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Residue of Tetracyclineand Penicillinn Antibiotic On Pork In Denpasar Bali

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Abstract. Humans need food from animals in quantity and good quality, so that the needs of the body are fulfilled. Meat products such as pork can contain certain ingredients such as antibiotic residues that have a negative impact on health. Monitoring antibiotic residues in pork must be carried out periodically to prevent and be aware of antibitics. Survey research on antibiotic residues in pork marketed in Denpasar, Bali. The goal is to find out tetracycline and penicilline antibiotic residues in pork which are marketed in Denpasar, Bali, Indonesia. Fifty pork samples from Badung, West Denpasar, East Denpasar, North Denpasar and South Denpasar markets. Each market is taken 10 samples purchased from different traders. Each weight is 100 grams. Antibiotic residue testing was carried out qualitatively using a screening test method.

Keywords: residue, antibiotics, meat, pork, screening test

I.INTRODUCTION

Humans need food of animal product not only the quantity, but also in quality. Meat sold on the market can contain of chemical material (for example antibiotic residues) that are not good for human health. Monitoring of antibiotic residues must be done to prevent and recognize the presence of antibitics in these animal products.

Antibiotics are substances that can kill or inhibit bacterial growth, there are various types or classes of antibiotics, among others: groups of Penicillin, Polypeptides and Cephalosporins, for example ampicillin, penicillin G works through bacterial cell wall synthesis inhibitors. Quinolone groups, for example rifampicin, actinomycin D, nalidixic acid work through transcription and replication inhibitors. Makrolides, Aminoglycosides, and Tetracyclines, for example gentamicin, chloramphenicol, kanamycin, streptomycin, tetracycline, oxytetracycline, erythromycin, and azithromycin work through protein synthesis inhibitors. Ionomycin group, valinomycin works through cell membrane function inhibitors. Sulfa or sulfonamide groups, for example oligomycin, tunicamycinand antimetabolites, for example azaserine [1].

Antibiotics are used for prevention (preventive) or killing (curative) diseases caused by bacteria. In the animal husbandry, which is the cultivation of pigs (fattening), antibiotics play an important

role in preventing and eradicating diseases caused by bacteria. Commonly used antibiotics are tetracycline and penicilline. Based on the Indonesian National Standard (SNI No. 01-6366-2000), the maximum limit of tetracycline antibiotics is 0.1 µg / g. Whereas Australia in its pollutant the meat consumed cannot contain any antibiotic residues at all.

Siswanto and Sulabda reported that antibiotic residues were still found in meat products in Bali. The presence of antibiotic residues in food of animal product related to the use of antibiotics for treatment of diseases [2]. The use of antibiotics asfeed additives is also often done by farmers. Other researchers get the results of antibiotic residues in chicken meat; chicken's liver; and beef each 4.25; 28.6; and 78.8% [3]. Yamaguchi et al. said that sulfonamides, fluoroquinolones, and tilmicosin were detected in some of the samples. Sulfaclozine and fluoroquinolones were mainly detected in chicken samples, and sulfamethazine was mainly detected in pork samples [4].

Antibiotic residues in meat if consumed for long periods of time have a negative effect on health, because bacterial immunity to antibiotics will emerge and as a result there will be resistance or reduced human immunity to a disease Muaz et al. (2018) [5]. Consuming antibiotic-contaminated meat for a long time, bacteria will emerge immune to antibiotics, and the effect will be on human resistance to diseases caused by bacteria. Antibiotic residues in meat have a bad effect despite warming up. Because the dangers of antibiotic residues in livestock meat products and the rare monitoring of antibiotic residues in pork in Bali, it is necessary to research the pork antibiotic residues in Bali.

The purpose of this study was to determine tetracycline and penicillin residues in pork marketed in several city markets in Denpasar, Bali. It is expected that the results of this study can provide information to the public, so that people are careful to consume pork. The urgency of this study is that it is positive for antibiotic residues in pork so it must be given understanding and education to farmers about the procedures and rules for using antibiotics.

The principle uses of antibiotics in animal productionwas the treatment, prevention and control of diseases as respiratory disorders, mastitis, gastrointestinal infections, arthritis and other infectious bacterial diseases. Antibiotics are classified as: the class of penicilline, polypeptide and cephalosporin, for example ampicillin, penicilline G works through bacterial cell wall synthesis inhibitors. The Quinolone group, for example rifampicin, actinomycin D,

nalidixic acid works through transcription and replication inhibitors. Makrolida, Aminoglycoside, and Tetracycline groups, for example gentamicin, chloramphenicol, kanamycin, streptomycin, tetracycline, oxytetracycline, erythromycin and azithromycin work through protein synthesis inhibitors. Ionomycin group, valinomycin works through cell membrane function inhibitors. Sulfa or sulfonamide groups, for example oligomycin, tunicamycin and antimetabolites, for example azaserine (Ashton. 2012 [6]; Gottlieb and Shaw, 2013[7]; Gualerzi et al. 2013)[8].

Antibiotics are used for therapeutic agents (treating clinical symptoms of bacterial infections) and as prophylactic agents (applied to prevent disease). This policy establishes guiding principles for the meat industry to ensure that the development of antibiotic resistance is minimized and meat is not contaminated by antibiotics. These feed additives are commonly used without medical prescription and forrelatively long intervals, either in large and small doses and It is practiced commonlyamong farm animal producers to treat all groups of livestock, such as birds, fish, orother animals even if there is only a few infected animals. This is due to impropermaintenance of treatment records or a failure to recognize treated animalsadequately may lead to skipping of these animals [9][10] (Beyene, 2016; Bayou and Haile, 2017).

The use of tetracycline antibiotics as a treatment for prevention of disease and as a food additive in broiler chicken food or beverages allows the presence of tetracycline antibiotic residues in broiler chicken meat. The misuse of antibiotics in the field of meat and egg breeding providers is that antibiotics are often used not only to prevent infection with animals / chickens by bacterial disease, but also for weight gain. The use of antibiotics is bad for livestock due to animal resistance to certain types of phatogenic micro-organisms. This has happened to poultry farms in North Carolina (United States) due to certain antibiotics, livestock resistant to Enrofloxacin which functions to eradicate the Escherichia coli bacteria. In other parts of the residue from antibiotics will be carried in livestock products such as meat, eggs and milk and will be dangerous for consumers who consume them. As reported by Rusiana, researching 80 broiler chickens in Jabotabek found 85% of broiler chicken meat and 37% of chicken liver were contaminated with tylosin antibiotic residues, penicillin, oxytetracycline and kanamycin (Patricia et al. 2012) [11]. Imtihan and Nur (2010) found that chicken meat marketed in several cities in Malang

also had positive antibiotic tetracycline residues [12]. The use of antibiotics in livestock rations has also been a heated debate by scientists due to the adverse effects not only for livestock but also for consumers who consume these livestock products through residues left on both meat, milk and eggs. Certain countries have limited the use of these additives in animal feed such as in Sweden in 1986, Denmark in 1995, Germany in 1996 and Switzerland in 1999. Furthermore, on January 1, 2006 the EU Society based on regulation number 1831/2003 set the milestone for the elimination of various types of antibiotics where for decades the back is a substance that is often used by farmers in various parts of the world (Australian Chicken MeatVederation INC. 2005) .

The fact, antibiotics usedas a growth promoter compound in animal feed, there has been an increase in farmer's income. However, the prohibition is not comprehensive only limited to certain types of antibiotics such as avoparcin (Denmark), vancomycin (Germany), spiramycin, tylosin, virginiamycin and chinoxalins (European Union). Until now, only four antibiotics are still allowed to be used in livestock rations in European communities, namely flavophospholipol, avilamycin, monensin-Na and salinomycin-Na (Australian Chicken MeatVederation INC., 2005). Antibiotic residues closely related to the time spent antibiotics in the blood circulation, so it is necessary to stop antibiotic administration. Downtime is the period from the time the last drug is given until the animal can be cut or the product can be consumed. Even though farmers are aware of the drug downtime, some of them do not comply. Approximately 50% of drug residue deviations in livestock products are due to non-compliance with drug downtime. A total of 15.60% of farmers in Australia do not comply with the provisions of drug downtime, while in Indonesia only 8.16% of dairy farmers are compliant with drug downtime by not selling fresh milk to the cooperative for 2−5 days after treatment. The use of drugs carried out by farmers themselves has caused drug residue deviations in livestock products by 63−65%. This situation is most likely related to the dose and downtime of drugs that are not followed. In Australia, around 35.40% of the use of antimicrobial drugs is not done properly. Such errors may also occur in Indonesia with a much higher percentage. Only 20% of dairy farmers in West Java know the types of drugs used by officials from the Livestock Service Office or cooperatives. Of the 20%, only 14.28% of farmers were aware of the drug downtime, while those who adhered to drug downtime by not selling milk to the

cooperative for 2−5 days after treatment were only 8.16%.

Oxytetracycline was found in 2 positivesof the 12 pork samples byELISA and in none of the 21 chicken samples. One sample contained levels higher than 100 µg/kg, which is the maximum residue limit for tetracyclines in muscle tissue. Doxycycline was found in 18 out of 19 inhibitor-positive, ELISA positive chicken samples. No tetracyclines were found in the remaining 3 samples, one of which was ELISA positive. The levels of doxycycline ranged from 63 µg/kg to 1033 µg/kg in a pork sample and from 10 µg/kg to 1680 µg/kg in a chicken sample. Levels higher than 100 µg/kg were found in 7 pork samples and in 6 chicken samples. In all samples with doxycycline or oxytetracycline levels. In all but one of the samples with doxycycline levels of 100 µg/kg or more. In others Results revealed that out of 202 cattle 41 (20.30%) tested positive for antibiotics in one or more of their organs. The meat of cattle from transhumance system, sick animals and older cattle was more likely to be contained with penicillin G and oxytetracycline residues. The average residues concentration in beef was 17.58 µg/kg for penicillin G and 240 µg/kg for oxytetracycline. In others, Ngom et al, (2017) found results revealed that out of 202 cattle 41 (20.30%) tested positive for antibiotics in one or more of their organs. The meat of cattle from transhumance system, sick animals and older cattle was more likely to be contained with penicillin G and oxytetracycline residues. The average residues concentration in beef was 17.58 µg/kg for penicillin G and 240 µg/kg for oxytetracycline. The others Wijaya and Herwin (2011) said that none of the 36 chicken samples were positive of penicillin, macrolides, aminoglycosides, and tetracyclines. Three samples from 24 samples of beef were positive of macrolides, those samples derived from Bandung City and Tasikmalaya District [13].

Wihtdrawal time depending on type of drug, animal species, animal genetic factors, local climate, method of administration, drug dosage, animal health status, livestock products produced, drug residue tolerance limits, and drug formulations. Therefore, it is only natural that every company that produces veterinary drugs includes a clear description of the drug downtime. The wihtdrawalfor giving non-compliant veterinary drugs causes animal drug residues in livestock products.

The percentage of the occurrence of antibiotic contamination in milk is quite high (more than 50%), and the type of antibiotic that most often contaminates milk is the class of penicilline and

tetracycline. There is chloramphenicol contamination in milk, in which case the drug is prohibited from being used in animals. Lots of chicken meat and liver are also contaminated with antibiotic residues, especially penicilline and tetracycline groups and contamination of the liver is higher than in meat, beef and liver also found antibiotic and hormone residues. Antibiotic residues in milk are estimated as a result of treatment of mastitis, because the prevalence of subclinical mastitis in Indonesia is very high at 87.10%. This situation is strengthened that there are quite a lot of dairy farmers who treat their own livestock, while farmers who understand the drug downtime are very few. The residual content of the drug that exceeds the specified residual maximum (BMR) will cause meat and milk to be unsafe because it can cause allergic reactions, poisoning, resistance to certain microbes or cause physiological disturbances in humans. The use of veterinary drugs must be in accordance with the applicable provisions by taking into account, among other things, downtime and dose suitability. In addition, animal drug storage must also follow the instructions. However, Yuningsih (2009) has observed that beef is relatively antibiotic under BMR [14].

Sulfaclozine and fluoroquinolones were mainly detected in chicken samples, and sulfamethazine was mainly detected in pork samples. High levels of sulfonamide residues, ranging between 2500 and 2700 μg/kg sulfaclozine and between 1300 and 3600 μg/kg sulfamethazine, were present in two chicken and three pork samples, respectively. Tilmicosin was detected at ranges of 150–450 μg/kg in 10 chicken samples. Positive percentages were 17.3, 8.8, and 7.4% for chicken, pork, and beef, respectively, for an average of 11.9%. The results suggest an appropriate withdrawal period after drug administration had not been observed in some livestock (Pavlov et al. 2008; Yamaguchi, et al., 2015) [15][16].

In the preproduction stage, the use of veterinary medicines is a necessity so that livestock productivity can be maintained or improved. From observations in the field, the use of antibiotics especially on broiler farms and laying tends to be excessive without regard to the correct usage rules. The inappropriate use of veterinary drugs is likely related to the pattern of marketing of veterinary drugs in the field, where 33.30% of small-scale laying hen farmers and 30.80% of small-scale broiler farmers who do not have veterinarians, get drugs directly from distributors or importers , so it is feared that the use of these drugs does not follow the rules. Farmers should have veterinarians who can deal directly with

drug distributors or importers. In addition, breeders often do not understand the withdrawal time of an animal drug, resulting in the emergence of residues in livestock products.

The survey results in the United States showed that around 77% of respondents were concerned about residual drug problems (especially antibiotics) in livestock meat. Some cases of disruption to Campylobacter bacterial resistance related to antibiotic residue problems in the United States were also reported. An assessment of meat, milk and eggs depends on the level and type of residue found in the product. Animal origin products containing drug residues above BMR should not be consumed or exported. However, in fact animal drug residues in meat and chicken eggs are much above BMR.

The use of veterinary drugs must be in accordance with the applicable provisions by taking into account, among other things, downtime and dose suitability. In addition, animal drug storage must also follow the instructions. The use of pesticides and other chemicals for environmental sanitation (cages) must also be careful not to contaminate feed or drinking water sources.

II. MATERIALS AND METHODS

The study used 50 pork samples from traditional markets in Badung, West Denpasar, East Denpasar, North Denpasar and South Denpasar. Each market is taken 10 samples purchased from different traders weighing 100 grams each. The method of testing the presence of antibiotics in meat is done qualitatively using the filter method (sreening test) at the Denpasar Veterinary Center LaboratoryStandar Nasional Indonesia (SNI) (2008) [17].

Materials and tools used include 8 mm diameter disc paper, 100 x 12 mm petri dish, 7 ml test tube, 100 ml volumetric flask, 50 ml centrifuge tube, 250 ml erlenmeyer, 1 ml and 5 ml volumetric pipette, 1 ml graduation pipette, and 5 ml, and a bottle of media (roux's bottle). Other tools are centrifuges, water baths, sterile cabinets, homogenizers, autoclaves, refrigerators, freezers, analytical scales, incubators, stirring magnets and pH meters. The supporting devices include micro-pipettes 50-300 µl, shovel, burner, ose, tweezers, and scissors.

Procedures

Make a tetracycline (as stock) comparative solution that is dissolving tetracycline into distilled water until a concentration of 1000 µg / ml is stored in the refrigenerator. Then make a series of

tetracycline and penicilline concentrations of 0.25; 0.50; 1.0; 2.0 and 4.0 µg / ml. Drop the raw solution into 75 µl of disc paper and the phosphate solution as a negative control, waiting for all to be absorbed. Entered into petri to grow bacteria and put it at room temperature for 1 to 2 hours. The presence of bacterial growth barriers indicates a positive sample containing antibiotics. Next measure the diameter of the resistance formed.

Sample preparation (sample)

10 grams of meat cut into small pieces put in erlenmeyer added 20 ml buffer solution, then homogenized using homogenizer. At a 3000-rpm centrifuge for 10 minutes and take the supernatant. This supernatant is ready to be used as a test sample solution.

Implementation of testing

Planting bacteria on the media so that by piping 1 ml of culture the bacteria are mixed into the agar medium. Then piping 8 ml of agar and bacterial mixture. into petridis, where each type of antibiotic uses three petri dishes. Left until it freezes. Next drip the comparative standard solution and buffer solution to each 75 µl disc until evenly distributed. Next put the disc paper into a petri dish and incubate at 36 0C for tetracycline and 55 0C for penicilline for 16 hours. Reading the results by measuring the diameter of the area of resistance formed around the disc paper. The same is done for positive control and negative control. Positive controls must form an obstacle area while negative control must not form an obstacle area.

III. RESULTS AND DISCUSSION

The test results from 50 samples of pork originating from several markets in Denpasar, only 4 samples positive containing antibiotics. Positive trachyclinic 2 samples (4%) and positive penicilline 2 samples (4%), so that overall, 8% are antibiotic positive.

Table 1. Test results for tetracycline and penicilline antibiotic residues in pork in Denpasar

No	Origin of meat	Total of sample	Positif		
			Tetracycline	Penicillin	
1	Badung	10	0	0	
2	North of Denpasar	10	0	0	
3	East of Denpasar	10	2	0	Positive total of antibiotic 8 %
4	South of Denpasar	10	0	2	
5	West of Denpasar	10	0	0	
	Total	50			
	Persentage (%)		4	4	

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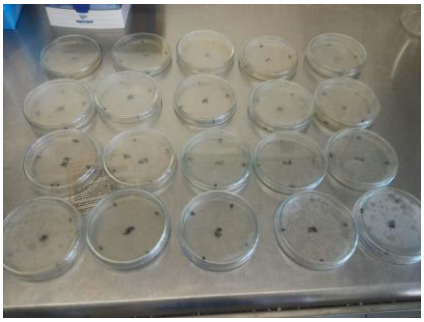
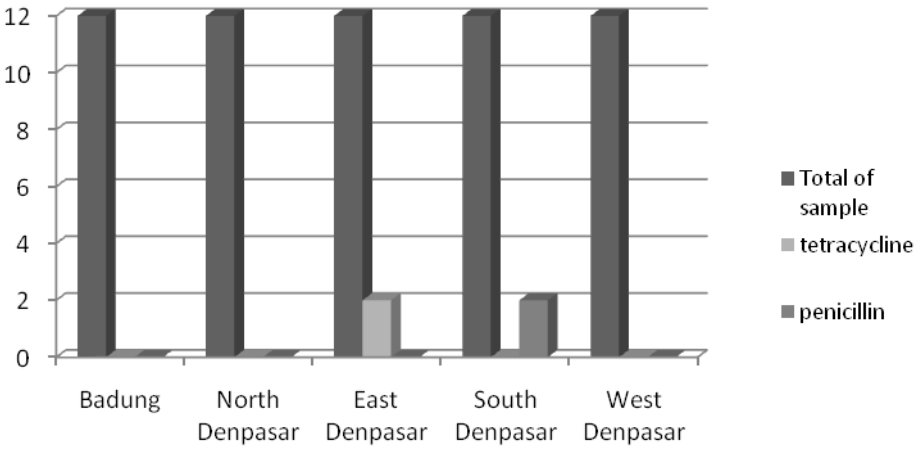


Picture 1. Agar on petridisc

Picture 2. Negative Secreening test

Picture 3. Negative Secreening test



The results above show that there are animal products that contain residues of tetracycline (4%) and penicilline (4%) antibiotics in Denpasar, Bali. Same with Liousia et al. [18] that 4% of the muscle swine samples were positive to aminoglycosides and macrolides. Although the percentage is small, that is 4

positive samples out of 60 total samples. These results illustrate no difference from findings elsewhere. It is understandable that antibiotic use in animals to be slaughtered is still present throughout Indonesia, even throughout the world. Generally developing countries have more cases than modern countries. The small percentage of positive antibiotic residues in meat, in Denpasar can be caused by pork which is marketed when cutting still contains antibiotics in the body. This can be caused by the pig undergoing treatment before being cut, and cutting is done before the circulation of antibiotics in the body (withdrawal time) is exceeded.

Unlike when compared to chicken farms, here is often done using antibiotics as a preventive bacterial infection as well as a weight booster. Weight boosters are expected to increase the selling weight of chickens, thus benefiting farmers. But pig farming in Bali is traditional, antibiotics are not used as a growth booster, but as a treatment for sick animals.

However, animals that are to be slaughtered should be free from antibiotics, so that the community is completely free from the consumption of antibiotic residues. If animals will be sold and will be slaughtered for consumption, then they must wait for the drug to run out in the body's circulation (withdrawal time).

IV. CONCLUSION

Pork marketed in several markets in Denpasar still contains 0.33% terracycline antibiotic residues and 0.33% penicilline. The small amount of antibiotic residue is inseparable from the function of the government of Bali province in this case the Livestock Service Office and related agencies that have been working well in providing counseling to farmers. Because there is still marketed meat containing antibiotic residues, it is still necessary to provide understanding to farmers (farmers) who will sell their cows to wait for 'antibiotic distribution' in the animal's body if animals need antibiotic treatment.

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