

ISOLATION, IDENTIFICATION, AND ANTIBIOTIC SENSITIVITY AGAINST *Salmonella* sp. FROM HATCHERY KUB CHICKEN IN YOGYAKARTA

[*Isolasi, Identifikasi, dan Sensitivitas Antibiotik Terhadap Salmonella sp. dari Penetasan Ayam KUB di Yogyakarta*]

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ABSTRACT

Salmonella sp., is responsible for bacteria that causes salmonellosis in produced hatchery local chicken (KUB) in Yogyakarta. The objective of this study was to isolation, identification and antibiotic sensitivity against *Salmonella* sp., from hatchery KUB chicken in Yogyakarta. This study was carried out in KUB chicken hatcheries in Yogyakarta province, specifically in the Sleman district (n=5), Bantul (n=3), Kulonprogo (n=3) and Gunungkidul (n=2). Isolation and identification *Salmonella* sp. were done from eggshells (n=17) and chick fluff of KUB chicken (n=13) based on conventional methods with the bacteriological analytical manual (BAM). Antibiotic sensitivity against *Salmonella* sp., determined by antimicrobial diffusion agar with a paper disc antibiotic. A total of 3 isolates *Salmonella* sp., were isolated from eggshells (n=2) and chick fluff from KUB chicken (n=1). *Salmonella* sp., was sensitive to tetracycline, oxytetracycline, enrofloxacin, sulfamethoxazole and resistant to ampicillin and erythromycin. The study showed that hatchery KUB chicken in Yogyakarta was contaminated with *Salmonella* sp., and had resistance to several antibiotics. Therefore, disinfection in incubator hatcheries KUB chicken should be done after hatches egg.

Keywords: Isolation, salmonella, KUB chicken, antibiotic, sensitivity

ABSTRAK

Salmonella sp., merupakan bakteri penyebab salmonellosis pada penetasan ayam kampung unggul Badan Litbang Pertanian (KUB) di Yogyakarta. Tujuan penelitian ini untuk isolasi, identifikasi, dan sensitivitas antibiotik terhadap *Salmonella* sp., yang diisolasi dari penetasan ayam KUB di Yogyakarta. Penelitian dilakukan pada tempat penetasan ayam KUB di propinsi Yogyakarta yaitu di kabupaten Sleman (n=5), Bantul (n=3), Kulonprogo (n=3) dan Gunungkidul (n=2). Isolasi dan identifikasi *Salmonella* sp., dilakukan terhadap cangkang telur (n=17), bulu anak ayam atau *fluff* (n=13) secara konvensional berdasarkan manual analitik bakteriologi (BAM). Sensitivitas antibiotika terhadap *Salmonella* sp., dilakukan dengan agar difusi menggunakan kertas cakram antibiotik. Sebanyak 3 isolat *Salmonella* sp., dari cangkang telur (n=2) dan *fluff* ayam KUB (n=1). *Salmonella* sp., sensitif terhadap tetrasiklin, oksitetrasiklin, enrofloksasin, sulfametoksazol dan resisten ampisillin dan eritromisin. Hasil penelitian menunjukkan bahwa tempat penetasan ayam KUB di Yogyakarta terkontaminasi *Salmonella* sp. dan resisten terhadap antibiotik. Oleh karena itu, desinfeksi pada tempat penetasan ayam KUB perlu dilakukan setelah digunakan.

Kata Kunci: Isolasi, salmonella, ayam KUB, antibiotik, sensitivitas

INTRODUCTION

Superior local chicken (KUB) is a type of superior local chicken produced through a series of native chicken selection studies throughout six generations in the Livestock Research Institutes (BALITNAK), Bogor, Indonesia (Iskandar, 2017). The advantages of KUB chicken are that, unlike local chicken, the genetic character of the feather color remains uniform, the body weight is up to 1.2–1.6 kg/head, the weight of the first laid egg is 30 g and the end production is 36 g, the age of the first laid egg is 20–22 weeks, the egg production reaches 160–180 eggs/head/year; heyday egg production is 50 %, peak egg production is 65 % and there is more disease resistance (Iskandar, 2017).

The important disease in KUB chickens is pullorum (Yunan *et al.*, 2023). In these moment,

pullorum disease in chicken is still problem in the poultry industry in Indonesia (Rini, 2018). In chicken, pullorum disease caused by *Salmonella* sp (ACMSF, 2016). *Salmonella* sp., is a Gram-negative bacterium, short, plump-shaped rod in the Enterobacteriaceae family that is non-spore forming, non-capsulated, aerobic and facultatively anaerobic (Fàbrega and Vilaa, 2013).

Salmonellosis is a disease which is caused by infection with salmonella bacteria in animals or humans (Fàbrega and Vilaa, 2013). Character of salmonellosis is a zoonotic disease (Pande *et al.*, 2016). It means, that it can be transmitted from human to animal and otherwise (Pande *et al.*, 2016). Salmonellosis in humans can occur through contaminated food, and it is called a foodborne disease (WOAH, 2016). This disease is related to personal hygiene and environmental conditions.

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Salmonellosis is linked to the consumption of *Salmonella* sp., contaminated food products (Chlebicz and Sliżewska, 2018). Salmonellosis is often characterized by gastroenteritis (ECDC, 2019; FDA, 2021). This illness is accompanied by nausea, vomiting, abdominal cramps, and bloody diarrhea (ECDC, 2019; FDA, 2021). It is also associated with headache, feverish conditions and myalgia (ECDC, 2019; FDA, 2021). Symptoms begin to appear 8–72 hours after bacteria enter and infect the intestine (ECDC, 2019; FDA, 2021). In general, symptoms usually last for 4–7 days (FDA, 2021). A total of 16 million cases of inflammatory fever includes salmonellosis, 1.3 billion cases of gastroenteritis, and 3 million deaths from *Salmonella* sp (Anderson *et al.*, 2016; ECDC, 2019; FDA, 2021). In 2019, there were a total of 926 salmonellosis foodborne outbreaks that were reported across 23 European countries (ECDC, 2019). These outbreaks resulted in 9169 cases, 1915 hospitalizations, and unfortunately seven deaths (ECDC, 2019). It's alarming to note that *Salmonella* sp., was responsible for 17.9 % (one in six) of all foodborne outbreaks in 2019 (ECDC, 2019).

Salmonellosis is one of the primary causes of foodborne disease, with 11% being due to animal contact (Teklemariam *et al.*, 2023). Infections can be acquired through both direct and indirect contact with animals (Teklemariam *et al.*, 2023). Indirect transmission may occur through interaction with the animals' environment or contaminated items at farms (Teklemariam *et al.*, 2023).

Salmonellosis in chickens can occur through two transmission routes, namely vertical and horizontal transmission (ACMSF, 2016). Vertical transmission is the transmission from hen to egg, which hatched into a day-old chick (DOC) infected with salmonellosis, while horizontal transmission is the transmission from sick chickens to other chickens (ACMSF, 2016). *Salmonella* sp., can infect and kill two-week-old chickens (ACMSF, 2016). The symptoms may vary and include weakness, loss of appetite, and poor growth. The animals are crowded close to heat sources and sit with drooping wings and their eyes closed (ACMSF, 2016).

In Yogyakarta, the breeding of KUB chicken is popping up Umi *et al.* (2019), but it is no control over salmonellosis (Yunan *et al.*, 2023). In KUB chickens, salmonellosis spread throughout DOC (Yunan *et al.*, 2023). Antibiotics is one of the alternative treatments for *Salmonella* sp., infections in chickens (ACMSF, 2016). In addition, the KUB chicken farmer in Yogyakarta always gives antibiotics when KUB chicken shows clinical signs such as chalky diarrhea (Untari *et al.*, 2021). It was caused by the availability of antibiotics on the

market, which are cheap and easy to obtain without a veterinary prescription, and the impact of antibiotic resistance will occur (Untari *et al.*, 2021). Antibiotics are used to fight bacterial infections (ACMSF, 2016). However, selective pressure gave rise to bacteria resistant to antibiotics (Mehdi *et al.*, 2018). Therefore, the objective of this study was to isolation, identification, and sensitivity antibiotic against *Salmonella* sp., from hatchery KUB chicken in Yogyakarta.

MATERIALS AND METHODS

KUB chicken hatchery

The study was conducted by a cross-sectional design. Number of hatcheries KUB chicken from Sleman, Bantul, Kulonprogo and Gunungkidul were calculated based on the assumption that the prevalence of *Salmonella* sp., in hatchery KUB chickens is 55.7 % and corrections have been made to avoid large estimation deviations (Martin *et al.*, 1987). Sample was selected by a double-stage technique with a proportional technique based on the number of KUB chicken hatcheries at the district level.

Collection sample

Samples were collected from hatchery KUB chicken in Yogyakarta. Chick fluff and eggshells samples were chosen because it can be source of *Salmonella* sp., infection in KUB chickens' hatchery. A total of 13 chick fluff and 17 eggshells samples were collected only from Sleman, Bantul, Kulonprogo and Gunungkidul districts because an others place such as in Yogyakarta city no present KUB chicken hatchery. Chick fluff and eggshell weighing up to 500 grams were collected from KUB chicken hatcheries. Furthermore, the sample was entered into a sterile container to be brought to the laboratory and further processed.

Salmonella sp., examinations

Salmonella sp., was detected by accordance method Sharan *et al.* (2020) with only minor changes to the media and culture. Each sample of eggshell and chick fluff from KUB chicken as much as 50 g was added to 500 mL of pre-enrichment buffer peptone water (BPW) (CM: 0509, Oxoid Ltd., Basingstoke, United Kingdom) and furthermore incubated at 37 °C for 24 h. Afterward, 25 mL of culture eggshell and chick fluff KUB chicken in BPW medium were added to 250 mL of enrichment mannitol selenite cysteine broth (MSCB) (CM: 0699B, Oxoid Ltd., Basingstoke, United Kingdom) and incubated at 37 °C for 24 h. After being incubated in enrichment medium, it was cultured on xylose lysine deoxycholate agar (XLD) (CM:0469, Oxoid Ltd., Basingstoke, United Kingdom) medium on petri

dish plate and incubated at 37 °C for 24 h. Colonies on XLD petri dish plate medium that appear red with black center were isolated and identified as *Salmonella sp.*, with a Gram stain and biochemical reaction (Fàbrega and Vilaa, 2013). Biochemical reaction of *Salmonella sp.*, revealed that no lactose fermented but H₂S gas was produced with black color.

Antibiotic sensitivity

Antibiotic sensitivity test was performed by diffusion agar method on mÜeller hinton agar plate medium (MHA; CM 0337, Oxoid Ltd., Basingstoke, United Kingdom) that recommended by the Clinical and Laboratory Standards Institute (CLSI, 2020). Antibiotic sensitivity toward *Salmonella sp.*, such as ampicillin (10 µg), erythromycin (60 µg), enrofloxacin (5 µg), oxytetracycline (30 µg), sulfamethoxazole (300 µg), and tetracycline (30 µg) were tested with agar diffusion methode. Briefly, *Salmonella sp.*, isolate was cultured on brain heart infusion medium (BHI: CM 1135, Oxoid Ltd., Basingstoke, United Kingdom) and incubation at 37 °C for 24 h. The next step was cultured in nutrient agar (NA: CM 0003, Oxoid Ltd., Basingstoke, United Kingdom) and incubation at 37 °C for 24 h. A separate *Salmonella sp.*, colony was taken with sterile *Ose* included in NaCl physiologist solution and made cell suspension with equal to Mac (MC) Farland solution five number. The MC Farland solution five number was prepared by mix 0.5 ml BaCl₂ 1 % with 9.5 ml H₂SO₄ 1 % solution (CLSI, 2020). Concentration solution of MC Farland five number was equivalent with bacteria content about 2 x 10⁹

cfu/ml (CLSI, 2020). A total of 1 ml cell suspension which equivalent solution MC Farland five number was dripped on surface of mÜeller hinton agar (MHA: CM 0337, Oxoid Ltd., Basingstoke, United Kingdom). The next step was flattend and dry in incubator for 10 minutes. The mÜeller hinton agar (MHA: CM 0337, Oxoid Ltd., Basingstoke, United Kingdom) have been inoculated with *Salmonella sp* and then affixed with paper disc antibiotic and incubation at 37 °C for 24 h. Interpretation of antibiotic sensitivity was carried out based on diameter of inhibitory zone in millimeter (mm), and sensitivity patterns were observed and classified as sensitive, intermediate, or resistant based on the diameter of the inhibition zone (CLSI, 2020).

Statistical analysis

Microsoft Excel was used to classify and code data describing hatchery KUB chicken, isolation, identification, and sensitivity antibiotic toward *Salmonella sp.* Descriptive statistics were used to analyze the total number of hatcheries KUB chicken in Yogyakarta, isolates of *Salmonella sp* and antibiotic sensitivity.

RESULTS

Hatcheries of KUB chicken in Yogyakarta

Table 1 shows that in Yogyakarta, hatcheries of KUB chicken are in four districts, namely Sleman, Bantul, Kulonprogo, and Gunungkidul. Among the other districts, in Sleman most occurred hatcheries KUB chicken. Hatcheries of KUB chicken in Yogyakarta were using electrical energy with an average capacity of 100–500 eggs.

Table 1. Hatcheries of KUB chicken in Yogyakarta. (*Penetasan Ayam KUB di Yogyakarta*).

District	KUB chicken hatcheries
Sleman	5
Bantul	3
Kulonprogo	3
Gunungkidul	2
Total	13

Isolation and identification *Salmonella sp*

Isolation and identification *Salmonella sp.*, from chick fluff and eggshells showed in Table 2.

Both chick fluff and eggshell more contained *Salmonella sp.*, in the Sleman district than in the other districts.

Table 2. Isolation and identification *Salmonella* sp., from chick fluff and eggshells. (*Isolasi dan identifikasi Salmonella* sp., dari bulu anak ayam dan cangkang telur).

District	Isolation of <i>Salmonella</i> sp.	
	n=13 chick fluff	eggshells (n=17)
Sleman	1 positive and 4 negatives	2 positive, 3 negatives
Bantul	3 negatives	4 negatives
Kulonprogo	3 negatives	1 positive, 3 negatives
Gunungkidul	2 negatives	2 negatives
Total	1/13 (7.7 %)	3/17 (17.6 %)

Antibiotic sensitivity

Antibiotic sensitivity test against *Salmonella* sp., from chick fluff and eggshells was presented in

Table 3. *Salmonella* sp., from chick fluff and eggshells was resistant to ampicillin and erythromycin, whereas the others antibiotic was sensitive.

Table 3. Antibiotic sensitivity against *Salmonella* sp., from chick fluff and eggshells. (*Sensitivitas antibiotik terhadap Salmonella* sp., dari bulu anak ayam dan cangkang telur).

Antibiotic	Minimum Inhibition Zone (mm)			Isolat source	
	S	I	R	Chick fluff (n=1)	Eggshell (n=2)
<u>Ampicillin</u> (10µg)	≥17	14–16	≤13	R	R
Tetracycline (30 µg)	≥14	13–14	≤9	S	S
<u>Erythromycin</u> (60 µg)	≥18	14–17	≤13	R	R
Oxytetraklin (30µg)	≥14	13–14	≤9	S	S
Sulfamethoxazole (300 µg)	≥14	13–14	≤9	S	S
Enrofloxacin (5 µg)	≥21	16–20	≤15	S	S

R: Resistance I: Intermediate : Sensitive

DISCUSSION

Currently, many KUB chickens are found in the Yogyakarta area such as in Sleman, Kulonprogo, Bantul and Gunungkidul district. Concomitant increase demand of KUB chicken in the market causes KUB chicken farmers to continue self-produce KUB chicken DOC. Furthermore, the price of KUB chicken DOC on the market reaches Rp 7.500. It is promising a business opportunity for KUB chicken farmers. Sleman is one of two districts in Yogyakarta to receive KUB chicken technical guidance from the Indonesian ministry program (Umi *et al.*, 2019). Therefore, the breeding KUB chicken in Sleman district is the most abundant among the other districts.

In Yogyakarta, KUB chicken hatcheries get their eggs from around of KUB chicken breeders. It was caused by the KUB chicken group purchasing the incubator, so that all KUB chicken farmers could use it at the same time. Because of the impact of this matter, the size of the eggs varies and the

product of DOC is not uniform. Consequently, KUB chickens DOC is ununiform also due to ununiform egg size. Production of KUB chicken DOC is also determined by egg quality (Iskandar, 2017). One of the successes in hatching is determined by the quality of the eggs. The good eggs for hatching are eggs, it has character such as having an oval shape with diameter ratio of 3:4 between the wide part and the long part, or an egg shape index of 75 percent (Iskandar, 2017). Normal hatching eggs have an egg shape index of 70–75 %. Hatchability and weight of DOC that do not meet SNI No. 8405–1:2017 standards for KUB chickens DOC will suffer if egg size is not uniform.

Salmonellosis in KUB chickens is an important problem disease, especially in chicks less than A week old (ECDC, 2019). In addition, salmonellosis in adult KUB chickens can affect productivity through decreased egg production and body weight. Salmonellosis weakens the immune system in adult chickens, making them more susceptible to other

diseases, particularly those caused by viruses. The situation is complicated, making it difficult to handle. Salmonellosis is typically difficult to eradicate from contaminated hatcheries, but substantial reductions in prevalence are possible with improvements to biosecurity, cleaning, and disinfection (Ekaterina *et al.*, 2020; Claire *et al.*, 2022).

One source of salmonellosis transmission in KUB chicken is through eggs (Yunan *et al.*, 2023). It spreads vertically through *Salmonella*-infected brood stock. Therefore, it is necessary to inspect chicken hatcheries by examining eggshells and chick fluff. Salmonellosis was discovered in chick fluff and eggshell in the Sleman district (Table 2). It was more than other districts such as Bantul, Kulonprogo and Gunungkidul. It was unknown, but KUB chicken hatcheries are more common in Sleman district than in other districts. The occurrence of salmonellosis may be influenced by many factors, such as the origin of the hen, feed, water consumption and environmental hygiene (Munck *et al.*, 2020). Originating hens from salmonella-free farms is one of the important things to do to reduce the occurrence of salmonellosis in chicks (Munck *et al.*, 2020). It was caused by salmonellosis transmitted vertically through chicken eggs produced from *Salmonella sp.*, positive brooders (Munck *et al.*, 2020; Simpson *et al.*, 2020).

Contamination eggs with *Salmonella sp.*, during the production process is a complex issue, influenced by many variables, including flock size, flock age, stress, feed, vaccination and cleaning routines (Harriet and Ross, 2015). Eggs that used to hatch KUB chickens come from a variety of farms with varying levels of biosecurity. Therefore, salmonellosis in KUB chicken is suspected to be transmitted through chicken fluff or eggshells. It is in line with Theva *et al.* (2013) that prevalence of *Salmonella sp.*, in chick fluff samples is 8.7 % in broiler breeders, 3.1 % in layer breeders, 13.2 % in turkey breeders and 11.9 % in other breeder birds, such as ducks, geese, quail, partridges and pheasants.

Using antibiotics in chicken farms is a common practice for farmers, especially for treatment and growth spurts. In Indonesia, antibiotics were used by farmers for disease treatment 83.3 %, disease prevention 36.7 %, prevention and treatment 26.7 % and production increase 10 % (CIVAS, 2016). The unintended consequence of routine antibiotic use is increasing antibiotic resistance. Antibiotic sensitivity testing against *Salmonella sp.*, is important to be precise when choosing an antibiotic. *Salmonella sp.*, is resistant to erythromycin and ampicillin because these antibiotics may be frequently used and then there is

a mutation. The other research shows that *Salmonella sp.*, from eggshells was isolated in as many as 3.6 % of cases and was resistant to erythromycin 32.8 % and ampicillin 17.2 % (Kilonzo-Nthenge *et al.*, 2016). All of isolates *Salmonella sp.*, from broiler and layer chicken in Bogor, Sukabumi, and Cianjur districts were resistant to ampicillin and erythromycin (Hanun, 2019; Nafilah, 2019). Ampicillin resistance is caused by damage to the beta-lactam ring due to degradation by the penicillinase enzyme produced by *Salmonella sp.* (Kakoullis *et al.*, 2021). In addition, ampicillin resistance is also caused by the presence of genes in encoding ampicillin resistance, such as the *blaSHV*, *blaPSE* and *blaTEM* genes (Munita and Arias, 2016). Antibiotic resistance in *Salmonella sp.*, also caused by chemical modification of antibiotics using enzymes that catalyze reactions such as acetylation, phosphorylation, and adenylation (Kakoullis *et al.*, 2021).

Erythromycin resistance can occur due to modifications in the 23S rRNA molecule, which is in the 50S ribosomal subunit (Munita and Arias, 2016). It was a modification in erythromycin that made it unable to bind with the ribosome, so the bacteria could be continue the process of protein synthesis (Munita and Arias, 2016; Kakoullis *et al.*, 2021). Erythromycin enters the bacterial body via the bacterial cell membrane and binds reversibly to the 23S ribosomal RNA molecule in the 50S subunit of the bacterial ribosome (Munita and Arias, 2016; Kakoullis *et al.*, 2021). This binding process will inhibit the activity of the peptidyl transferase enzyme, thereby interfering with the amino acid translocation process during translation and protein synthesis. The character of erythromycin is bacteriostatic, so it only stops the growth of bacteria but does not destroy them (Munita and Arias, 2016).

Salmonella sp., which obtained from KUB chicken fluff and eggshell, were still sensitive to tetracycline, oxytetracycline, enrofloxacin and sulfamethoxazole (Table 3). It demonstrates that antibiotics can be used at the KUB chickens farm in Yogyakarta. In KUB chicken farms, antibiotics such as tetracycline, oxytetracycline, enrofloxacin, and sulfamethoxazole are still possible to use, but their use needs to be changed to reduce the occurrence of multidrug resistance in *Salmonella sp.* Routine change of antibiotic is decrease of multidrug resistance occur especially in *Salmonella sp.* (Rafael *et al.*, 2020). If occur of *Salmonella sp.*, multidrug, it will be difficult to overcome. As a result, biosecurity control, cage cleanliness and disinfection of the incubator hatchery KUB chicken are all efforts to reduce the occurrence of salmonellosis.

CONCLUSION

Salmonella sp., was found in hatcheries KUB chicken in Yogyakarta sensitive tetracycline, oxytetracycline, enrofloxacin, sulfamethoxazole and resistant ampicillin and erythromycin. Salmonellosis in KUB chicken can be reduced by increasing biosecurity controls such as disinfectant spraying of incubator hatchery KUB chicken after hatches egg.

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