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HPLC- Ms/Ms-based Estimation of Oxytetracycline, Ciprofloxacin and Gentamicin Residues and Assessment of the Effect of Boiling and Frying on their Reduction in Table Eggs

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Abstract

A total of 180 eggs (20 batches each represented by 3 eggs) of the farm, balady, and organic egg samples were collected in October 2021 from markets in Ismailia governorate, Egypt, and examined for detection of the oxytetracycline, ciprofloxacin, and gentamicin residues by using HPLC-Ms/Ms. The farm and balady eggs contained 20% with mean values of 69.95±1.78 and 43.6±2.65 ppb, respectively, and all of them were below the permissible limits and accepted. They contained 20% and 60% with mean values of 26±0.21 and 39.58±5.58 ppb, respectively, and all positive samples were unaccepted. Both egg types were free from gentamicin residues, meanwhile, all organic eggs were clear from the three antibiotic residues under investigation. The study also revealed the distribution of oxytetracycline and ciprofloxacin in albumin and yolk after oral administration and their residues in the 6th-day eggs were (750 and 500 ppb) and (500 and 319 ppb), respectively. The effect of boiling for 15 minutes on oxytetracycline and ciprofloxacin residues in albumin and yolk was also investigated and showed a reduction% of (88 and 90) and (90.5 and 87.5), respectively. Meanwhile, the frying of whole eggs for 5 minutes reduced their concentration by 94.3% and 94.5%, respectively. In conclusion, the antibiotic residues in edible eggs are a serious risk that should be monitored, and it's significant to highlight the importance of efficient boiling and frying of eggs in reducing these residues and relieving their hazards to the consumer's health.

KEYWORDS
Oxytetracycline, Ciprofloxacin, Gentamicin, Antibiotic residues, Egg

INTRODUCTION

Table egg is considered a complete, economic with high nutritive values food that is suitable for a healthy diet, infants, and patients. The predictable worldwide egg production will be 85 and 91 million tons by 2030 and 2050, respectively (FAO, 2018). Eggs contain high-quality and digestible protein which includes essential amino acids. They also contain essential fatty acids such as lecithin and choline which are important for the nervous system and brain development. They are rich in fat-soluble vitamins (A, D, E, and K) and other vitamins such as B1, B2, B12, and folic acid but poor in vitamin C. Eggs are a good source of minerals such as phosphorus, iron, calcium, copper, iodine, magnesium, potassium, sodium, zinc, chloride, and sulfur (Gbylik- Sikorska *et al*., 2021).

Antibiotics are compounds that are natural, semi-synthetic, or synthetic and possess antimicrobial properties. They are used in poultry production for therapeutic or prophylactic purposes and may also enhance growth and egg production and allow the consumer to purchase eggs at a reasonable price (Nagiub *et al*., 2021). Their use is quite common in developing countries and oxytetracycline, ciprofloxacin, and gentamicin are examples of the predominately used antibiotics (Sara *et al*., 2021).

The administration of antibiotics may result in residue accumulation in meat and eggs causing public health hazards including allergic manifestation, gastrointestinal disorders, development of antibiotic-resistant bacteria, and damaging the normal microflora of the digestive tract. Thus, the withdrawal period must be observed until no residues are discharged in the edible eggs (Al-Aboudi *et al*., 2021).

MATERIALS AND METHODS

Egg sampling

A total of 180 eggs from 20 batches each represented by 3 eggs of a farm, balady, and organic egg samples were collected in October 2021 from local markets in Ismailia governorate, Egypt. All eggs were transported to the food control unit, Animal health research institute, Dokki in Giza for analysis.

Residues of Oxytetracycline and ciprofloxacin in egg components after oral administration by laying hens (Billah *et al*., 2015)

Three groups of native balady laying hens (2 groups for the experiment and a control group; 6 hens for each) were 6 months of age and 1.750-1.800 kg in weight. Hens were fed on an antibiotic-free ration and kept for 2 weeks before treatment. The 1st group received 40 mg/kg bw oxytetracycline and the 2nd group administrated 10 mg/kg bw ciprofloxacin, the dose given once

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daily orally for 5 days. The 3rd group was used as a control group. The eggs of the 6th day were subjected to the estimation of antibiotic residues in albumin and yolk separately. The eggs on the 7th day were exposed to boiling and frying processes.

Preparation of boiled eggs samples according to Al-Aboudi et al. (2021)

Examined egg samples were boiled at 100°C for 15 minutes and the albumin and yolk were collected separately, blended, and 3gm of each were weighed and transferred into a centrifuge tube. 5 ml EDTA solution (pH 4) was added to the tube and the mixture was homogenized by using T25 Digital Ultra Turrac Homogenizes, and become ready for the extraction procedures.

Preparation of fried egg samples by frying

Fried eggs were prepared for for 5 minutes according to Mosha (2017).

Analysis of antibiotic residues

Methods of analysis

The samples were analyzed as a procedure described by Darko *et al*. (2015) using high-performance liquid chromatography (HPLC–MS/MS Agilent 1260) operating at 270 nm wavelength with a 50 nm bandwidth.

Chemical and reagents

Acetonitrile (MeCN), methanol (MeOH), hexane, potassium dihydrogen phosphate, and sodium dihydrogen phosphate were of HPLC grade. Moreover, the standards of oxytetracycline, ciprofloxacin, and gentamicin antibiotics were required.

Standard solution

Stock solutions (100 mg/ml) of each 3 antibiotics standards were prepared by dilution with MeCN: MeOH (30:70) solution. Working solutions of standards (100 ug/ml-10 ug/ml) were freshly prepared through dilution of an appropriate aliquot of the stock solutions with MeCN: MeOH (30:70) and stored in the refrigerator. The blank standard was the dilution solution of MeCN: MeOH (30:70) without adding the drug. All solutions prepared for HPLC were filtered through a 0.45-um nylon filter before usage.

Sample preparation

Whole eggs or the egg components were extracted by homogenized in an ultra-turra T25 basic homogenizer for 1 minute at 7000 rpm. A 2 ml aliquot of homogenized egg sample and 20 ml of MeCN were blended at high speed in homogenized for 5 minutes and the supernatant was filtered through Whatman 0.45 um filter paper. Two additional homogenizations with MeCN and filtration were performed. The combined filtrate was transferred into a separatory funnel containing a 30 ml portion of MeCN - saturated hexane and the mixture was shaken for 5 min. The MeCN layer was collected into a concentration bottle and reduced to dryness under a vacuum.

Sample clean-up

Extracts were cleaned up using C18 sorbent columns. The absorbent was pre-conditioned by sequential washing with 10 ml of methanol and 10 ml of 0.05 M sodium dihydrogen phosphate solution. The semi-dry residues coming from the egg extraction were re-dissolved in 10 ml of sod. dihydrogen phosphate and applied in the SPE cartridge. The eluate was collected into a 15 ml polypropylene centrifuge tube and evaporated to dryness at 40°C. The dry matter was reconstituted with 1 ml of MeCN/H_2O(3/7 v/v) and spiked with 0.5 ml of MeCN-saturated hexane. The resulting solutions were centrifuged at 3000 rpm for 5 minutes. The acetonitrile layer was collected and filtrated through a 0.45 um nylon membrane before HPLC analysis (Kao *et al*., 2001).

HPLC analysis

Five calibration standards were prepared from their working stock solutions by using MeCN/KH_2Po_4 buffer. Calibration standard solutions and reagent blanks for each antibiotic were HPLC and analyzed in the same way as the sample. The concentration of antibiotics residues in samples was determined by interpolation from a five-point calibration curve generated via measurement of the HPLC peak area, where the calibration curve of different standards was used to convert the samples' peak area to concentration.

Calculation

A standard curve of three examined antibiotic standard solutions (concentration versus peak area) was made and from the measured peak area of test samples, the antibiotic concentration was calculated by using the line equation as follows Y= MX, where: Y= peak area, X= antibiotic concentration (ppb), M= slope of curve intercept of Y.

Statistical analysis

Data were statistically analyzed by a one-way analysis of variance (ANOVA) using the SPSS Ver. 18 (Chicago, IL, USA). At a 95% confidence interval, the P-value was calculated and $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Eggs may incorporate antibiotic residues after illegal or extra-label use of the drug, failure to meet the withdrawal times, use of feed unintentionally cross-contaminated during feed mixing, and mislabeled use (Sara *et al*., 2021). In this study, the antibiotic residues were determined by Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) which is efficient due to its selectivity and sensitivity at low concentrations (Ji *et al*., 2021).

Oxytetracycline residues in Egg samples

Twenty percent of the examined farm and balady eggs contained oxytetracycline residues, as the results revealed in Table (1). Their values in farm eggs were 60.5-88 ppb with an average of 69.95±1.78 ppb, and in balady eggs were 31-69.5 ppb with a mean of 43.6±2.65 ppb. According to the Codex Alimentarius Commission (2012), all examined egg samples were below the recommended limit (200 ppb) and were accepted.

These findings agreed with the results reported by Yoshimura *et al*. (1991) and Darko *et al*. (2015). On the other hand, higher concentrations were stated by Furusawa (2001); Fath El-Bab (2012); Nagiub *et al*. (2021) and Sara *et al*. (2021). Nevertheless, Widiasih *et al*. (2019) and Kamali *et al*. (2020) reported lower oxy-

tetracycline residues. All examined organic egg samples had no oxytetracycline residues. Similar results were recorded by Dipeolu *et al*. (2005) in Japan, also Hafez *et al*. (2013) and Ubayd *et al*. (2021) in Egypt. However, the joint FAO/WHO Expert Committee on Food Additives (JECFA) stated that the acceptable daily intake (ADI) of tetracycline compounds is 0-0.03 mg/kg body weight (WHO, 2019), their consumption may lead to poor development or teratogenic effects in fetuses, staining of teeth in young children, hypersensitivity, gastrointestinal and renal disorders, and cytotoxic and Immuno-pathological effects (Idowu *et al*., 2010).

Ciprofloxacin Residues in Egg Samples

The ciprofloxacin residues were detected in 20% and 60% of examined farm and balady egg samples, respectively. The minimum and maximum values for the ciprofloxacin residues in farm eggs were 25- 28 ppb with a mean value of 26±0.21 ppb, while in balady eggs were 26.6- 60 ppb with a mean value of 39.58±5.58 ppb (Table 1). No ciprofloxacin residues were detected in the organic eggs. All samples containing ciprofloxacin residues were unaccepted according to the European Commission (EU, 2010) which declared that the presence of any ciprofloxacin residues in eggs is not allowable. These results are nearly like those stated by Huang *et al*. (2006). Nevertheless, Gorla *et al*. (1997) detected lower levels, while a ciprofloxacin higher concentration was reported by Fath El-Bab (2012) and Al-Aboudi *et al*. (2021). Treiber and Knaner-Berank (2021) failed to detect any ciprofloxacin residues in examined egg samples. Consumption of food containing ciprofloxacin residues may lead to chondrogenic effects and the production of drug-resistant bacteria (Al-Aboudi *et al*., 2021).

Gentamicin Residues in Egg Samples

Gentamicin was not detected in any examined farm eggs, balady eggs, and organic eggs, and egg samples were accepted (100%). Similar findings were confirmed by the Australian government, the Department of Agriculture (2019), the Canadian food inspection agency (CFIA, 2006) and Treiber and Knauer - Beranek (2021). High levels of gentamicin residues were informed by Olatoye *et al*. (2019) and Cornejo *et al*. (2020). Gentamicin may be mutagenic, nephropathic, and hepatotoxic or may cause reproductive abnormalities or bone marrow toxicity (Cornejo *et al*., 2020).

Distribution of oxytetracycline and ciprofloxacin residues in albumin and yolk separately after oral administration by laying hens

The eggs of the 6th day after oral administration were collected for detection of the oxytetracycline and ciprofloxacin residues in the egg's albumin and yolk separately. As revealed in Table (2), the average oxytetracycline residues in albumin was 750 ppb. Lower levels were recorded by Roudaut (1989); Yoshimura *et al*. (1991); Furusawa (1999) and Kamali *et al*. (2020). The oxytetracycline residues in the yolk were 500 ppb that is matching the findings reported by Munoz *et al*. (2014). A higher concentration was recorded by Roudaut *et al*. (1989), meanwhile, Yoshimura *et al*. (1991); Furusawa (1999) and Kamali *et al*. (2020) estimated a lower level of oxytetracycline in yolk samples. Concerning the ciprofloxacin residues in albumin and yolk were 500 and 319 ppb, respectively. A higher level of ciprofloxacin residues in albumin was obtained by Billah *et al*. (2015) and a lower value was detected by Gbylik-Sikorska *et al*. (2021). Higher values of ciprofloxacin residues in yolk were detected by Gorla *et al*. (1997) and Billah *et al*. (2015), meanwhile, lower concentration was distinguished by Gbylik- Sikorska *et al*. (2021).

Nagy *et al*. (1997) mentioned that oxytetracycline residues appear more rapidly in albumin than in yolk, they were detected until the 9th and 12th day in albumin and yolk, respectively. Billah *et al*. (2015) found that ciprofloxacin increased through the first 5 days of the medication period in albumin and yolk and depleting these residues in them, it's required a withdrawal time of 8 days and 14 days, respectively.

Table 1. Incidence and concentration of oxytetracycline, ciprofloxacin and Gentamicin (ppb) in examined table egg samples (n= 60).

Antibiotic residue	Examined egg	Incidence (%)		Concentration (ppb)			Accepted according to MRL	
		+ve	-ve	Min	Max	Mean±SE	Accepted (%)	Unaccepted (%)
Oxytetracycline	Farm	20	80	60.5	88.0	69.95±1.78[a]	100	Zero
	Balady	20	80	31.0	69.5	43.6±2.65[b]	100	Zero
	Organic	Zero	100	N. D	N. D	N. D.	100	Zero
ciprofloxacin	Farm	20	80	25.0	28.0	26.0±0.21[c]	80	20
	Balady	60	40	26.6	60.0	39.58±5.58[d]	40	60
	Organic	Zero	100	N. D	N. D	N. D.	100	Zero

ppb: part per billion; MRL of Oxytetracycline residues are 200 ppb according to Codex Alimentarius Commission (2012); MRL of Ciprofloxacin residues are not allowed to be present according to European commission (2010); The means with different letters show significant difference where p-value < 0.05 at confidence interval 95%.

Table 2. level of oxytetracycline and ciprofloxacin concentration in albumin and yolk after oral administration and their reduction after boiling (15 min) and frying (5 min).

Egg section	Antibiotic residues after oral administration (ppb)		Level of reduction after boiling for 15 minutes			
	Oxytetracycline	Ciprofloxacin	Oxytetracycline		Ciprofloxacin	
Albumin	750	500	Concentration (ppb)	Reduction	Concentration (ppb)	Reduction
			93	88%	49	90.20%
Yolk	500	319	50	90%	40	87.50%
Whole egg (Total)	1250	819	143	88.50%	89	89%
			Level of reduction after frying for 5 minutes			
			71.5	94.30%	45	94.50%

The different distribution of the antibiotic residues in albumin and yolk may be conveyed by the differences in solubility and physicochemical properties, such as molecular weight and binding capacity to albumin (Furusawa, 2001).

Effect of boiling and frying on oxytetracycline and ciprofloxacin residues

The eggs of the 7th day were collected and thermally heated to detect the efficacy of this treatment in deteriorating the oxytetracycline and ciprofloxacin residues. As demonstrated in Table (2), the boiling of the egg for about 15 minutes reduced the oxytetracycline residues in albumin from 500 to 93 ppb with an 88% reduction rate. The oxytetracycline residues in the yolk were diminished from 500 to 50 ppb with a reduced rate of 90%. Meanwhile the ciprofloxacin residues in albumin and yolk degraded from 500 to 49 ppb and from 319 to 40 ppb with a reduced rate of 90.2 % and 87.5%, respectively. Consequently, the reduction rate in the whole egg after boiling was 88.5% and 89% for oxytetracycline and ciprofloxacin, respectively. The oxytetracycline reduction rate after boiling for 15 minutes was also estimated by Kamali *et al*. (2020) and Fath El-Bab (2012) and their results were a 20% and 52% reduction in the oxytetracycline residues, respectively. Nearly similar ciprofloxacin results were revealed by Al-Aboudi *et al*. (2021).

The frying of the whole eggs for 5 minutes could diminish the oxytetracycline residues from 1250 to 71.5 ppb with a 94.3% reduction rate (Table 2). A relatively similar result was reported by Al-Aboudi *et al*. (2021), while Mosha (2017) recorded a lower reduction rate. The ciprofloxacin residues declined after frying from 819 to 45 ppb with a 94.6% reduction rate which matched the findings of Al-Aboudi *et al*. (2021). Subsequently, it is noticeable that frying for 5 minutes could degrade the oxytetracycline and ciprofloxacin residues in the examined eggs more than boiling them for 15 minutes.

CONCLUSION

The presence of antibiotic residues in table eggs is considered a serious health hazard that must be controlled and minimized by decreasing the usage of antibiotics for prophylaxis and treatment through controlling infectious diseases and substitution the use of natural substances and probiotics to enhance the growth and immunity of the laying hens. Strict observation of withdrawal time in laying hens and eggs should be applied and cooking time and temperature can play roles in the antibiotic residue's reduction.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Al-Aboudi, A., Al Mashhadary, D. A., Basha E.A., Musallam, I., 2021. Enrofloxacin and ciprofloxacin residues in Table eggs: Distribution and heat treatment effect. Bulletin of University of Agriculture Science and Veterinary Medicine. Cluj Napoca. Animal Science and Biotechnologies 78, 10-16.

Australian Government, Department of Agriculture, 2019. Eggs residue testing annual datasets 2018-2019. National residue Survey, pp. 1-4.

Billah, M.M., Rana, S.M.M., Hossain, M.S., Ahmed, S.K., Barik, S., Hassan, M., 2015. Ciprofloxacin residues and their impact on biomolecule in eggs of laying hens following oral administration. Int. J. Food Contam. 2, 13.

CFIA (Canadian Food Inspection Agency), 2006. National chemical residue monitoring program annual report.

Codex Alimentarius Commission, 2012. Maximum residues limit (MRLS) and risk management recommendations for residues of veterinary. Drugs in foods CAC/MRL2.

Cornejo, J., Pokrant, E., Figueroe, F., Riquelme, R., Galdames, P., Dipillo, F., Jimerez- Bluhm, P., Hamilton- West, C., 2020. Assessing antibiotic residues in poultry eggs from a backyard production system in Chile, the first approach to a non–addressed issue in farm animals. Animal 10, 1056.

Darko, G., Mensah, J.K, Dapaah, S.S., Odei, J., 2015. Estimated dietary exposure to veterinary residues in chicken and eggs. International Journal of Food Contamination 2, 16.

Dipeolu, M. Eruvbetine, D., Oguntorq, E, Bankole, O.O., Sowunmi K.S., 2005. Comparison of effects of antibiotics and enzyme inclusion in diets of laying birds. Archivos de Zootenria 54, 3-11.

EU (European Commission Regulation), 2010. No.37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Off. J. Eur. Comm. 1. 15/1-72.

FAO (Food and Agriculture Organization of the United Nations), 2018. The future of food and agriculture alternative pathways to 2050 supplementary material. https://knowledge4policy.ec.europa.eu/publication/future-food-agriculture-alternative-pathways-2050_en

Fath El-Bab, G.F., 2012. Residues of some antibiotics in Table eggs in some private farms, J. Egypt. Vet. med. Assoc. 72, 69-80.

Furusawa, N., 1999. Spiramycin, Oxytetracycline, and Sulphamoromethoxine content of eggs and egg-forming tissue of laying hens. Zentralbl Veterinarmed A. 46, 599-603.

Furusawa, N., 2001. Transference of dietary veterinary drugs into eggs. Vet. Res. Commun. 25, 651- 662.

Gbylik- Sikorska M., Lebkowska- Wieruszewska, B., Gaida, A., Nowacka-Kozak, E., Lisowski, A., Po Syniak, A., 2021. Transfers of enrofloxacin, ciprofloxacin, and lincomycin into eggs shells and residue depletion in egg components after multiple administrations to laying hens. Poult Sci. 100, 101341.

Gorla, N., Chiostri, E., Ugnia, L., Weyers, A., Giacomelli, N., Davicino, R., García Ovando, H., 1997. HPLC residues of enrofloxacin and ciprofloxacin in eggs of laying hens. Int. J. Antimicrob. Agents 8, 235-6.

Hafez, A.T, El- Gayaty, A.H, Megahed, A.A., 2013. A Comparison between organic and conventionally produced eggs. Assiut Vet. Med. 59, 55-59.

Huang, J.F., Lin, B., Yu, Q.W., Feng, Y.Q., 2006. Determination of fluoroquinolones in eggs using tubes solid–phase microextraction coupled to high–performance liquid chromatography. Anal. Bioanal Chem. 384, 1228–1235.

Idowu, F., Junaid K., Paul, A., Gabrial, O., Paul, A., Sati, N., Maryam, M., Jarlath, U., 2010. Antimicrobial Screening of commercial eggs and determination of tetracycline residue using two Microbiological Methods. Inter. J. Poult. Sci. 9, 959-962.

Ji, X., Xu, Y., Wang, J., Lyu, W., Li, R., Tam, S., Xiao, Y., Tang, B., Yang, H., Quian, M., 2021. Multiresidue determination of antibiotics in ready – to eat duck eggs marketed through e-commerce stores in China and Subsequent assessment of dietary risks to consumers. J. Food Sci. 86, 2145-2162.

Kao, M.Y., Charg, M., Cherg, C., Chou, S., 2001. Multiresidues determination of veterinary drugs in chicken and swine muscles by high-performance liquid chromatography. J. Food Drug Anal. 1, 84-95.

Kamali, A., Mirlohi, M., Etebani, M., Sepahi, S., 2020. Occurrence of tetracycline residue in Table eggs and genotoxic effect of row and heated contaminated egg yolks on hepatic cells Iran J Public Health 49, 1355–1363.

Ubayd, N. A., Abduh, M. S., Abbas, M.E., 2021. Microbial Quality and residues of some antibiotics in conventional and organic Table eggs at retail stores in Kafer El-Sheikh governorate. Alex. J. Vet. Sci. 69, 98-112.

Mosha, J.C., 2017.Tetracycline residues in raw and cooked chicken eggs in Tanzania. The University of Agriculture. Morogoro, Tanzania.

Munoz, R., Cornejo, J., Maddaleno, A., Araya Jordan, C., Iraguen, D., Pizarro, N., Martin, B.S., 2014. Withdrawal times of oxytetracycline and tylosin in eggs of laying hens after oral administration. J. Food Prot. 77, 1017-1021.

Nagiub, Hagar Amir, Mohamed Rlsherbieny El Sayed, Hassan ali Maarof, 2021. Prevalence of Antibiotic Residues in Table Eggs. Mansoura Vet. Med. J. doi: 10.21608/mvmj.2021.46884.1006.

Nagy, J., Sokal, J., Turek, P., Korimova, L, Rozanska, H., 1997. Residues of oxytetracycline in egg white and yolk after medication of laying hens. Bull. Vet. Inst. Pulawy 41, 141–147.

Olatoye, O.I., Ojomo, T,O., Adeseko, Y.J., 2019. antibiotics use and gen-

tamicin residues in commercial poultry and chicken eggs from Oyo and Lagos States, Nigeria, Revue d'elevage et de medicine veterinaire, des pays tropiacux 72, 161-165.

Roudaut, B., 1989. Residues of aminoglycoside antibiotics in eggs after medication of laying hens. British Poult. Sci. 30, 265-271.

EMEA, 2000. Gentamicin summary report (2). The European Agency for the Evaluation of medicinal products, the Committee for veterinary medicinal products. https://www.ema.europa.eu/en/documents/mrl-report/gentamicin-summary-report-2-committee-veterinary-medicinal-products_en.pdf

Sara, A.M., Hala, A.E., Sahar, M.N., Samia, A.A., Hassan, Inaam El- Adwad, Hadeel, A. AMabyie, Samia, H. Abdulrahman, Iahraga, G., 2021. Assessment of Oxytetracycline Residue in Table Eggs from Khartoum State's Market. Amer. J. Biomed. Sci. Res. 14, 1-5.

Treiber. F.M, Beranek- Knauer, H., 2021. Antimicrobial residues in food from the animal organ – A. Review of the literature focusing on products collected in stores and markets worldwide. Antibiotics 10, 534.

WHO, 2019. Evaluation of the joint FAO/WHO expert committee on food additives (JECFA). https://www.who.int/publications/m/item/89th-meeting-joint-fao-who-expert-committee-of-food-additives-(jecfa)

Widiasih, D., Drastini, Y., Yudhabuntara, D., Maya, F., Lini, P., Susetya, H., Nugroho, W., Putri, K., Primatika, R., Sumiarto, B., 2019. Detection of Antibiotic Residues in Chicken Meat and Eggs from Traditional Markets at Yogyakarta City Using Bioassay Method. Acta Veter. Indonesiana Special Issue 1–6.

Yoshimura, H., Osawa, N., Rasa, F. S.C., Hermawati, D., Werdinirgish, S., Isriyanthi, N.M.R., 1991. residues of doxycycline and oxytetracycline in eggs after medication via drinking water to laying hens. Food Addit. Contam. l8, 1-3.