteritidis (12).

There are very few studies on the incidence of Salmonella in ducks in Indonesia. However, there is a need for continuous monitoring of the presence of *Salmonella spp*. in ducks, especially in Surabaya, Indonesia, to help authorities protect the public and minimize the risk of foodborne outbreaks. This study aimed to determine the presence of Salmonella in Surabaya duck farms.

METHODS

Design Study

This research was conducted at a duck farm in Surabaya City. The design of the study was an observational laboratory.

Population and Sample

A total of 48 samples were taken from duck farms from breeders in the city of Surabaya, consisting of 24 egg samples and 24 cloacal swab samples.

Data Collection

Sampling used sterile equipment, including a cotton swab and a tube containing enrichment tetrathionate broth media. Isolation of bacteria using Salmonella Shigella Agar (SSA) OXOID media. Microscopic examination using Gram staining with materials such as Crystal Violet, Lugol, Alcohol acetone, and Safranin. The biochemical tests used include Triple Sugar Iron Agar (TSIA), Simons Citrate Agar (SCA), Sulphide Indole Motility (SIM), and Urease.

Sampling was carried out in duck breeders in Surabaya. Before sampling, the cage was observed with the criteria that the cage had a temperature between 27°C to 30°C. Egg sampling was carried out by meeting the criteria for newly hatched with the characteristics of still sticking feces from the eggshell and then putting it in a plastic bag. It was labelled to prevent bacterial contamination from other eggs. After that, the fecal samples from the cloacal swab were taken from the mother ducks that had just laid eggs. Then samples were taken using a sterile cotton swab and put in the Tetrathionate broth solution. Eggs taken from the farm were immediately taken to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Wijaya Kusuma University, Surabaya. The egg whites were then taken using a 1 mL syringe and placed in a solution of Tetrathionate broth. The two samples were dissolved in Tetrathionate broth and incubated for 24 h at 37°C. Bacteria were isolated by streaking on SSA media. The enrichment process was carried out, and the colony morphology was observed after incubation at 37°C for 24 h. Only colonies with rounded, transparent edges with black spots in the middle were purified. A microscopic examination was carried out to examine the morphology of Salmonella bacteria cells and the bacteria's purity. Bacterial colonies whose purity was confirmed were subjected to biochemical tests to determine the species of Salmonella.

Data Analysis

The data collected in this study were analyzed using descriptive statistics. The descriptive analysis involves summarizing and describing the collected data, such as calculating measures of central tendency (mean, median) and measures of dispersion (standard deviation, range).

RESULTS

The study's results on detecting Salmonella in duck egg whites at three farms in Surabaya showed 0 %. There was no Salmonella. In contrast, the results of the study on cloacal swab samples contained three of the 24 samples identified as Salmonella. The data is presented in Table 1.

Table 1. Results of detection of Salmonella enterica on egg whites and cloacal swabs.

Duck Farmer	Number of samples	Egg whites		Cloacal Swab	
		Positive (+)	Negative (-)	Positive (+)	Negative (-)
Duck Farmer 1	6	0 % (0/6)	100 % (6/6)	0 % (0/6)	100 % (6/6)
Duck Farmer 2	6	0 % (0/6)	100 % (6/6)	16 % (1/6)	84 % (5/6)
Duck Farmer 3	12	0 % (0/12)	100 % (12/12)	16 % (2/12)	84 % (10/12)
	24	0 % (0/24)	12.5 % (3/24)		

Figure 1 showcases the colony morphology of Salmonella on Salmonella-Shigella Agar (SSA). The image displays multiple colonies of Salmonella, which appear as small, opaque, and colorless colonies with a dark center. The colonies have a smooth and slightly raised appearance, indicating the presence of Salmonella on the agar plate. Figure 2 illustrates the biochemical reactions of *Salmonella enterica* using various test media. The leftmost panel represents Triple Sugar Iron Agar (TSIA) test, where Salmonella shows an alkaline/alkaline reaction. The middle panel represents Sulphide Indole Motility Agar (SIM) test, indicating the production of hydrogen sulphide (H_2S) gas and motility. The rightmost panel represents the Urease test, with Salmonella demonstrating a positive reaction by turning the medium pink. Lastly, the bottom panel represents Selenite-Cystine Agar (SCA) test, showing the growth of Salmonella colonies on the medium.

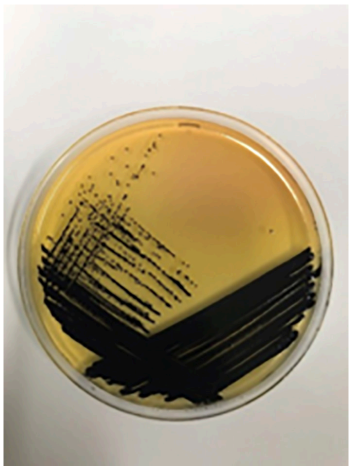

Figure 1. Salmonella colony morphology on SSA.

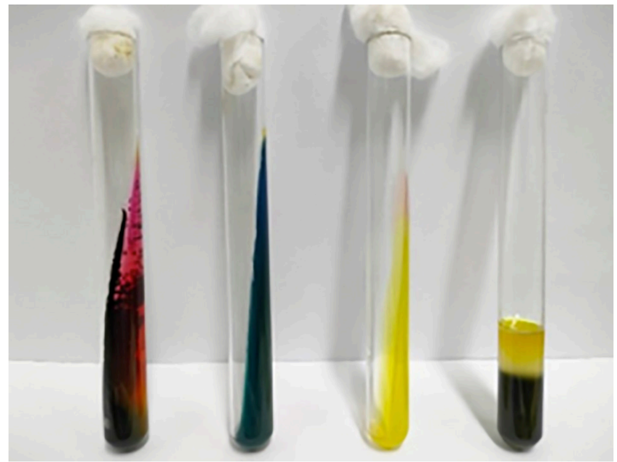

Figure 2. Biochemical reactions of *Salmonella enterica* from left to right: TSIA, SCA, Urease, SIM.

DISCUSSION

Biochemical assays are necessary for identifying *Salmonella enterica* species and serovars (13). While serotyping is required for precise serovar identification, many biochemical features are shared by Salmonella strains (14). These include alkaline slant and acid butt reactions in the Triple Sugar Iron Agar (TSIA) test, hydrogen sulphide (H2S), and gas generation. The Sulphide Indole Motility (SIM) test usually yields positive findings, indicating motility and H_2S generation. Furthermore, *Salmonella enterica* strains typically show positive results in the Selenite-Cystine Agar (SCA) and urease tests (15). However, specifically Salmonella enterica serovars may have distinct biochemical profiles. In the TSIA test, for example, Salmonella typhi exhibits alkaline slant and acid butt reactions, H_2S generation, a negative SCA test, motility, a positive urease test, and indole negativity. *Salmonella paratyphi* A, on the other hand, exhibits alkaline slant and acid butt reactions with gas production in the TSIA test but lacks H_2S production, it is motile but does not make H_2S in the SIM test, and is motile but does not produce H_2S in the SCA and urease tests (16,17).

The present data show that 12.5 % of Salmonella bacteria were found in duck cloacal swabs (3 of 24 samples). While, Adzitey et al., 2012 (18) showed that 28 of 100 (28.0 %) intestinal contents and 15 of 75 (20.0 %) cloacal swabs obtained from ducks in wet markets and farms were positive for Salmonella. The prevalence of Salmonella in duck intestine and a cloacal swab was not significantly different. However, the dominant Serovar was *S. typhimurium*, followed by *S. hadar* and *S. enteritidis*, *S. braenderup* and *S. Albany* (15,19). Another bacteriological examination also resulted in 94 positive samples from 630 isolates, of which 28 were from 33 sick ducks and 66 isolates from 72 recently died ducks. Three Salmonella serogroups were obtained by serological identification, *Salmonella typhimurium*, *Salmonella enteritidis*, and Salmonella blegdam (20).

Characterization of Salmonella isolated from duck farms and slaughterhouses in Shandong province, from a total of 49 strains of Salmonella isolated from 2 342 samples from four duck farms and one duck slaughterhouse in Jinan and Tai'an, Shandong province, China, showed the presence of *S. enteritidis* 20/49 samples (40.8 %) and S. anatum 10/49 (20.4 %), which were the most common (2). Another study that took samples from the cecum, liver, spleen, and gall bladder identified Salmonella by 7 % in 50 samples. Salmonella from waste samples around the farm was also found to be 6 % (3).

Samples taken from the processing environment can also be positive for Salmonella. Salmonella species can survive well in feces, soil, pond water, drinking water, feed, transport crates, eggshells, floor treatment, cutting tables and washing water. This shows that Salmonella is proven to be able to contaminate the environment around farms. Pathogens can survive in duck farms and slaughter areas and infect other animals (18). In effect, in a study was shown that Salmonella was found in feces (92 %), followed by eggshells (34 %) and cloacal swabs (4 %), and there was no Salmonella sp. present in egg contents (21), which is line with our present results. Overall, a total of 180 Salmonella isolates (25.7 %) were obtained from the duck production chain, 82 (35.7 %) isolates were from hatchery samples, followed by 64 (29.2 %) from market samples, 17 (23.6 %) from livestock samples, and 17 (9.4 %) from abattoirs. All isolates were divided into nine serotypes, of which *S. typhimurium, S. anatum*, and *S. enteritidis* were the dominant serotypes (7).

In contrast to other studies, the main Salmonella serotypes detected from 155 isolates were S. pullorum (82.6 %) and S. enteritidis (17.4 %). Salmonella species are considered intracellular pathogens and carry several virulence factors for entry and survival in the intracellular environment (15,22). Salmonella can spread not only horizontally but also vertically through embryonic chicken eggs. Salmonella can settle in poultry's reproductive tract, contaminate fresh eggs, and infect chicken embryos. Chicken embryos that do not die will still carry Salmonella after hatching, which will cause healthy chicks to become infected with salmonella disease, such as *Salmonella pullorum* (23). Zhao et al., 2016 (24), on native chickens in China, showed that 38 out of 300 samples (12.7 %) were identified as positive for Salmonella, and the most common serotype found was *S. enteritidis*. Pullorum disease caused by *S. pullorum* is strongly associated with direct vertical transmission from egg contamination in the genital tract or indirectly from chicken contact in hatcheries. In this study, no *Salmonella pullorum* or *Salmonella gallinarum* were found. *S. pullorum* is not excreted extensively in feces, unlike many other Salmonella serotypes, which are more commonly associated with human food poisoning (25). Salmonella infection in humans usually occurs when contaminated food products are ingested, resulting in gastroenteritis. Contaminated food such as milk, ice cream, cheese, butter, eggs, pork, beef, poultry, fruits, and vegetables have been implicated as sources of human infection (26).

CONCLUSIONS

Salmonella is a significant cause of foodborne disease in humans. Salmonella infection in poultry is humans' most important source of salmonella-related food poisoning. Although ducks are highly resistant to systemic infections caused by Salmonella, ducks are highly penetrating and become potential reservoirs of Salmonella and can release microorganisms in feces, thereby contributing to environmental contamination.

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