

Residues Of Antibiotic In Chicken Meat Imported From Brazil And United States Of America Through Quarantine Stations In Dili, Timor Leste

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Abstract: The aims of this research are to investigate some residue of antibiotics in chicken meat imported from Brazil and America over the quarantine station in Dili Timor Leste at 2016. The sample used was chicken meat imported from Brazil and America with totally of 40 samples. The research was done at Nasional Veterinary Laboratorium Biosafety level 2 in Dili Timor Leste, using Enzyme-Linked Immunosorbent Assay (ELISA) competitive method, in accordance with the standard kit Maxsignal Bioscientific crop, which resulted in optical density OD value with the concentration of fluoroquinolone residue and tetracyclines residue in chicken meat imported from Brasil and America to Timor Leste. The research showed that 9 (36%) of 40 samples from America and Brazill contained positive fluoroquinolone residues which all came from Brazil. The results of the examination of tetracyclines residues, were found in 1 (6.6%) of 15 samples positive Tetracilin residues from America while from Brazil are found in 8 (32%) of 25 samples positive Tetracilin residues. Antibiotic residues in chicken meat imported from Brazil and America which entered through the quarantine station in Dili Timor Leste still contain low concentrations of antibiotic residues between 0.1 ng/ml to 0.25 ng/ml for fluoroquinolone antibiotic and concentration of ng/ml 0.05 to 0.1 ng/ml for tetracyclines antibiotic.

Keywords: Antibiotic Residues, Chicken Meat, Brazil And United States, ELISA

Date of Submission: 14-06-2019

Date of acceptance: 29-06-2019

I. Introduction

Chicken meat is one of the animal products which is consumed, and it's gradually increasing from time to time in Timor Leste. The needs of meat have especially not been able poultry meat fulfilled in Timor Leste. This condition leads the government to import poultry frozen meat from Brazil and America of over 180,000 kg/month of regularly through the quarantine station in Dili Timor Leste (Timor Leste Quarantine Report, 2009).

The food safety issue is a priority of WHO, FAO, OIE and Codex Alimentarius which play a role in global public health. The country is obliged to provide safe food for the public (FAO / WHO, 2010). The chicken meat should be a special concern to the Timor Leste government, especially on the food security program and food safety. Poultry meat business is still having difficulty in controlling the use of antibiotics as growth promoters, prevention, and treatment of disease. Antibiotics are still the main option for poultry broilers in many countries. It is still very dependent on the use of antibiotics, either as growth promoters, prevention, and treatment from bacterial diseases.

Antibiotic residues on the animal product such as beef, pork and chicken meat is still a problem in some countries such as in Vietnam. It reported the existence of residual amounting to 150 - 450 mg/kg or 17.5 % discovery of the total sample tested (Yamaguchi et al 2015). Buket, et al (2013) in Turkey reported that (45.7 %) of local chicken meat containing residue of quinolone. It also founded residues of tetracycline 8 % in chicken meat in Vietnam (Nhiem at all, 2006) It also reported that residues of tetracycline antibiotic 18 % in chicken meat muscle in India (Ramakant 2014). Brazil is one with the best poultry production but still faces many obstacles, especially a disease such as salmonellosis and colibacillosis (Patricia, 2013). Moreover, even the American also face antibiotic residues problem in a certain case.

Uncontrolled use of antibiotics regarding the rules would be a very serious problem for consumers and the environment. Antibiotics in the tissues, especially muscle meat will persist as residue within 3 - 4 days before it is gradually washed way (Castañon, 2007). Several studies of these cases have been reported (Cabelo 2006; Ezenduka et al, 2014; Ramakant et al, 2014). The impact of antibiotics residues would be very serious for the consumers since people who consume that is food contaminated with antibiotics residues in the long term will result in antibiotic resistance and hypersensitivity reactions reaching from slight to severe (Phillips et al,

2004; Crawford, 1994). Some of developing countries for economic reasons do not have policies and carry out minimal efforts to control the use and content of antibiotic residues in the livestock and food from the animal, including Timor Leste (MRL) (EU, 2006). Under these conditions, it is extremely urgent to conduct an examination of antibiotic residues in food products of animal origin, especially chicken meat imported from abroad that come to Timor Leste, as for safeguard consumers.

II. Research Method

This research is based on observation with cross-sectional study for chicken meat samples which taken from four supermarkets whom imported chicken meat from Brazil and the United States of America to Timor Leste. Samples collected are proportionally by taking every chicken meat from musculus pectoralis breast and thigh femoralis musculus.

Location and time research. The research was conducted at the National Veterinary Diagnostic Laboratory in Dili, Timor Leste. The study was conducted in two stages, the sample of chicken meat from four supermarkets in Dili capital of Timor Leste who imports chicken meat from Brazil and America which controlled by the quarantine station in Dili, Timor Leste from January to February 2016. Furthermore, the second stage will be carried out using the ELISA test kit.

This study sources of data which obtained from tissue samples of chicken meat imported from Brazil and America to Timor Leste. The main sample of this study came from every company that imported chicken meat. Samples are taken from eight types of chicken meat. Five types from Brazil are (Super Frango, Sera, Sadia, Ferdigao, Farangsul) and 3 types from America are (Tyson, Mountaire and Golden phones). There is a total of 40 samples used in two groups between Brazil and America. The 25 samples from Brazil and 15 samples from America. The eight kinds of samples as mentioned above so that each type take 5 samples in accordance with duplication ELISA kits. Materials used it is, ELISA test kit, OXYTET extraction buffer, balance TET buffer, wash solution, meat extraction buffer, stop buffer, TMB substrate, imported chicken meat, negative and positive control reagents, Elisa KIT which consists of a strip of 96 well. Research equipment. The equipment used for ELISA test is serum tubes, centrifuge, micropipette, multichannel pipette, 9 ml tubes, boards, mortar, gloves, masks, plastic, refrigerate, and ELISA Reader machine.

Samples preparation for antibiotic Tetracycline test: Preparation of 1x OXYTET Buffer, mix 1 volume of 5 x OXYTET extraction buffer with 4 volumes of distilled water. Preparation of 1x TET Buffer, mix 1 volume of 10 x TET sample diluted with 9 volumes of distilled water. Preparation of 1x TET balance buffer Combine 300 µL of TET Balance Buffer concentrate and 29.7 ml of 1x TET sample diluent. Preparation of 1x feed Extraction Buffer mix 1 volume of 30 x feed Extraction Buffer with 29 volumes of distilled water to 1 g of homogenized sample add 3 ml of 1 OXYTET Extraction Buffer and 1 ml n-hexane. Vortex for 10 minutes in a multi-tube vortexer or shake 30 minutes on a shake. Centrifuge for 10 minute at 4,000 x g. Transfer 200 µL of the supernatant to a new tube containing 300 µL 1 X TET sample balance Buffer. Vortex for 1 minute, use 75 µL per well in the assay. Elisa method applied is Maxsignal Bioscientific methods.

Samples Preparation for antibiotic fluoroquinolone test. Prepare 1 x Extraction buffer with a mix of 1 vol 10 x Extraction buffer with 9 volume of distilled water. Prepare 35% Methanol/Extraction buffer, mixed 6.5 vol for 1 x sample extraction buffer with 3.5 volumes of 100% methanol. 1 x Extraction buffer with 3.5 volumes of 100% methanol. Remove fat from the sample homogenizes the sample with a suitable mixed. Weight out 1.0 g gram of chicken meat and homogenizer sample and add 4 ml of 35% Methanol/sample Extraction buffer and 50 µL of meat extraction buffer I Vortex 3 minutes with maximum speed. Centrifuge for 10 minutes at 4, 000 rpm temperature (20-25 Co). Transfer 1 ml of supernatant to a serum tube, add 50 µL of meat extraction buffer II and vortex 30 seconds. Centrifuge for 10 minutes with 4000 x g rpm at room temperature (20-25 Co). Use 50 µL of supernatant for the assay. Data analysis. ELISA test result with antibiotics tetracycline and fluoroquinolones at residues data of the meat samples presented by descriptively.

III. Result And Discussion

The testing results of fluoroquinolone residues taken form 40 samples of chicken meat imported from Brazil and the United States of America into Timor Leste can be seen in Table 1.1. From the table give information that 9 sample chicken meat from Brazil contain fluoroquinolone residue. There are no samples from America identified contain fluoroquinolones residue

Table 1.1 The results of Fluorouinolone residues in chicken meat From Brazil and America

Originally Samples	number of Samples	Residues Fluoroquinolone
America	15	0
Brazil	25	9

The testing results of tetracycline residues taken from 40 samples of chicken meat imported from Brazil and the United States of America into Timor Leste can be seen in Table 1.2. The percentage of ELISA test for fluoroquinolone residues were positive detected can see in Table 1.3. The percentage of ELISA test for tetracycline antibiotic residues were positively detected can be seen in Table 1.4. the result of the ELISA test to detect Antibiotics residue shows that none of the Chicken meat samples from America detected contained residues fluoroquinolone residue but there 1 chicken meat sample detected contains tetracycline residue. Different results are found in chicken meat samples from Brazil. There are some positive samples containing good antibiotic residues both fluoroquinolone or tetracycline

Table 1.2.The results of Tetracycline residues in chicken meat From Brazil and America

Originally Samples	number of Samples	Residues tetrasiklin
America	15	1
Brazil	25	8

Table 1.3 The results of ELISA for Fluorouinolone residues in chicken meat 5 type Brazil and 3 type from America

Origin Company	Number of Samples	+ / _	Percentage
America (Company Initial)			
T	5	0	0 %
M	5	0	0 %
GP	5	0	0 %
Brazil (Company Initial)			
SF	5	3	12 %
SA	5	1	4 %
SE	5	0	0 %
FO	5	0	0%
FR	5	5	20 %
Total	40	9	36 %

Table 1.4 The results of ELISA for Tetracycline residues in chicken meat 5 type Brazil and 3 type from America

Origin Company	Number of Samples	+ / -	Percentage
America	15	-	6 %
T	5	-	-
M	5	-	-
GP	5	1	6 %
Brazil	25	-	32 %
SF	5	1	4 %
SA	5	3	12 %
SE	5	3	12 %
FO	5	1	4 %
FR	5		
Total	40	9	36%

Based on ELISA test results in this research, there are 40 samples that taken from Brazil and America which took from 4 supermarkets that imported chicken meat from Brazil and America which entered through the quarantine station in Dili, Timor Leste. It was randomly taken 15 samples of American and 25 samples from Brazil. Samples test taken from meat muscular pectoralis (chest) and muscle femoralis (thigh) to detect fluoroquinolone and tetracycline in Table 1.1 and Table 1.3. seen a total of 9 samples (3.6 %) that had detected positive fluoroquinolone residue and 31 samples (77.5 %) was not detected positive. There are 9 samples which detected positive fluoroquinolone, mostly chicken meat imported from Brazil, while American was not found positive. These five types of chicken meat imported from Brazil with a total of 25 samples which were ELISA tested for the fluoroquinolone residues which detected positive (36 %). The most commonly found fluoroquinolone residues positive has come from FR 5 samples (20 %), SF 3 samples (12 %) and SA 1 sample (4 %).

ELISA test for Tetracycline residues from 40 samples of chicken meat that come from Brazilian and America are showed in Table 1.2 and Table 1.4 that there are also 9 samples chicken meat from Brazil and

America which had detected positive (3.6 %) tetracycline and 32 samples (80 %) which had not any detected tetracycline residue negative. Nine samples detected positive tetracycline residue which was mostly chicken meat imported from Brazil, while the chicken meat from the United States, found one sample positive. ELISA test for Tetracycline residue also used 25 samples from Brazil and 15 from America, the total of 40 samples, Brazilian chicken meat were detected positive (32 %) and are most commonly found in chicken meat variety of SA and SE, each 3 samples (12 %) positive, SF , FR each 1 sample (4 %) positive and one sample positive from America is GP (6 %). This research corresponds to research in Sudan which shows that antibiotic residues detected in musculus meat reached 29.2 % (Hind at all, 2014), with 45.7 % positive quinolone antibiotic residues in meat of local chicken (Buket, at al. 2013). According to (Phillips et al, 2004). fluoroquinolones and tetracyclines antibiotic are the drugs commonly used in livestock to prevent and treat diseases caused by Salmonella, E coli, Streptococcus, Staphylococcus. The number of residual drugs or chemical in the tissue of animals as a result of treatment or prevention of animal diseases. According to (Donkor et al., 2011), found that the presence of residues in chicken meat can be caused by several factors, among others, are farmers do not understand about the future of stopping (withdrawal time), the livestock was butchered before the antibiotics stopped circulating in animal body exhausted and yet completely excreted.

The concentration of fluoroquinolone residue in Brazilian and American chicken meat, based on the ELISA test in table 1.5 residual concentration of detected was 0.1 ng/ml, 0.25 ng/ml and there also one sample was detected more than 5 ng/ml. From the test of fluoroquinolone shows that 7 samples detected concentrations of 0.1 ng/ml, 1 sample contains 0.25 ng/ml and 1 sample exceeds the limit of 5 ng/ml. In accordance with maxsignal Bio Scientific (2015) for sensitive detection limit or limit detection maximum for meat is 5 ng/ml for fluoroquinolone. This research shows that the optical density (OD) ELISA test results of samples found 8 samples that are below the limit of detection. But one sample exceeds the limit is likely to occur because of contamination during the test or production time. This is according to research conducted by (Kim at all, 2015) in Vietnam which also found Fluoroquinolone concentration of 0.5 ppm is still categorized as the normal range but the exceeds to the maximum limit because of contamination. In the European Union, maximum fluoroquinolone residue in meat is 0.1 ppm (Ramakant, 2014). Also found fluoroquinolone residue levels in samples of local chicken meat ranging 0.45 ± 30.81 pg/kg (Buket, at al. 2013).

In the ELISA test for tetracycline showed also that the concentration of tetracycline in Table 1.4 tetracycline antibiotic residues concentrations detected was 0.05 ng/ml, and 0.15 ng / ml. In accordance with maxsignal Bio scientific (2015) for sensitive detection limit or limit detection maximum for meat is 1.6 ng/ml for tetracycline. This research shows that the optical density (OD) ELISA test results of samples found 3 samples detected concentrations of 0.05 ng/ml and 6 samples contain concentrations 0.15 ng/ml. In according to the maximum residue limited (MRL) and technical guidelines SNI 01-6366-2000 number of contaminant and residue limits of tetracycline in meat is 0.1 ppm, and compared with other countries such as Europe, 0.1 ppm, Canada 0.2 ppm, Australia 2 ppm and America 0.1ppm (Ramakant, et al, 2014). class of tetracycline is widely used in animal farming for treatment and as an animal food additive. Irregular use of antibiotics can result in residues in meat tissues and can cause allergic reactions and resistance to bacteria. The cause of their residues in meat is due to lack of education about the good and right use of antibiotics on the farm. Another factor is possible due to economic pressures. The farmer cut their animal to meet their needs. Whereas if the farmer know about knowledge and how to use antibiotics (Murdiati, 1999). The use of antibiotics for animals in developing countries are becoming a common problem that is increasing due to the occurrence of many cases of infectious disease (Kim, et al, 2015). The existence of fluoroquinolone and tetracycline residues in chicken meat because the farmers understand about management using the antibiotics in accordance with the time stopping of the drug (withdrawal time) and in the United States they obey the rules and know to use instructions veterinary medicines in animal husbandry practices (Ramakant, 2014). In the United States, food drug administration (FDA) regularly conduct monitoring, surveillance to detect a controlled sample of animals from a slaughterhouse (Dan, 2003)

IV. Conclusion

This research concluded that fluoroquinolone and tetracycline residues are still detected in chicken meat that is imported from Brazil in low concentrations but did not find antibiotic residues in chicken meat imported from the United States. Concentration of fluoroquinolone are found at between 0.1 ng/ml and 0.25 ng /ml whereas concentrations of tetracycline are detected; 0.05 ng / ml and 0.15 ng/ ml. Further studies should be done to a finding residues contained in chicken meat from Brazil and other countries that enter Timor Leste.

Acknowledgements

The authors of this research would like to thank the Ministry of finance and section human resources development in Timor Leste for funding this Research.

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Hapsari Mahatmi" Residues Of Antibiotic In Chicken Meat Imported From Brazil And United States Of America Through Quarantine Stations In Dili, Timor Leste" *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)* 12.6 (2019): PP- 39-43