



## **Detection Of Antibiotic Residues in Eggs of Layer Chickens and Knowledge of Animal Officers About Antibiotics**

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### **Abstract**

The chicken farming industry in Indonesia cannot be separated from the use of antibiotics as a curative effort in controlling the disease. Its use is either via injection, soaking, or as a feed additive (growth promoter) mixed in feed. Inappropriate use of antibiotics causes allergic reactions or resistance and the possibility of causing poisoning, residues in livestock tissues or organs that are harmful to human health when consuming them. This study aimed to determine the antibiotic residues in eggs and the level of knowledge of livestock workers on the use of antibiotics in livestock. The detection of antibiotic residues was conducted using the agar plate diffusion method by Kirby Bauer with the disk diffusion technique using *Escherichia coli* and *Salmonella sp.* As indicator bacteria. The results were analysed using a qualitative descriptive approach.

The samples were 76 eggs taken from the Regional Technical Implementation Unit of the Non-Ruminant Livestock Center of the Aceh Livestock Service in Blang Bintang and Saree Aceh Besar. Chloramphenicol antibiotic was used as a test medium and as control by calculating the diameter of the inhibition zone formed on MHA media. furthermore, a cross-sectional study design was used and questionnaires were distributed to 14 livestock officers to understand the knowledge of officers on the use of antibiotics in livestock by using google form. The data were analyzed using the chi-square test through the SPSS 22.0 program.

The results showed that the eggs and feed samples were negative from antibiotic residue. There was no relationship between feeding and antibiotic residues in eggs and laying hens feed. However, only 35.7% of officers had good knowledge about proper antibiotic usage. This means continuing awareness to the officers regarding the use of antibiotics either for treatment of diseases or as feed promoter are essential.

*Keywords: Laying hens, antibiotic residue, antibiotic resistance*

### **Background**

Chicken eggs as a source of protein of animal origin are widely consumed by Indonesian people due to being relatively affordable and easily obtained and so the farmers are trying to increase production regularly to meet the market demand. The egg production of laying hens in 2019 was 4.7 million tons compared to the previous year. This means the production increased by 1.37 percent (BPS, 2019). The chicken farming industry in Indonesia cannot be separated from the high use of antibiotics as a curative effort in controlling the disease. The increasing incidence of antibiotic residues and antibiotic resistance is likely due to their inappropriate use (Sholih *et al.*,

2015). Not only for the treatment of bacterial infections in humans but antibiotics are also used in the livestock sector (Suharsono, 2010). Antibiotics are used to control bacterial infectious diseases in farm animals. Antibiotics are administered either by injection, soaking or as a feed additive (growth promoter) mixed in feed (Nurhasnawati *et al.*, 2016). The use of the same types of antibiotics in humans and animals could potentially lead to transferring the bacteria that are resistant to the same antibiotics used by animals or animal products to humans (Nghiem *et al.*, 2017). This is exacerbated by the widespread use of antibiotics by the community without legal

prescriptions from medical doctors or veterinarians (Insany *et al.*, 2015).

The use of antibiotics as a growth promoter or feed additives in Indonesia has been prohibited based on the Minister of Agriculture no. 14/ 2017 concerning the classification of veterinary drugs which states that in animal feed there should be no mixture of antibiotics in it (Ministry of Agriculture, 2019). If the downtime of antibiotic drugs used in the food of animal origin is ignored, it can cause antibiotic residues (Meutia *et al.*, 2016). Allergic reactions or resistance and the possibility of causing poisoning due to the use of antibiotics in an irregular or inappropriate dose and not following with the diagnosis of the disease can cause residues in the tissues or organs of livestock that are harmful to human health when consuming them (Yuningsih, 2005). From the public health point of view, antibiotic residues can have implications for the health and economic sector, resulting in allergic reactions, microbial resistance, carcinogens, toxicity, and product rejection (Asredie and Engdaw, 2015).

The negative impact of antibiotic residues on animal products, in general, is the health impact due to toxicological, microbiological, and immunopathological hazards (Dewi *et al.*, 2014). Irrational use of antibiotics, excessive use of antibiotics, excessive use of new antibiotics, and long-term use of antibiotics can lead to resistance of microorganisms to various antibiotics (multidrug-resistance). One of the bacteria that has been resistant to several antibiotics in *Escherichia coli* which is a normal flora in the large intestine of animals and humans. Currently, *Escherichia coli* has become resistant to many groups of antibiotics, such as  $\beta$ -lactam groups, fosfomycin, and quinolones (Noviana, 2004). The resistance level of *Escherichia coli* isolated from the feces of layer chickens to doxycycline was 36.36 percent and ciprofloxacin was 14.77 percent (Ariyani *et al.*, 2018). The use of antibiotics in feed is closely related to the incidence of resistance to three strains of pathogenic bacteria such as *Salmonella*, *Campylobacter*, and *Escherichia coli*. These bacteria are three of the five main causes of

*foodborne disease* or the cause of death due to foodborne pathogen infection in the United States which are around 90% (Walyani, 2019). Kusumawardani (2016) reported the results of sensitivity tests to chloramphenicol antibiotic in which 2 from 17 strains of *E. coli* showed resistance. Therefore, it is important to pay attention to the withdrawal time of treatment with antibiotics in animals and to routinely examine the residues of antibiotics in animal products. The application of good *hygiene* and management practices will ensure food safety for a more secure public health (Priyanka *et al.*, 2017).

Based on the description above, to provide food of animal origin that is safe, healthy, whole, and halal (ASUH) requires strict supervision starting from cultivation, feeding and medicine, post-harvest handling, storage and distribution to consumers so that there is no antibiotic residues and antibiotic resistance in animal products and microbial contamination in food of animal origin, especially in relation to the protection of health and consumer safety. The purpose of this study was to detect antibiotic residues in layer chicken eggs and the level of knowledge of livestock officers on the use of antibiotics in laying hen's farms of the Aceh government in Aceh Besar.

## Materials and Method

This research was conducted from October 2020 to March 2021. The testing of antibiotic residues was carried out at the Veterinary Public Health Laboratory and the Microbiology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University. Egg samples and interviews with livestock officers were taken and carried out at the Aceh government farms UPTD BTNR Blang Bintang and Saree Aceh Besar.

The population in this study were 7000 chicken eggs on laying hens farms of the Aceh government, UPTD of the Blang Bintang and Saree Aceh Besar Non-Ruminant Livestock Center. Determination of the number of samples in this study used the Slovin formula. Then about 20% of egg samples were taken by *purposive sampling*

with standard egg criteria were 50 g to 60 g (SNI, 2008).

$$\text{Slovin's formula: } n = \frac{N}{1+Ne^2}$$

N = Population Quantity

n = Number of Samples

e = Error tolerance limit (*error tolerance*)

Then:

$$n = \frac{N}{1+Ne^2} = \frac{7000}{1+(7000 \times 0,05^2)} = \frac{7000}{1+17,5} = \frac{7000}{18,5}$$

= 378 egg samples

20% of 378 eggs = 76 eggs.

Fourteen animal husbandry officers at UPTD BTNR Blang Bintang and Saree Aceh Besar were asked questions through a questionnaire using google forms and in-person related to farmers knowledge regarding the use of antibiotics in animals. This type of research was conducted in a qualitative descriptive manner based on a cross-sectional study. The design of this study used the Agar plate diffusion method by Kirby Bauer method with disk diffusion technique using *Escherichia coli* and *Salmonella* sp. as indicator bacteria. The egg samples and feed samples of laying hens dipped in blank disks were used as test media, and chloramphenicol antibiotic disks were used as a control by calculating the inhibition zone diameter formed on MHA media. Moreover, online questionnaires via google forms were then distributed to livestock officers followed by a direct interview to find out the education level of the officers, regarding the use of antibiotics in animals.

## Results

Based on the results of the analysis of antibiotic residues on 76 egg samples at the UPTD BTNR Blang Bintang and Saree Aceh Besar farms, the results showed negative results with an inhibition zone of 0 mm on the test media for *Escherichia coli* and *Salmonella* sp. That can be seen in Table 1 and in Figure 1.

Table 1. Antibiotic residue test results

| Bacteria Indicator                            |             |          |          |
|---|-------------|----------|----------|
| <i>Escherichia coli</i> <i>Salmonella</i> sp. |             |          |          |
| No  | No Test     | Results  | Results  |
| A1  | ST 01 to 40 | negative | negative |
| A2  | ST 41 to 76 | negative | negative |

Note: A1=Egg samples of UPTD BTNR Blang Bintang; A2 = Egg samples of UPTD BTNR Saree

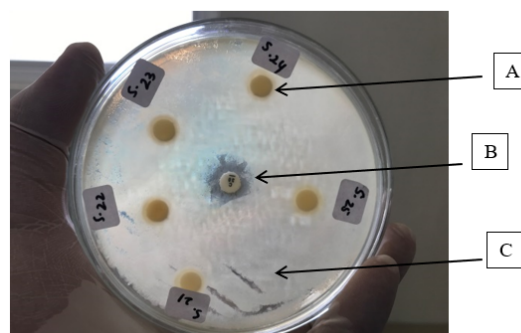


Figure 1. Results of testing egg samples on storage media for *E. coli* and *Salmonella* sp.

Note: A= sample of chicken eggs; B= positive control disc chloramphenicol disc; C= storage media for *Salmonella* sp.

Table 2. Knowledge of livestock officers on the use of antibiotics

| Variable  | Category | Frequency (%) |
|-----------|----------|---------------|
| Knowledge | Good     | 5 (35.7%)     |
|           | Not good | 9 (64.3%)     |

Based on Table 2, it can be seen that the level of knowledge of livestock officers regarding the proper use of antibiotics were still low (only 35.7% of respondents had good knowledge).

## Discussion

The results of qualitative egg samples testing using agar plate diffusion method using Kirby Bauer method with disc diffusion technique using *Escherichia coli* and *Salmonella* sp. as a storage medium showed negative results indicated by the absence of an inhibition zone around the paper disc. This shows that the chicken eggs produced from the UPTD BTNR Blang

Bintang and Saree Aceh Besar farms do not contain antibiotic residues, so they are safe for public consumption.

This finding follows the regulation of veterinary drugs, especially regarding the prohibition of AGP (Antibiotic Growth Promoter) in feed based on the regulation of the Minister of Agriculture No. 14/2017 concerning the classification of veterinary drugs. The negative result is likely to happen because the period of stopping the drug has been achieved and antibiotics were used was appropriate dosage. Based on research (Meutia *et al.*, 2016). Factors that affect the duration of antibiotic residues in eggs include the dose and types of antibiotics.

Chemical residues can be found due to the use of drugs, including antibiotics, feed additives, or animal growth-promoting hormones. Drug compounds that enter livestock body cannot be entirely excreted from the tissues and retained in body tissues as residues (Rahayu, 2014). Palupi *et al.* (2009) observed that using drugs with excessive doses, long-term administration, and inappropriate drug discontinuation caused drug residues in the carcass and visceral organs. In contrast to the positive control of chloramphenicol, an inhibition zone was seen around the paper disc. The formation of an inhibition zone around the paper disc in this positive control was the effect of antibiotics that inhibited bacterial growth.

Meanwhile, based on the results of interviews with 14 livestock officers using google forms and livestock officers who do not have smartphones and so using a manual questionnaire directly revealed that 5 (35.7%) livestock officers had a good level of knowledge on antibiotic residues and 9 (64.3%) farmworkers still had have poor knowledge about antibiotic residues.

The research results conducted in the period 2011-2016 conducted by Wijaya (2011) and Yulianti *et al.* (2016) showed that cases of antibiotic contaminations in chickens tended to decrease, even some studies did not find any antibiotic residue contamination. Most farmers were likely quite to understand the rules for using antibiotics. In some studies, it is still found

that there are residues of tetracycline antibiotics, although in small amounts. Supervision by the government will improve the quality and safety of food of animal origin from farms to consumers, such as monitoring the use of antibiotics in livestock which must be carried out under the supervision of a doctor (Etikaningrum and Iwantoro, 2017). If antibiotic residues were not found, it is likely because farmers and livestock officers already understood the appropriate use for the withdrawal time and the right dose of antibiotics to cure certain poultry diseases (Donkor *et al.*, 2011). On the sixth day, post-slaughter antibiotic residues were no longer found. The food products were safe to be consumed because the antibiotic withdrawal time at a dose of 2.5 mg/kg body weight intramuscularly mainly was on the sixth day. (Davis, Foster and Papich, 2007).

It has been reported that most livestock workers in Subang district, as many as 59% of respondents had common knowledge of antibiotic resistance. As many as 41% of farmer respondents, had a high level of knowledge (Walyani, 2019). The results of a survey on farmer perceptions of antibiotics on farms in West Kalimantan were conducted on 293 commercial broiler breeders. More than half of the population of poultry farmers in West Kalimantan still did not have an adequate basic understanding of antimicrobial resistance, and yet 48% (140/293) of commercial broiler farmers in West Kalimantan already had a basic understanding of the risk of antimicrobial resistance (Sumambang *et al.*, 2019). It is thought that attitudes and knowledge influence a person's practice, but there are factors of intention, subjective norms, and perceptions of behavioral control (Purnawarman and Efendi, 2020). Breeders who apply good farming methods have significantly different attitudes than farmers who do not apply good farming practices (Thongpalad *et al.*, 2019).

## Conclusion

In conclusion, the testing for antibiotic residues on layer chicken egg samples from laying hens farm owned by the government of Aceh Regional Technical

Implementation Unit for Non-Ruminant Livestock Center Blang Bintang and Saree Aceh Besar showed negative results. However, there was still a lack of understanding about the officers' proper use of antibiotics in poultry farms. More socialization to the farmers is necessary to eliminate the case antibiotic residues in poultry products.

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