Residual effect of amoxicillin in broiler

Mst. Shumiya Khatun[1], Dolan Das[1,5]*, Mst. Sharmin Akter[1], Md. Ashraf Zaman Faruk[2], Sobrata Das[3], Md. Rizwanul Islam Tuhin[4], Md. Shafiqul Islam[1]

[1]Department of Pharmacology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Department of Anatomy and Histology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[3]Department of Physiology, Sylhet Agricultural University, Sylhet-3100, Bangladesh
[4]Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh
[5]Livestock Extension Officer, LDDP, DLS, Natore, Bangladesh

ARTICLE INFO

ABSTRACT

This study was designed with the aim to determine the impact of residual antibiotics on poultry muscle (breast and thigh), kidney, spleen, fat & liver and effect of discriminate and indiscriminate use of antibiotic on haematological constituents in broiler chickens. The chickens were properly reared and on the day of 16, chicks were randomly divided into three groups namely control group (group A), discriminate antibiotic group (group B), indiscriminate antibiotic group (group c). Each group consists six chicks. In case of discriminate group, the chicks were treated with amoxicillin antibiotic followed by maintaining withdrawal period one week before the sacrifice of the birds. On the other hand indiscriminate group of chicks was continued without maintaining any withdrawal period and birds were sacrificed for sampling. TLC method was used for screening detection of amoxicillin residues in poultry tissues and hematological parameters were investigated with appropriate methods such as hemoglobin determination, packed cell volume and total erythrocyte count. In case of control group (group A): No positive sample found. In case of discriminate group (group B): the residues of amoxicillin were 100% in livers, 66.67% in kidneys, 0% in thigh muscles, and 0% in breast muscles, 33.34% in fat and 33.34% in spleen. In case of indiscriminate group (group c): the residues of amoxicillin were 100% in livers, 100% in kidneys, 16.67% in thigh muscles, and 16.67% in breast muscles, 50% in fat and 50% in spleen. Among the poultry tissues, liver and kidney had the highest level of antibiotic residues in comparison to other samples. It was observed that indiscriminate use of antibiotic represent the antibiotic residues in different edible tissues. Evidence suggests that more judicious use of antimicrobials in food animals will reduce the selection of resistant bacteria and help to preserve these valuable drugs for both human and veterinary medicine. National authorities should adopt a proactive approach that promotes programs aimed at reducing the need for antimicrobials in food animals and ensuring their prudent use. Further investigation is required for the quantitative determination of antibiotic residues in food product.

INTRODUCTION

An important sub sector of livestock production, the poultry industry in Bangladesh plays a vital role in economic growth and simultaneously creates numerous employment opportunities. The poultry industry, as a fundamental part of animal production, is committed to supply the nation which a cheap source of good quality nutritious animal protein in terms of meat (Akter et al., 2009).

According to WHO–FAO joint survey, meat consumption per head in Bangladesh is 15.23 kg per year while the requirement is 43.8 kg per person. So there is a deficit of 65.23 % to meet our domestic requirement. It may be noted that poultry contributes 35.25% of total meat supply (Akbar et al., 2013).

Antibiotics are used largely for three purposes in poultry, therapeutic use to treat sick poultry, prophylactic use to prevent infection in poultry and as growth promoters to improve feed

utilization and production for their growth promoting properties they are routinely used at sub-therapeutic levels as poultry feed additives (Okerman et al., 1998).

Amoxicillin is broad-spectrum, pharmacologically active beta-lactam antibiotic effective against gram-positive and gram-negative bacteria. In poultry, amoxicillin is used for the treatment of susceptible infections of the alimentary, urogenital and respiratory tracts (APVMA, 2007).

Therefore, amoxicillin is seen to be a valuable antibiotic in terms of therapeutic effect for treating bacterial infections in poultry. Despite the important therapeutic use of amoxicillin in veterinary practice, relatively little has been published on the pharmacokinetic behaviour and bioavailability of this drug in birds (Dorrestein et al., 1986; Lashev and Pashov, 1992).

The consequences of the use of antibiotics are very diverse and appear as a failure of individual organs and systems in general. Tissues may be contaminated with harmful concentration of drug residues due to continuous and improper antibiotic usage in poultry industry (Shareef et al., 2009).

Human consumption of toxic levels of antibiotic residues in food of animal origins (meat) had caused several pathological defects in man that are of public health importance (Dipeolu, 2004; Doyle, 2006; Nisha, 2008; Shareef, 2009; Muhammed, 2009).

One of the most sensitive systems is the haematopoietic system. The process of haematopoiesis in the body is carried out continuously and the young dividing cells are very sensitive to the action of antibiotics. Toxic effects of antibiotics on the process of haematopoies are causing a change of blood parameters. For example, the toxic effect of amoxicillin (which is a part of the synthetic antibiotic amoxystin) showed neutropenia and eosinophilia (Stolker and Brinkman, 2005).

Microbial resistance to antibiotics is a worldwide problem in human and veterinary practices. It is generally accepted that the main risk factor for the increase in antibiotic resistance is an extensive use of antibiotics. The antimicrobial agents used in animal care are also significant, not only in increasing the resistance in animal pathogen but also in bacteria transmitted from animals to humans.

From the above facts, it is mentioned that this antibiotic residues have potential hazards for human as well as animal health. In this context, this research work was undertaken to detect amoxicillin antibiotic residues in broiler liver, kidney, spleen, fat, breast muscle and thigh muscle by thin layer chromatography (TLC) test, to assess the impact of antibiotics used as growth promoters on the haematological parameters of broiler chickens and to know the withdrawal period of drug.

MATERIALS AND METHODS

Experimental layout

The experiment was conducted in the Department of Pharmacology, Bangladesh Agricultural University (BAU), Mymensingh.

Preparation for experimental samples

Selection of house

The birds were reared in an isolated poultry shed, Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.

Cleaning and disinfection

First the shed was thoroughly washed by sweeping and washing with tap water. The room was disinfected with a phenolic disinfectant (phenyl) and allowed to dry, leaving the room, unused with the electric fan and the bulb switched on.

Experimental birds and feed

Eighteen apparently healthy day-old broiler chicks were purchased from CP Hatchery Ltd. The chicks were already vaccinated with Marek's disease vaccine in the hatchery. All chicks were brooded in the same brooder up to 7 days of age under strict management. Vitamin preparation (Rena-C,

Reneta Pharmaceuticals Ltd.) & Glucose was administered to the birds daily mixing with water. BCRDV (Newcastle L-36) & Gumboro vaccine was given to all chicks in eye (one drop) at 7 and 12 days respectively.

Broiler starter mash and broiler grower pellet were obtained from Quality Feeds Ltd. The birds were given *ad libitum* feed twice daily. Clean & cold water was provided for 24 hours. Amoxicillin BP 30% was obtained as solution from Navana pharmaceuticals Limited.

Grouping of birds

Chicks were randomly divided on the 16 days of age into three groups. Group A (6 birds) was kept as untreated control group received non medicated water. Group B (discriminate group contain 6 birds) were administered with Amoxicillin at recommended therapeutic dose@ 1gm/5L through drinking water for seven consecutive days from 16th to 22th days of age. Group C (indiscriminate group contain 6 birds) were administered with Amoxicillin at recommended therapeutic dose@1gm/5L, through drinking water for fourteen consecutive days from 16th to 29th days of age. Birds received their freshly prepared daily medication in the morning hour of each day. The concentration of Amoxicillin in the water to give the required dose per kilogram of body weight was calculated by determining the water consumption and body weight of each bird on the day of medication. Drinking water was free from pesticides, heavy metals and coliform count. Drinking water medication was used because oral administration of drugs is the most practical way for treating birds being followed in Bangladeshi field condition.

Sampling

The weights of every bird were taken individually in the morning and the results were recorded.On day 30th, before sacrifice; all the birds of three groups (Group- A, B and C) were weighed individually and the results were recorded. After slaughtering of the birds, the breast muscle, thigh muscle, kidney and liver were collected for the estimation of antibiotic residue. All samples were marked separately and preserved at -20º C in polythene bags for their extraction and analysis. Blood samples were also collected and preserved for haematological analysis.

Blood samples were collected from wing vein into sterile heparinised vials and test tubes from all groups (Control, discriminate & indiscriminate). Blood smears were prepared simultaneously at the time of blood collection and used for differential leukocyte count.

Extraction of antibiotic

For extraction of antibiotic residue from samples thin Layer Chromatography was performed. Samples (Muscle tissue, liver and kidney) were blended with a food processor properly for three to five minutes. These mashed/blended samples were taken into properly cleaned and sterilized petridishes with proper care as well as covering. From this 4 g of aliquot sample was taken into beaker with the help of electric balance and spatula. Then homogenization was done with addition of 10 ml phosphate buffer (pH 6.5). After proper mixing, protein contents of these samples were precipitated with the addition of 2 ml trichloroacetic acid (30%) maintaining sufficient care and attention. Then the mixed samples were taken into properly cleaned and sterilized test tubes for centrifugation. Then centrifugation was done at 7000 rpm for 15 minutes with the help of automatically time regulated centrifuge machine. 2 ml supernatant was mixed 100 μL of formaldehyde for 45 minutes at 100º C water bath. The supernatant was extracted with equal volume of diethyl ether and mixing was done properly in order to perform defamation. Then the mixture was kept for 10 minutes to become a separate layer, an upper oily layer and bottom layer. Then by using cleaned and sterilized separating funnel, these mixtures were separated from each other and upper oily was discarded and only bottom layer was collected. This extraction of supernatant was repeated twice with diethyl ether. Then the extracts were evaporated until dryness. The dried sample was reconstituted within 2ml of mobile phase methanol acetone. Then, extracts were collected into screw capped vial with proper care and kept into refrigerator for further advanced analysis. Total procedure was performed as the reference cited by (Poppelka et al., 2005).

Amoxicillin BP 30% was used as reference antibiotic.

Preparation of the solvent system

In order to perform Thin Layer Chromatography (TLC) along with the stationary phase or solvent preparation was done as directed in the references Here 10 ml of methanol and 10 ml of acetone was mixed properly and was used as mobile phase (Thangadu et al., 2002).

Pointing, running and detection

A volume of 50 μl of methanol dissolved deposits was pointed on silica plates. Treated plates transferred to TLC tank containing acetone-methanol (1:1) as mobile phase. After reaching of solution front to end of plates, chromatograms observed on ultraviolet light at 256 nm.

Examination under UV detector

The chromatogram was examined under the ultraviolet lamp at 256 nm for spots that fluorescence. The outlines of the spots were marked with a series of dots using sharp pencil for calculation of retention factor (R_f).
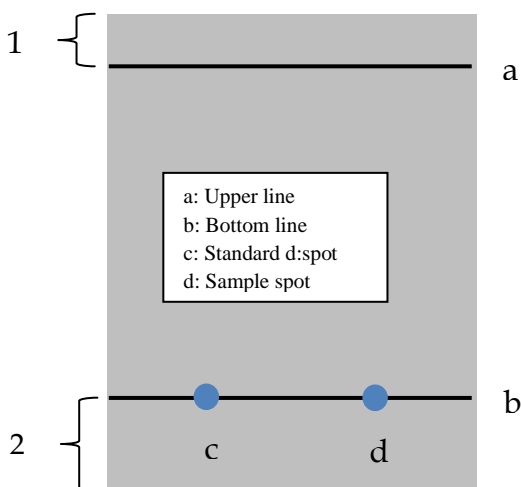

Figure 4: Marking and spotting on TLC plate

Calculation of R_f values

To define the relative migration rate of substances under various conditions retardation factor is determined It is the ratio of distance moved by the substance and distance moved by solvent For this,

the distance that each spot had traveled from the start line was measured in cm, taken from the center of the spot Also the distance of the solvent was measured from the start line not from the bottom of the chromatogram. Then calculation of R_f values was done using the following equation

$$Rf = \frac{\text{Distance travelled by sample (a)}}{\text{Distance travelled by solvent (b)}}$$

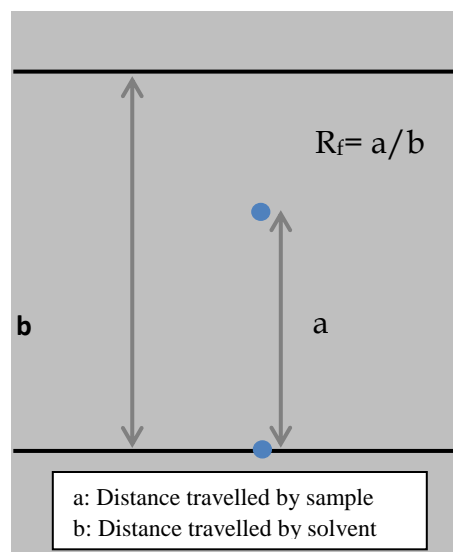Results of all RF values were recorded on a paper in tabular form.

Figure 5: Calculation of Rf values

Interpretation of results

A substance was positively identified in the unknown solution when it behaved identically as the reference substance. Sample was considered positive if same color under UV light, same color with the spray reagent and same RF value as those of the reference sample were observed.

Hematological examination

Hemoglobin concentrations (Hb) was determined by the Helligehemometer method, total erythrocyte count and Packed cell volume (PCV) was determined by using the following formula as described by Lamberg and Rothstein (1977).

RESULTS

Detection of Amoxicillin residues in different samples of broiler

The research was carried out on investigation of amoxicillin antibiotic residues in broiler both as discriminate and indiscriminate manner. Any antibiotic residues in non-treated control group were not found. But, antibiotic residues in liver, kidney, fat and spleen were found in discriminate antibiotic group. On the other hand, positive sample found in liver, kidney, breast muscle, thigh muscle, fat and spleen in indiscriminate group of birds (Plate 1- 3).

Table 1: Detection of Amoxicillin residues in different samples of group-B (Discriminate group) by TLC

Name of Sample	Total No. of Sample	Number of sample		% of sample	
		Positive	Negative	Positive (%)	Negative (%)
Liver	6	6	0	100	0
Kidney	6	4	2	66.67	33.34
Breast muscle	6	0	6	0	100
Thigh muscle	6	0	6	0	100
Fat	6	2	4	33.34	66.67
Spleen	6	2	4	33.34	66.67

Table 2: Detection of Amoxicillin residues in different samples of group-C (Indiscriminate group) by TLC

Name of Sample	Total No. of Sample	Number of sample		% of sample	
		Positive	Negative	Positive (%)	Negative (%)
Liver	6	6	0	100	0
Kidney	6	6	0	100	0
Breast muscle	6	1	5	16.67	83.83
Thigh muscle	6	1	5	16.67	83.83
Fat	6	3	3	50	50
Spleen	6	3	3	50	50

Positive results showed 100% in liver, 66.67% in kidney, 33.34% in fat, 33.34% in spleen in discriminate group of birds & 100% in liver, 100% in kidney, 16.67% in breast muscle, 16.67% in thigh muscle, 50% in fat and 50% in spleen in indiscriminate group of birds (Table 1 & Table 2).

Table 3: Different hematological parameters of broiler

Day	Blood Parameters	Name of group	Average blood parameters value (Mean ± SEM)	P Value	Level of Significance
30th Day	Hb (g/ml)	Group-A (Control group)	8.17 ± 0.07		
		Group-B(Discriminate group)	7.37± 0.13	<0.01	**
		Group-C(Indiscriminate group)	7.18 ± 0.09		
	PCV (%)	Group-A (Control group)	24.17 ± 0.48		
		Group-B(Discriminate group)	22.5 ± 0.43	0.0068	**
		Group-C(Indiscriminate group)	22 ± 0.37		
	TEC (million/mm^3)	Group-A (Control group)	3.20 ± 0.06		
		Group-B(Discriminate group)	2.70 ± 0.03	<0.01	**
		Group-C(Indiscriminate group)	2.54 ± 0.05		

Table 4: Body weight of broilers at different age

Variables	Group of Broilers	Average weight (g) (Mean ± SEM)	P Value	Level of significance
Live weight(g) on 16th day	Group-A	613.7 ± 38.77		
	Group-B	658 ± 20.4	0.4146	ns
	Group-C	660.8 ± 18.09		
Live weight(g) on 22th day	Group-A	987.5 ± 17.31		
	Group-B	1080 ± 26.06	0.0156	*
	Group-C	1105 ± 33.16		
Live weight(g) on 28th day	Group-A	1611 ± 28.99		
	Group-B	1717 ± 16.87	0.0003	***
	Group-C	1769 ± 15.19		
Live weight(g) on 30th day	Group-A	2082 ± 28.66		
	Group-B	2145 ± 73.2	0.1445	ns
	Group-C	2285 ± 92.19		

Determination of different hematological parameters of broiler

Blood is a very important body fluid in humans and other animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from the body cells. In my study, assess the impact of amoxicillin antibiotic residues on hematological parameter of broiler. The results showed that highest hemoglobin, PCV and TEC are present in control group of broiler (Table 4).

Body weight of broilers at different age

In the study, the results showed that highest body weight found in indiscriminate group of broiler and relatively lowest body weight found in control group of broiler (Table 4).
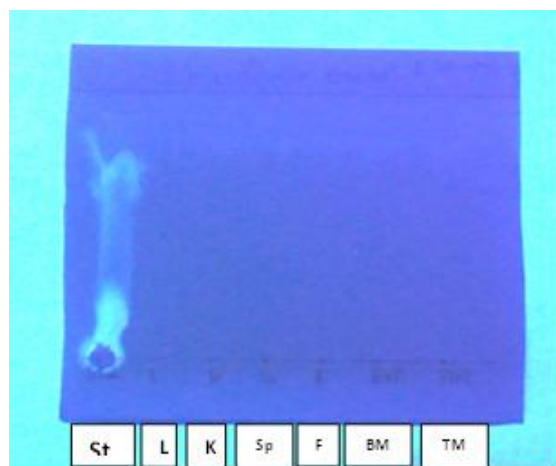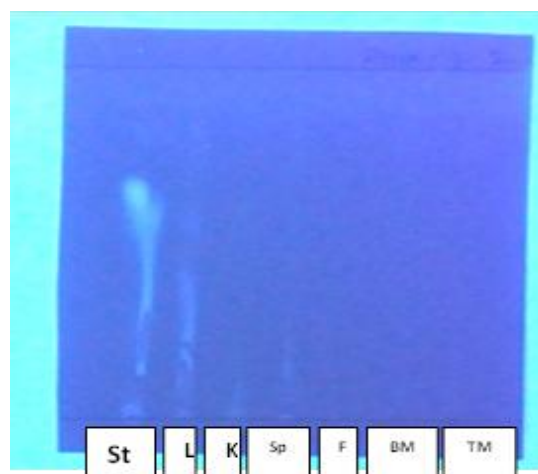

Plate 2: TLC plate showing the amoxicillin residue status for group B (discriminate)

Plate 1: TLC plate showing the amoxicillin residue status for group A (control)
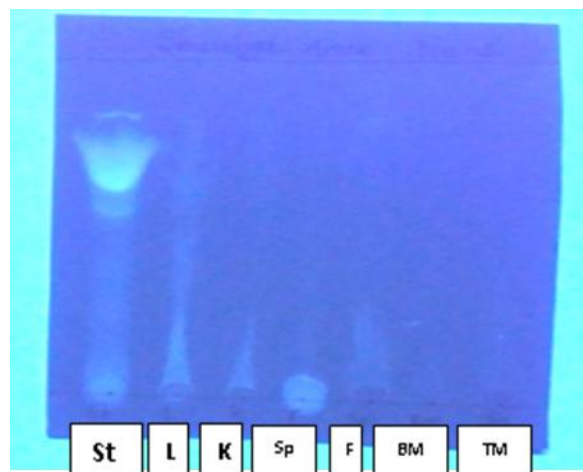

Plate 3: TLC plate showing the amoxicillin residue status for group C (indiscriminate)

DISCUSSION

In the study, a total sample of 108 was collected from control, discriminate and indiscriminate group of broiler. Among them 31.48% of tested tissue samples gave positive results for the presence of antibiotics compared to 68.51% that were negative. The main edible part is the muscles and to a lesser extent liver and kidney. In this study high positive samples were detected in liver (100%), kidney (66.67%) in case of discriminate antibiotic group and incase of indiscriminate antibiotic group liver, kidney showed (100%) positive results with a lower percentage in thigh and breast muscle. Also assess the impact of amoxicillin antibiotic residues on hematological parameter of broiler. The results showed that the level of blood parameter in the control group, discriminate antibiotic group, indiscriminate antibiotic group of broiler chickens did not deviate from normal & highest hemoglobin, PCV and TEC are present in control group.

Recently, scientific community are being carried out to investigate the presence of the different veterinary dugs including antibiotic residues in animal products due to the side effects they impose on the health of human consumers. The main objective of this study was to screen for the presence of antibiotic residues in poultry meat. In a previous study, it was shown that antibiotics (namely tetracycline and penicillin) are used extensively in poultry farms either for prevention (50 % of visited farms) or for treatment (37.5%) (Salman et al., 2012).

These results are different to what was reported previously where lower percentage of positive sample was detected in muscle and liver (11.47% and 10%, respectively) compared to 24.6% in the kidneys for poultry tissues in Khartoum (Hala and Salih, 2006).

The higher results obtained under the present study may be attributed to the extensive use of antibiotics by farm owners. The levels of antibiotic residues may vary within the different tissues. When choosing muscle for example, there remains a choice between breast or thigh muscle. In this study the breast muscle was chosen. According to previous studies, there was no significant difference of antibiotics residues between the different sections of breast tissues (Reyes and Donoghue, 2008).

But it's clear from the result that, antibiotic residue already exist in our food chain, especially in broiler. However, a comprehensive study require in Bangladesh to detect and estimate all the antibiotics used in broiler chicken to take potential steps to protect the mankind and environment from antibiotic residues hazards.

CONCLUSION

Indiscriminate use of amoxicillin antibiotic in poultry revealed detectable level of antibiotic residues in liver, kidney, breast muscle, thigh muscle, fat and spleen which is dangerous for human consumption. Liver and kidney contained the highest percentage of antibiotic residues. In the study, the level of blood parameter in the control and experimental groups of broiler chickens did not deviate from indices of the control group chickens.

Once antibiotics are administered to animal body, antibiotic residues are present in high or low concentrations in their products. However, it mainly depends on the duration of the administration of antibiotics. After the administration of antibiotic, concentrations of their residues gradually reduces in meat and other meat products and mostly after 4-5 days of administration secretion of residues are negligible.

The antibiotics were identified on the basis of their retention times (RTs) as compared to the standards and by adding known quantities of the standards to the sample and re-chromatography which showed complete overlap of analytic peaks. Hence, the withdrawal time of different drugs should be strictly followed and during this period meat and other animal products like liver, spleen, fat and kidney should not be used for human consumption. Use of antibiotic as growth promoter should be strictly prohibited and whenever they are used for therapeutic purpose must be used in proper doses and for proper time. Thus, by observing proper scientific guidelines and precautions we can minimize the harmful effects of antibiotic residues.

REFERENCE

Akbar MA, Amin MR, Ali MA, Bhuiyan MSA, Kabir AKMA, Siddiki SR (2013). Animal Husbandry- A business education for today and tomorrow, 3rd Annual Conference and Seminar, Bangladesh Society For Animal Production Education and Research (BSAPER).

Akter A and Uddin S (2009). Bangladesh Poultry Industry. Journal of Business and Technology (Dhaka)*, *4(2): 97-112.

APVMA(2007).Vetrimoxin.50.Soluble.Powder.Http:// www. Apvma.Gov.Au/Advice_Su Mmaries/40946.

Dipeolu (2004). Problems and prospects of antibiotics residues in meat products in Nigeria. Journal of Veterinary Science, 1(1): 63-67.

Dorrestein GM, Rinzemaj D and Burrelaar MN (1986). Tissue distribution of amoxicillin after oral and intramuscular administration to pigeons (*Golum balivia*). Avian Pamology, 1s: 663-676.

Doyle (2006).Veterinary drug residues in processed meats-Potential Health Risk. A review of the scientific literature*, *food research institute (Fri Briefings), University of Wisconsin–Madison, March 2006. Last Accessed [15/3/2014], Https://Fri.Wisc.Edu/Files/Briefs_File/Fribrief_Vet drgres.Pdf.

Elnasri SHA and Osman IAM (2012). Detection of antibiotic residues in milk using delvo test kit and disc assay methods in Khartoum State*. *Journal of Veterinary Medicine and Animal Production, 3: 3-15.

Hala I and Mohammed Salih (2006).Residues of antibiotic in poultry meat.Msc. Department of Preventive Medicine.University of Khartoum, Khartoum.

LambergSland Rothestein R (1977).Laboratory Manual Of Haematology And Urinalysis.1stEdn.,Avi Publishing Company Inc.,Westport Connecticut, USA.

Okerman L, Van HoofJ, Debeuckelaere W (1998). Evaluation of the european four-plate test as a tool for screening antibiotic residues in meat samples from retail outlets. Journal - Association of Official Analytical Chemists*, *81:51-56.

PoppelkaP, Nagy J, Germuska R, Marcinca KS, Jevinova P and Derijk A (2005). Comparison of various assays used for detection of beta-lactam antibiotics in poultry meat. Food Additives & Contaminants*, *22(6): 557-562.

Shareef AM, Jamel ZT and Yonis KM (2009). Detection of antibiotic residues in stored poultry products. Journal of Veterinary Science*, *23(1): 45-48.

Stolker AAM and Brinkman UT (2005). Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals. Journal of Chromatography, 1067(1): 15-53.

Thangadu S, Shukla SK and Anjaneyulu Y (2002). Separation and detection of certain $- lactams and floroquinolone antibiotic drugs by thin layer chromatography. Analytical Sciences, 18: 97-100.