TERNAK TROPIKA
Journal of Tropical Animal Production
Vol 25, No. 2 pp. 131-140, Desember 2024

DOI: 10.21776/ub.jtapro.2024.025.02.4
OPEN ACCES Freely Available Online

DETECTION OF ANTIBIOTIC RESIDUES IN BEEF AND BEEF LIVER IN GORONTALO CITY

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Submitted 29 April 2024, Accepted 10 Desember 2024

ABSTRACT

This study aims to determine the presence of antibiotic residues in beef and beef liver sold at the slaughterhouse (TPH) in Gorontalo City. The total of the samples are 34 samples, there are 20 samples of beef and 14 samples of beef liver in Gorontalo City. Testing of beef and beef liver samples using agar diffusion method. The presence of antibiotic residues can be seen from the formation of an inhibitory zones around the disc paper. The results of the study found that in sampling from the slaughterhouses in Gorontalo City, samples of beef liver from TPH Andalas gave a diameter of inhibitory zones about 11.37 mm. Beef liver samples from Padebuolo TPH gave an inhibitory zones about 8.12 mm, while beef liver samples taken from TPH Moudu gave a diameter of inhibitory zones about 15.39 mm and 10.99 mm. Then the second sample, the samples of beef from TPH Andalas gave a diameter of inhibitory zones about 12.68 mm and 9.95 mm. Beef samples from Padebuolo TPH gave inhibitory zones about 9.12 mm and 12.44 mm, while beef samples taken from TPH Moudu gave a diameter of inhibitory zones about 11.69 mm and 8.42 mm. As for TPH Biau, beef samples gave inhibitory zones about 9.86 mm and 8.11 mm. The results at each time of collecting the samples showed that most of the samples contained antibiotic residues with a weak category with the average diameter about 12.3 mm. The conclusion from the laboratory tests on 26 samples, they are 14 samples of beef and 12 samples of beef liver which were analyzed qualitatively using methanol solution showed that some of the samples were identified as antibotic residues, which were shown by the formation of inhibitory zones in each sample.

Keywords: *Agar diffusion method; antibiotic residues; beef; beef liver*

How to cite : Taha, S. R., Datau, F., Rokhayati, U. A., & Unggango, Y. (2024). Detection of Antibiotic Residues in Beef and Beef Liver in Gorontalo City. TERNAK TROPIKA Journal of Tropical Animal Production Vol 25, No 2 (131-140)

INTRODUCTION

Nurwanto et al (2012) beef has nutritional content which is quite complete, such as water, protein, fat, minerals and low carbohydrate content. The carbohydrate content can be used as a medium for the growth of good bacteria so that meat will easily be damaged. Bahri (2008) states, food ingredients livestock products will become dangerous and useless if they are meat It is not safe, therefore there is a need for absolute security inside food safety so that it is useful for the body.

Meat is very beneficial for health, growth, and human intelligence. Commonly consumed meat can be obtained from large and small ruminants (cows, buffalo, sheep, goats), poultry (chickens, ducks), and various livestock (rabbits, horses, deer, pigs)

The number of Indonesian traditional cuisines that use meat as the main ingredient, for example beef soto, rendang, rawon, empal, and satay, make meat as one of the foods that people like in Indonesia. The meat can be processed or cooked in various ways such as boiled, fried, baked, grilled, or dried.

Meat is categorized as perishable food and potentially hazardous food (Lukman et al. 2009). Meat can contain biological, chemical and physical hazards. One of the chemical hazards that can be found in meat is antibiotic residues. Judging from the aspects of public health, antibiotic residues in food from animals can threaten public health. Public health threats due to antibiotic residues in animal-derived foods include bacterial resistance, consumer health problems such as allergies or poisoning.

One of the chemicals that can contaminate animal products are residues antibiotics. Antibiotic residues in food can threaten public health. This threat takes the form of a negative impact that impact society, One of the occurrences is resistance bacteria, food allergies and also poisoning. The problem of antibiotic residues arises in animal food products caused by inappropriate application of antibiotics in livestock. Antibiotics used for treatment and also livestock growth promoter, so if antibiotics are not used pay attention to the drug withdrawal period, then it will Causes the formation of antibiotic residues in animal food products (Dewi, 2014).

Surveillance of microbial residues and contamination in animal-derived foods is very important, especially in relation to consumer health and safety protection. In this regard, efforts to provide safe, healthy, whole and halal (ASUH) animal-derived foods continue to be carried out. One of them is by monitoring through a program of monitoring and surveillance of residues and microbial contamination.

The problem of antibiotic residues in animal-derived foods is related to poor practices in antibiotic use on farms. Antibiotics are currently widely used for treatment and growth promoters. The use of antibiotics that do not pay attention to the drug stop period (withdrawal time), will cause antibiotic residues in animal products (Donkor et al. 2011). According to Agustina et al. (2000) bioassay monitoring and surveillance of antibiotic residues showed that on average 80% of the samples examined contained microbial contamination. Contamination of antibiotic residues in livestock products in chicken meat; chicken's liver; and beef 4.25; 28,6; and 78.8% contain tetracycline antibiotic residues and production processes related to food safety from livestock mainly occur in slaughterhouses, the home slaughtering for beef.

Antibiotics that are often used in livestock include Penicillin (Penicillin G, Potassium Penicillin G), Tetrasildin (Tetracycline, Chloretracycline), Aminoglycosides (Gentamicin Sulfate, Noomisin) and Macrolides (Ecitromycine), Chloramphenicol. If this antibiotic is used beyond the limit, it will cause antibiotic residues.

The residual effects of antibiotics on food farms initially get less attention, this is understandable because generally the residual concentration is very low, so the effects that are caused will not be seen

immediately, the clinical symptoms that are caused are sometimes not real. Widiastuti, R., T. B *et al* (2000), states that any residues will be lost in a farm product within a week after the last administration. So far, research efforts on antibiotic residues on beef in Gorontalo Province in general and Gorontalo in particular have never been reported, so that researchers want to conduct this study with the title "Antibiotic Residues Test on Beef Sold traditional market and supermarket in Gorontalo ".

Objective of Research

To determine the presence of antibiotic residues on beef in Gorontalo City, Gorontalo Province.

Method of Research

Antibiotic Residue Test was conducted at the Microbiology Laboratory at Faculty of Mathematics and Natural Science (FMIPA) in Universitas Negeri Gorontalo.

Tools and Materials

This study are, Petri Dish, Paper Disc, Measuring, Pipette Test, Tube, Measuring Cup, Erlenmeyer, Drop Pipette, Autoclave, Centrifuge, Niddle Ose, Oven, Bunsen Lamp, Filter Paper, Gauze, Newsprint, Funnel Vertical Burner Stirring Rod Inkubator The maTools and materials used for terials used in this study are: Beef Media NA(Nutrient Agar) Aquadest Bakteri *E. Coli*

Procedure of Research

1. Sterilization of Tools and Materials

The tools used are first washed and dried. Tools made of glass (test tube, Erlenmeyer, and measuring pipette) sre covered with cotton and then wrapped in newspaper, disc paper is inserted into one of the petri dishes and all petri dishes are wrapped in newspaper. All tools are sterilized using an oven at 160° C for 2 hours. Medium NA and aquadest contained in the erlenmeyer are clogged with cotton, sterilized using an autoclave at 121° C for 15 minutes. An oil needle is sterilized by annealing directly over the flame every time you use it.

2. Basic Media Preparation

Basic media is prepared by weighing 23 grams of Nutrient Agar (NA) media, then dissolved in 1,000 ml of distilled water in Erlenmeyer and heated using a hot plate until it boils and dissolves completely. Then sterilized in an autoclave at 121° C for 15 minutes.

Preparation of Test Bacteria

Escherichia coli from rejuvenated supply culture by transferring 1 or 2 oses planted on NA media, then put into a test tube and incubated for 24 hours at 37° C.

Making Test Bacterial Suspension

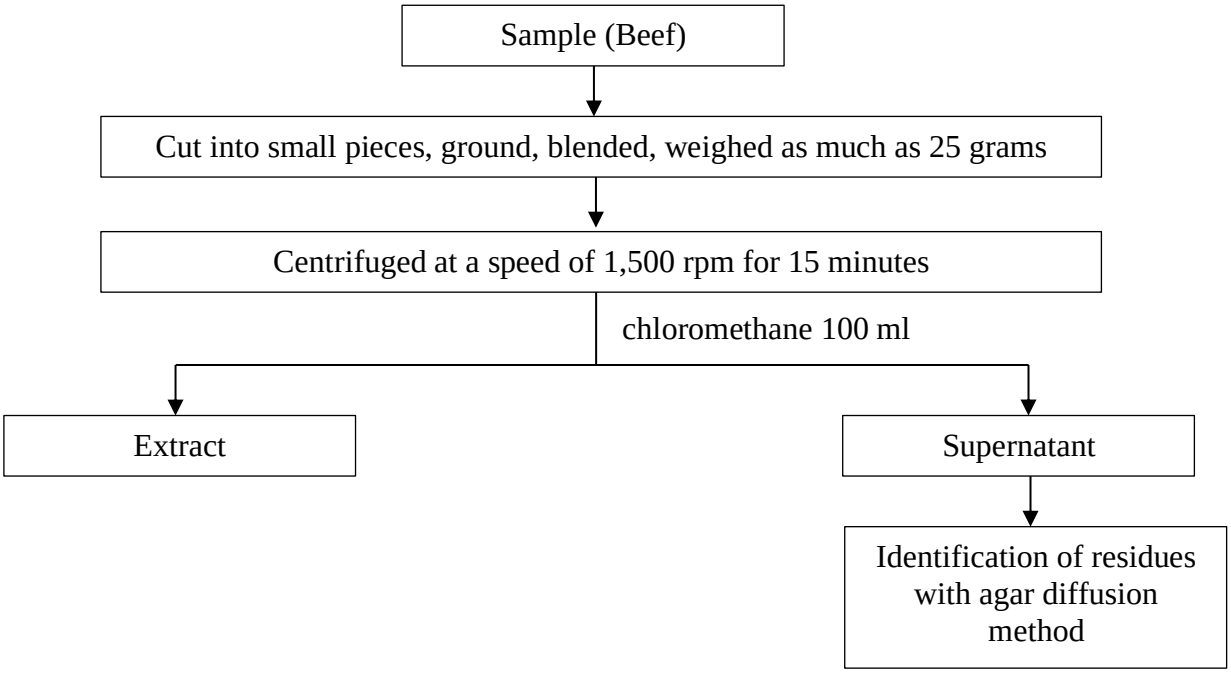
Rejuvenated test microbes were scratched as many as 3 to 4 scratches and then put into a test tube which contained sterile aquadest. Then homogenized by vortexing. Turbidity from the suspension was measured by UV-Vis spectrophotometer to obtain a suspension with 25% trasmitan at a wavelength of 580 nm.

Identification of Residues with Agar Diffusion Method

Samples (beef) are cut into small pieces, then ground until smooth and blended then weighed as much as 25 grams and added chloromethane solvent as much as 100 ml. Then centrifuged at a speed of 1,500 rpm for 15 minutes until a clear supernatant is obtained. The supernatant formed is taken. Inoculation of 0.1 ml pipette bacterial suspension is put into a petri dish that has been sterile and has been marked. Media NA which has been liquefied and cooled is poured into a petri dish, the petri dish is rotated slowly so that the media is well mixed with the bacterial suspension. Disc paper that has been dripped with a sample of 0.01 ml using tweezers and then put into a petri dish that has contained media and bacteria. Incubate at room temperature for 48 hours and observe the diameter of the inhibitory area formed (Rahmah, 2010).

1. Sampling and Sample Amount

The number of samples is determined non-randomly (purposively) from the traditional market which has been determined by researchers in each market and supermarket, namely 2 samples of beef for each traditional market in Gorontalo City and 1 sample for each supermarket. The total number of samples examined were 24 samples of beef. The weight of beef samples taken is a minimum of 300g.



Observed Variables

The variables observed were the existence or absence of antibiotic residues in beef sold at the central and supermarket markets of Gorontalo City. Testing of antibiotic residues using diffusion method to fit the observation by looking at changes on beef.

Measurement of Inhibition Zone

The inhibitory effect is determined based on the diameter of the inhibition zone (a clear zone or area without microbial growth) that forms. If an inhibition zone is present, the test sample is considered to contain antibiotic residues.

Conversely, if no inhibition zone is observed, the sample is deemed free of antibiotic residues. A sample is considered positive for antibiotic residues if the diameter of the inhibition zone formed is ≥2 mm (based on SNI No. 01-6366-2000). The sample is considered negative if the inhibition zone diameter is 0–2 mm. According to SNI No. 01-6366-2000, the Maximum Residue Limit (MRL) for amoxicillin is defined as an inhibition zone diameter of <13 mm.

Table 1. Standard Interpretation of Amoxicillin Inhibition Zone Diameter (CLSI, 2012)

Antibiotic	Disk Content (µg)	Standard Interpretation of Inhibition Zone Diameter (mm)
		S*
Amoxicillin	25	≥18

Notes: S = Susceptible, I = Intermediate, R = Resistant

Data Analysis

The data obtained were analyzed descriptively qualitatively by determining whether or not the results were obtained, explaining the object or subject under study according to what it was, with the aim of systematically describing, the facts, and the characteristics of the object under study precisely.

RESULTS AND DISCUSSION

The Existence of Antibiotic Residues

Testing of antibiotic residues in beef in this study was carried out by the agar diffusion test method in bioassay, in accordance with SNI 7424: 2008 which discusses the method of diffusion of antibiotic residues on meat, eggs and milk by bioassay. The diffusion test is a way to test to detect antibiotic residues qualitatively according to certain detection limits in meat, eggs and milk.

Bioassay is a test that uses microorganisms to detect active antibiotic compounds. The principle of testing, if there is an antibiotic residue, it inhibits growth. Microorganisms in agar media. Inhibition can be seen by the formation of a barrier area (clear zone) around the disc paper. The diameter of the inhibitory area shows the concentration of antibiotic residues (Pikkemaat *et al*. 2009). The advantage of antibiotic residue testing with screening test method is bioassay, namely: (1) easy to use and handle, (2) costs are not too expensive, (3) the processing time is short and fast, (4) allows automation, (5) has good sensitivity and specificity, (6) detection capability (CCβ) with probability error (β) <5% (Reig & Toldra 2008). The diffusion test aims to prove the presence of antibiotic residues in beef meat and liver marketed in Gorontalo City and its surroundings. Detection results of antibiotic residues in each sample of beef meat and liver from slauhterhouse (TPH) in Gorontalo Ciity are presented in Table 1.

Use of antibiotics as treatment or therapy or as a feed additive livestock, can increase production so it can catch up desired goal of Breeder. But then again antibiotics can be used cause something problems when giving irregular antibiotics, where may cause residue in the network and animal organs. This residue dangerous for health society as consumers like cause allergic reactions, resistance reaction due to consuming food that has residues over a long period of timAntibiotics have been widely used in livestock farming to improve productivity and animal health. However, inappropriate practices such as overdosage, failure to comply with withdrawal periods, or misuse of certain types of antibiotics can result in antibiotic residues remaining in animal tissues such as meat and liver, World Health Organization. (2020). These residues can enter the human food chain and pose various health risks Codex Alimentarius. (2019).

The average diameter of the inhibitory zone formed on NA base media indicates the presence of antibiotic residues. When compared with control doses, the average size of the beef and beef liver inhibition zones of the 10 cows used in the study turned out to be 50% of the inhibition zone exceeding the maximum residual limit set by SNI 2000 (0.1 ppm) which is greater than 10 ppm.

Antibiotic residues in beef and cattle liver result from improper administration of antibiotics. Antibiotics that are not fully metabolized in the animal's body tend to accumulate, particularly in organs such as the liver, which serves as the central site of metabolism (Ali, M., et al., 2018). If animals are slaughtered before the drug withdrawal period is complete, these residues can enter the human body through the consumption of meat or liver.

One of the most significant risks of consuming antibiotic residues is the increase in antibiotic resistance. Chronic exposure to low doses of residues can promote the selection of antibiotic-resistant bacteria, making bacterial infections more difficult to treat (Ventola, C. L., 2015; Laxminarayan, R., et al., 2013). Antibiotics in food may also disrupt the balance of the human gut microbiota, which is linked to various health

disorders such as diarrhea, inflammation, and metabolic diseases (Nicholson, J. K., et al., 2012). Furthermore, the consumption of beef or liver containing antibiotic residues may trigger allergic reactions, including skin rashes, respiratory issues, and severe cases of anaphylaxis (Tang, C., 2017). Some antibiotics used in animals have carcinogenic or toxic potential if accumulated in the human body. This is particularly concerning for individuals sensitive to certain antibiotics, such as penicillin or sulfonamides. Although research on this subject remains limited, these risks should not be overlooked (European Food Safety Authority, 2018).

Tabel 2. Average Diameter of Inhibitory Zones in Beef and Beef Liver Samples

Types of Samples	Sampling Location	Average Diameter of Inhibitory Zones (mm)		
		Samples	Positive Control	Negative Control
Beef	A.1	12.68	12.28	0
	A.2	9.95	12.28	0
	B.1	8.43	5.03	0
	B.2	7.03	5.03	0
	C.1	8.12	7.35	0
	C.2	8.44	8.02	0
	D.1	7.69	11.38	0
	D.2	6.42	0.00	0
	E.1	8.11	9.94	0
	E.2	9.86	9.09	0
	F.1	6.91	3.00	0
	F.2	7.28	5.08	0
	G	0.00	6.34	0
	H	0.00	6.34	0
Beef Liver	A.1	11.37	6.74	0
	A.2	0.00	*6.74*	0
	B.1	0.00	9.09	0
	B.2	0.00	9.09	0
	C.1	8.12	7.51	0
	C.2	8.44	7.51	0
	D.1	5.39	13.15	0
	D.2	7.12	10.99	0
	E.1	9.62	7.43	0
	E.2	7.2	6.87	0
	F.1	6.91	10.72	0
	F.2	6.63	10.72	0
	G	0.00	0.00	0
	H	0.00	0.00	0

The use of positive control is as a comparison of the diameter of the inhibitory area. This antibiotic has a large inhibitory area of more than 10 ppm. Pada kontrol positif digunakan Streptomycin, dimana Streptomycin ini merupakan antibiotik kelas penisilin (antibiotik beta-laktam). Obat ini diketahui memiliki spektrum antibiotik yang luas terhadap bakteri Gram positif dan Gram negatif pada manusia maupun hewan (Kaur *et al*., 2011). Antibiotik ini memiliki daerah hambat yang luas yaitu lebih dari 16mm. Penghambatan dapat dilihat dengan terbentuknya daerah hambatan (zona bening) di sekitar kertas cakram. Besarnya diameter daerah hambat menunjukkan konsentrasi residu antibiotik (Pikkemaat *et al.* 2009).

Selection of dichloromethane solvent in sample extraction because this solvent can attract antibiotics perfectly even though it is bound to protein and has a low boiling point of 4O° C. The use of negative control with extraction pellets aims to eliminate the

influence of solvents on the formation of clear areas around the disc with diameter of the inhibitory area 0. Therefore, disc paper must be dried before placing it on the test media. Antibiotic residues were identified microbiologically based on the diameter of the inhibitory area given by antibiotics contained in the sample against bacteria. The extracted antibiotics will diffuse through disc paper into the media that has been implanted with bacteria.

The results at each time of collection showed that most of the samples contained antibiotic residues with a weak category, ie an average diameter of 12.3 mm. According to Greewwood (1995), the classification of the inhibitory response to the growth of bacteria that have a bright zone diameter of more than 20 mm, the growth inhibition response is categorized as strong, bright zone diameter 16-20 mm categorized as moderate, bright zone diameter 10-15 mm categorized as weak and diameter of bright zone 0 categorized there is no inhibitory response to growth.

In sampling from slaughterhouses in Gorontalo City, samples of beef liver from TPH Andalas gave a diameter of inhibition area of 11.37 mm. Beef liver samples from Padebuolo TPH gave an inhibition zone of 8.12 mm, while beef liver samples taken from TPH Moudu gave a diameter of inhibition area of 15.39 mm and 10.99 mm.

In the second sampling, beef samples in Gorontalo City, for beef samples from TPH Andalas gave a diameter of 12.68 mm and 9.95 mm in inhibition zone. Beef samples from TPH Padebuolo gave inhibition zone of 9.12 mm and 12.44 mm, while beef samples taken from TPH Moudu gave a diameter of inhibition zone of 11.69 mm and 8.42 mm. As for beef samples from TPH Biau gave a diameter of inhibition zone of 9.86 mm and 8.11 mm.

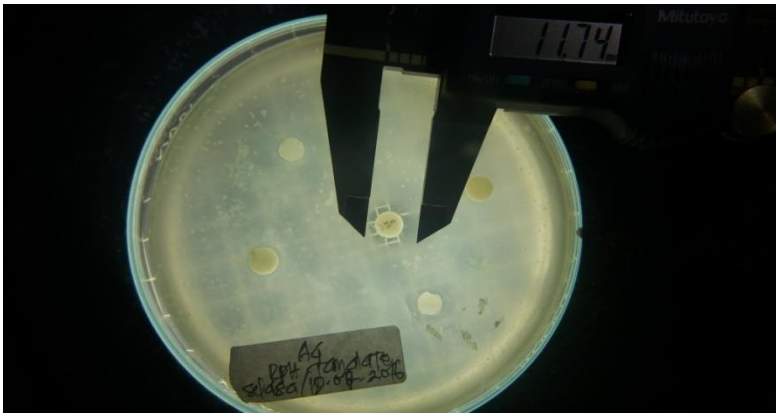


Figure 1. Observation image

In the identification of antibiotic residues in beef and beef liver samples, most of the samples produced antibiotic residues with the diameter of the inhibitory region formed in the sample, this was because the stockmen gave antibiotics to livestock for treatment, reducing the risk of death and inhibiting the spread of disease to other animals.

Based on observations made at 8 sampling locations, it was shown that 70% of beef thigh meat was positive or contained antibiotic residues so that it was not suitable for consumption by the people of Gorontalo City. The most commonly detected antibiotics in meat are streptomycin (including ampicillin), tetracycline (including chlortetracycline and oxytetracycline), sulfonamides (including sulfadimethok-sin, sulfamethazine and sulfamethoxazole), neomycin, gentamicin, and penicillin (Phillips *et al.*, 2004).

The irregular administration of antibiotics can lead to residues in the tissues or organs of animals. These residues may pose health risks to humans who consume them. Such risks include allergic reactions that can increase sensitivity and resistance

reactions resulting from prolonged consumption of low concentrations (Wijaya, 2011). The potential health hazards of antibiotic residues have prompted the establishment of regulations or limits on the permissible levels of antibiotic residues. In Indonesia, the Maximum Residue Limit (MRL) values for antibiotics in livestock products are regulated according to the Indonesian National Standard (SNI, 2001). The SNI specifies a list of antibiotics and their metabolites, as well as the MRL values for each livestock product (meat, milk, and eggs) that are considered safe for human consumption. These regulations allow for the assessment of health impacts on humans. If the residue levels are below the MRL, the product is deemed safe for consumption. However, if the levels exceed the MRL, the product is considered hazardous to human health.

Meat, milk, and eggs, as food sources, can be contaminated not only by microorganisms but also by various drugs, chemicals, and toxins, either during the pre-production process or during the production process itself (Bahri et al., 2005). Antibiotic residues are compounds and/or their metabolites found in the tissues of animal products, including residues resulting from the breakdown of these substances. All methods of antibiotic administration can lead to residues in animal-based food products such as meat, milk, and eggs (Phillips et al., 2004). At certain levels, antibiotic residues pose risks to human health. According to the technical guidelines of the Indonesian National Standard (SNI) number 01-6366-2000 on the Maximum Limit of Microbial Contamination and Maximum Residue Limits in Animal-Based Food, drug or chemical residues refer to the accumulation of drugs or chemicals and/or their metabolites in the tissues or organs of animals following their use for prevention, treatment, or as feed additives for growth promotion. The results of the study in Jabotabek conducted by Rusiana (2003), stated that out of 80 samples of broiler chickens, 85% of meat and 37% of liver were threatened with the residues of antibiotics tylosin, penicillin, oxcytetracycline and kanamycin. In addition, studies conducted in Semarang City from 47 samples taken, they are 33 samples from traditional markets and 14 samples from modern markets, proved that 3 samples from traditional markets positively contained residues of Oxytetracycline, they are 0.869 ppm (Pasar Johar), 0.271 (Sampangan Market) and 0.366 (Dammar Market) which exceeds the Maximum Residue Limit (BMR) which is more than 0.1 ppm (Faizah, 2011).

In addition, to find out the presence of antibiotic residues can be tested chemically and biologically. Chemical tests that can be used include gas-chromatography, spectrophotometry and electrophoresis. This method is used more to determine the characterization of antibiotics. Meanwhile, biological tests that can be used include yogurt tests, so that the test loch, so that the pilot test is a test, and a reduction test. The principle of these tests is using bacteria that have known sensitivity to certain antibiotics as an indicator. To avoid the occurrence of antibiotic residues in milk in terms of public health, several precautions can be taken, including routine checks, and dairy cows that are on antibiotic therapy should be under the supervision of a veterinarian, especially to determine the dose and frequency of administration. In terms of industrial scale, milk that is known to contain antibiotic residues can be used as processed milk such as milk powder, condensed milk and sterile milk. According to Bahri (2008), controlling biological diseases by avoiding excessive use of chemicals or dangerous drugs can also be done to avoid the occurrence of antibiotic contamination.

In addition, the quality control of circulating feeds needs to be improved, including animal medicines mixed in livestock rations. Likewise the use of veterinary drugs given directly to livestock needs to be monitored, both for treatment and prevention. Supervision at the same

time followed by controlling the use of veterinary drugs in the field. Meanwhile, according to Martel *et al* (2006), the prevention of antibiotic contamination can also be done through the use of veterinary drugs that must be in accordance with the applicable regulations by taking into account, among others, downtime and dose suitability. In addition, the storage of veterinary medicines must also follow the instructions.

Preventive and control measures for antibiotic residues include antibiotic type policy in veterinary medicine (not using antibiotics used by humans for animals), monitoring of antibiotic use, application of good practices along the food chain (from farm to table), application of food safety guarantees in business units of animal-derived foods, as well as monitoring and surveillance of antibiotic residues in animal-derived foods (Yuningsih, 2004).

CONCLUSIONS

a. Based on laboratory tests of 34 samples that were analyzed qualitatively using methanol solution showed that all antibody residues were identified, although the inhibition zones shown were different.
b. Residues in 3 beef samples showed a threshold.
c. Residues in 4 beef liver samples showed a threshold.

Suggestions

a. Food and Drug Supervisory Agency (BPOM), Gorontalo Provincial Health Office and Veterinary Public Health Supervisor at the Directorate General of Livestock and Animal Health which is a government device that technically deals with food safety issues and pays attention to public health, so that it can always monitor beef and beef liver sold in Gorontalo city so that the security is maintained, and always provide knowledge about antibiotic residues, and the dangers to producers, traders and the public as consumers.
b. People are suggested to remain vigilant and more careful about choosing safe and healthy meat consumption.
c. Further research is needed for quantitative tests on chemical contamination of antibiotic residues in beef and beef liver in Gorontalo City.

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