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Full Length Article

Dietary Exposure and Risk Assessment of Chloramphenicol Residues in Animal-Derived Foods, Marketed in Faisalabad (Pakistan)

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Abstract

The use of chloramphenicol (CAP) is prohibited in food producing animals since 1994 in Europe and many other countries due to harmful side effects in humans. In developing countries like Pakistan, it is still in use illegally. So, the present study was conducted to evaluate CAP residues intake through animal-derived food in humans and their health risk assessment. Regarding this, 165 samples (including 40 bovine milk and 25 each of bovine kidney, beef, mutton, poultry and fish meat) were collected from different dairy farms and markets of District Faisalabad. The immunosorbent assay (ELISA) was performed after extraction in methanol (for milk samples) and ethyl acetate (for tissue samples) by using in-house and commercial ELISA, respectively. Overall, results indicated that 51 (30.9%) samples containing CAP residues with 25 (15.2%) samples having residues above the MRPL (0.3 μg kg^{-1}). Among CAP containing samples, 22.5% bovine milk, 16% bovine kidney, 20% beef, 24% poultry meat and 4% mutton samples were found positive. The CAP concentration in positive samples was ranged from 0.35 to 1.57 ng g^{-1}. However, all fish samples were found negative with 16% samples containing CAP residues below the MRPL. Health risk index exceeded 1 (the cut off value) for CAP residues in 25 samples, indicating the possibility of health risk associated with the consumption of contaminated milk and meat. © 2021 Friends Science Publishers

Keywords: Chloramphenicol residues; Dietary exposure, Milk, Meat, Immunosorbent assay; Risk assessment

Introduction

Agriculture plays a vital role for economy of Pakistan as it contributes 19.2% to the GDP. More than 65–70% population depends on agriculture for its livelihood. Livestock have 60.07% share in agriculture and 11.53% in GDP. Poultry is important sub-sector of livestock as it contributes 1.3% to GDP and provides employment to more than 1.5 M people in country (Hussain *et al.* 2015). Although the share of fisheries in GDP is negligible (0.39%), it contributes to the national income through export earnings. According to Pakistan Economic Survey (2020–2021), the gross production of milk (from cow, buffalo, sheep, goat and camel) is 63684 thousand tonnes and meat (including beef, mutton and poultry meat) is 4955 thousand tonnes.

Antibiotics are widely used in food-producing animals to control diseases and as growth promoter to enhance meat, milk and egg production (Laven *et al.* 2012). The inappropriate and overuse of veterinary drugs has become a common practice in recent years (Granelli and Branzell 2007). The drug residues if present in animal-derived food can cause serious health hazards; due to this reason, food safety has become a major issue all over the world (Macarov *et al.* 2012). The main group of drugs used as veterinary medicine are tetracyclines, amphenicols, aminoglycosides, macrolides, nitrofurans, sulfonamides and Quinolones (Samuel *et al.* 2011) and their illegal use can increase the chances of food contamination instead of their benefits (Penney *et al.* 2005). The World Health Organization (WHO) reported public health problems emerging from microbial resistance due to excessive use of antibiotics. The Food and Drug Administration (FDA) also set criteria for the approval of new antibiotics to perform risk assessments (FDA 2003). Drugs are evaluated as "low", "medium" or "high" risk on the basis of possible resistance gained by bacteria in animal population, transfer of these resistant bacteria to humans through food products and their adverse health effects (Garofalo 2007).

Chloramphenicol (CAP) is widely used as prophylactic and chemotherapeutic agents. It is used in veterinary medicines to treat different infections (Rocha *et al.* 2009). Its side effects in humans are aplastic anaemia, bone-marrow depression and syndrome of cyanosis (Takino *et al.* 2003). While in livestock, chromosomal aberrations in lymphocytes is reported in calves treated with 20 to 100 mg kg^{-1} of body weight (EFSA 2014). As per European Community Regulation 1430/94, the CAP is banned in food producing animals (Nicolich *et al.* 2006) but still it is being used illegally in some developing countries for treatment of some infectious diseases in livestock (Ye *et al.* 2008).

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For substances not classified as "allowed substances", Reference Points for Action (RPAs) may be established to comply with Union legislation regarding the food products of animal origin. For a number of compounds, the minimum required performance limit–MRLs are established on the basis of RPAs (EFSA 2014). The MRPL value for CAP is 0.3 μg kg^{-1} for all food matrices (Commission Decision 2003). In 2014, EFSA has evaluated the RPA for CAP and found it suitable for public and animal health. A strict surveillance system is enforced in the European Union by Council Directive 96/23/EC for screening of veterinary medicines.

For the monitoring of CAP residues in animal-derived food, different analytical methods have been published such as milk (Rodziewicz and Zawadzka 2008), equine, porcine muscles (Gantverg *et al.* 2003), shrimp (Xu *et al.* 2006), chicken, beef and fish tissue (Gikas *et al.* 2004; Yibar *et al.* 2011). Among these methods include chromatographic (Tajik *et al.* 2010), microbiological (Angelovski *et al.* 2011), enzymatic (Datta and Majumdar 1985) and immunological assays (Mehdizadeh *et al.* 2010). All these methods have detection limit at or below the permissible limits and validated in accordance with the Council Directive 2002/657/EC. However, Enzyme-linked immunosorbent assay (ELISA) is mostly used for screening and quantification purpose as it is highly sensitive, cost-effective and reliable method with high sample throughput. This assay can be performed in direct format in which antibodies are coated on a surface of microtiter plates and an indirect format in which analyte derivative is coated. ELISA is basically colorimetric detection of a product, produced from an oxidative reaction of substrate catalysed by an enzyme. Many researchers have developed ELISA for the detection of CAP in different matrices at MRPL levels (Samsonova *et al.* 2012). Commercial ELISA kits are available for CAP detection in different food matrices with false complaint results less than 5% (Scortichini *et al.* 2005).

Keeping in view the importance of food quality for human health, immunosorbent assay (in-house and commercial ELISA) was performed to monitor CAP residues in bovine milk and edible tissues (bovine kidney, beef, mutton, poultry and fish meat) collected from different dairy farms and local markets of 35 km radius from the city centre of District Faisalabad (Punjab), Pakistan. For this purpose, commercial ELISA kits were standardized and validated to use in surveillance studies along with in-house developed ELISA. The generated data was applied for health risk assessment. This base-line data may be useful for consumers, farmers/producers, health specialists, policy makers and other associated stakeholders.

Materials and Methods

Apparatus and chemicals used

Absorbance microplate reader (ELx808, BioTek), Microplate strip washer (ELx50, BioTek), Tissue homogenizer (HG-15D, DAIHAN scientific), Freezer (Bio-Medical, Sanyo), Vortexer (Lab-Line), TurboVap system (Biotage), Centrifuge (5340R, Eppendorf), Double distillation unit (Fistreem Cyclon), Micropipette (Eppendorf), Falcon Tubes (50 mL capacity, VWR), Glass test tubes (Kimax), ELISA plate sealers, ELISA Kits (Cat. #. W81113, Quicking Biotech), Chloramphenicol standard (C0378), Ethyl acetate (VWR), n-Hexane (VWR), Methanol (VWR), Sodium acetate (VWR).

Study site and collection of samples

For the present study, total 165 samples of different food matrices were collected from 31 sites including dairy farms and local markets of district Faisalabad, Pakistan (*latitude 30° 25' 45 N and longitude 73° 4' 44 E*) during 2017–19 as shown in Fig. 1.

Bovine milk and tissue samples were taken in falcon tubes and zip bags, respectively and recorded sample number, location and sampling date for traceability. These samples were shifted to Food Safety Laboratories (ISO/IEC 17025:2017 accredited) of Nuclear Institute for Agriculture & Biology (NIAB), Faisalabad in chilled condition (4–6ºC) and stored at -20ºC to for further analysis. Detail of samples is given Table 1.

Preparation of samples

Milk samples were extracted by adopting in-house ELISA protocol (Chughtai *et al.* 2017) while tissue samples were extracted by following kit manual.

Bovine milk

Defatted milk samples (2.5 ± 0.05 mL) were taken in 50 mL falcon tubes and added phosphate buffer (2.5 mL). The tubes were allowed to stand for about 5 min. The SPE cartridges (Strata) were used for extraction by using SPE assembly (Phenomenex) under vacuum. The filtrate was removed while the samples were eluted with 99.9% methanol (1.5 mL). The extracts were (totally or partially) dried at 65ºC in TurboVap system under nitrogen. The dried samples were reconstituted with sodium acetate (250 μL) and further used for assay development.

Tissue (bovine kidney, beef, mutton, poultry and fish meat)

Fat free tissue samples were cut down in to small pieces and homogenized at 10000 rpm for 1 min. Homogenized samples (2 ± 0.05 *g*) were taken in 50 mL falcon tubes and then added 8 mL ethyl acetate in each tube. After shaking for 10 min, samples were centrifuged at ~4000 *g* for 10 min. Two millilitres of upper ethyl acetate layer (supernatant) was collected accurately in glass tubes and dried under nitrogen at 50ºC. These dried samples were reconstituted with 0.5 mL

Table 1: Collection of milk and meat samples in Faisalabad, Pakistan

Sampling matrix	No. of samples	Sample identification code	Sample quantity
Bovine milk	40	BMI-17-001 to BMI-17-015 & BMI-18-001 to BMI-18-025	100 mL
Bovine kidney	25	BKD-18-01 to BKD-18-025	100 *g*
Bovine meat (beef)	25	BMT-18-001 to BMT-18-025	100 *g*
Ovine meat (mutton)	25	OMT-18-01 to OMT-18-025	100 *g*
Poultry meat	25	PMT-18-001 to PMT-18-025	100 *g*
Fish meat	25	FMT-19-001 to FMT-19-025	100 *g*

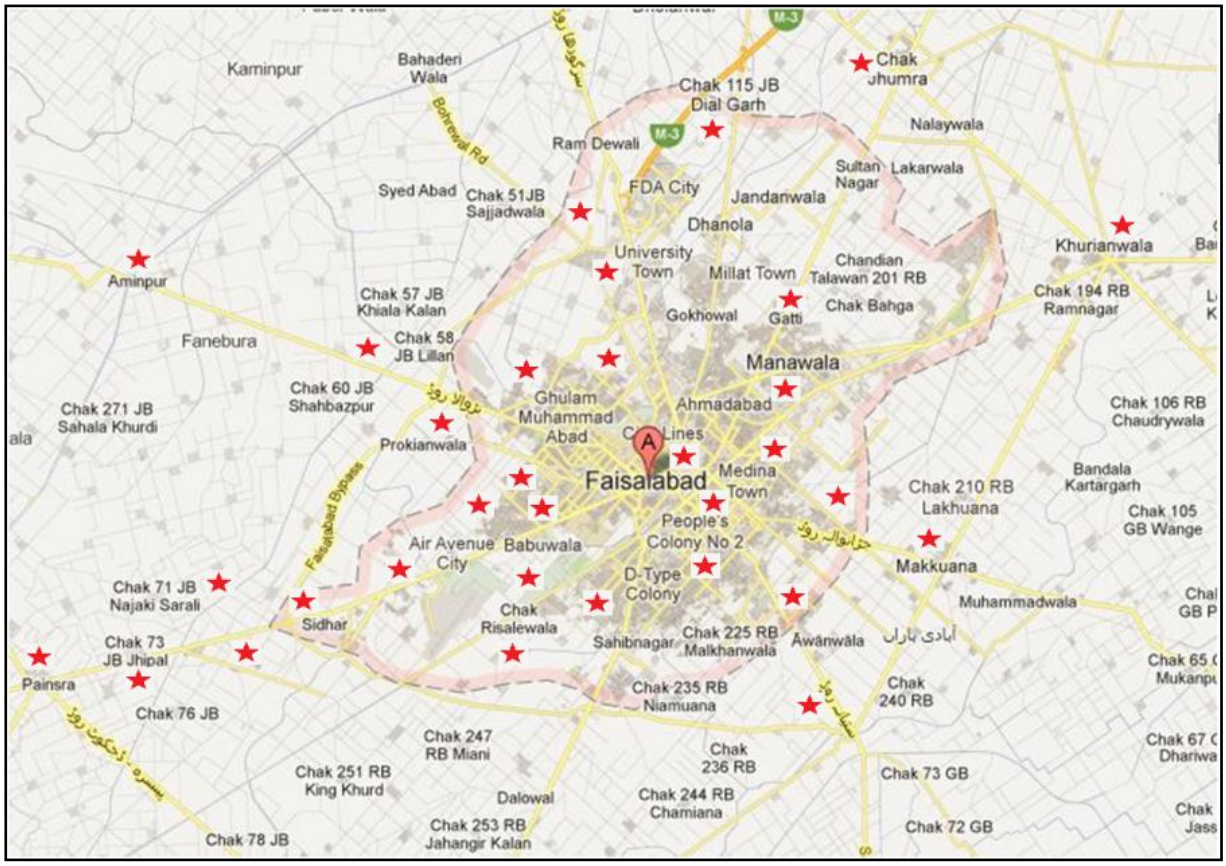

Fig. 1: Milk and meats sampling sites in district Faisalabad (Punjab), Pakistan

of each assay diluent and n-hexane. After vortexing, samples were again centrifuged at ~4000 *g* for 10 min. Upper n-hexane layer was removed and 80 μL from the lower layer was used for assay development.

Standardization and validation of ELISA

The ELISA kits (Cat. #. W81113, Quicking Biotech) were first standardized by using standards of 0, 0.025, 0.1, 0.2, 0.4 and 1.6 ng mL^{-1} and calculated inhibition concentrations (IC_{20} and IC_{50}) from the calibration curves. Then, these kits were validated by spiking known milk and tissue samples with working dilutions of CAP standards including 10, 15, 25, 50 and 75 ng mL^{-1} equivalent to 0.2, 0.3, 0.5, 1.0 and 1.5 ng mL^{-1}, respectively. Recovery percentages were calculated to check the efficiency of the kit and repeated these validation studies with one month gap up to the expiry date ELISA kits. These kits were further used in surveillance studies along with in-house developed ELISA.

Assay development

For assay development, all reagents were brought to room temperature (20–25ºC). Different CAP standards (0, 0.025, 0.1, 0.2, 0.4 and 1.6 n*g* mL^{-1}) were used for preparation of standards curve. Plate layout was designed for standards and samples placement and added 80 μL of both on plate in triplicate. The wells on edges of the plate were left empty to avoid edge well effect which may be due to the temperature variations and related differential evaporation from wells. After adding standards and samples, 50 μL of enzyme-label was added in all used wells, covered the plate with plate sealer and incubated at room temperature for 40 min. The plate was washed for 4

times with wash solution by using ELISA washer. After washing, 50 μL of each substrate-A and B solutions was added in all used wells and placed in incubator for 15 min. Finally, 50 μL of stopping solution was added to stop the reaction. The optical density was measured in microplate reader at 450 nm within 5 min after adding stop solution.

Calculations

The relative absorbance (RA) was calculated for both standards and samples by using the formula given below and Microsoft Excel was used to construct standards curve point by point. The RA of unknown samples was interpolated in standards curve to calculate the concentration of unknown samples.

$$\text{Relative absorbance (\%)} = \frac{\text{Absorbance of standards (or samples)}}{\text{Absorbance of zero standard}} \times 100$$

Health risk assessment

Health risk for CAP residues in milk and meat were estimated as the entire population approximately utilizes both products. Health risk index (HRI) was calculated by using estimated daily intake (EDI) and acceptable daily intake (ADI). For EDI estimation, mean respective food intake per person (kg day^{-1}) was multiplied by the concentration of CAP residues (μg kg^{-1}) and divided on individual average body weight (60 kg) (Balkhair and Ashraf 2016). Pakistan Economic Survey (2020–2021) reported the average consumption of bovine milk 382.2, beef 29.6, mutton 9.5, poultry meat 22.5 and fish meat 6.9 g day^{-1} capita^{-1}. As there is no information about average consumption of bovine kidney, the HRI was not calculated. There is no acceptable daily intake for CAP as it is zero tolerant that's why we have considered its MRPL 0.3 μg kg^{-1}. Health risk was measured by calculating HRI using following equations given by Hamid *et al.* (2017).

$$\text{Estimated daily intake} = \frac{\text{Residual CAP conc. x Food consumption rate (kg/day)}}{\text{Body weight for an adult (60 kg)}}$$

$$\text{Health risk index} = \frac{\text{Estimated daily intake}}{\text{Acceptable daily intake}}$$

Results

Inhibition concentrations (IC_{20} & IC_{50})

In order to generate reliable data, in-house and commercial ELISA kits (Cat. #. W81113, Quicking Biotech) were used to detect CAP residues in meat and milk samples as it is quick, cheap and method. The validation data of commercial ELISA indicated the detection limit 0.025 ng mL^{-1} with detection range from 0.025 to 1.6 n*g* mL^{-1}. The cross-reactivity with CAP is 100% and overall recovery rate is 85 ± 15%. Precision calculated as intra-assay CV < 8% and inter-assay CV < 15%. Before use in surveillance studies, these kits were first standardized by using different standards including 0.025, 0.1, 0.2, 0.4 and 1.6 ng mL^{-1} and then evaluated with approximately two months gap by calculating their inhibition concentrations IC_{20} and IC_{50} (criteria for the test performance). The values of IC_{20} were ranged from 0.05 to 0.13 n*g* mL^{-1} while IC_{50} from 0.30 to 1.0 n*g* mL^{-1} (Fig. 2). Overall, results indicated good performance of kits (as the MRPL value found between the IC_{20} and IC_{50}) while their efficiency becoming low with the passage of time towards their expiry.

Decision limit (CCα) and detection capability (CCβ)

After standardization, kits were further validated by calculating CCα and CCβ for both bovine milk and tissue samples. Forty samples for bovine milk and twenty-five of each tissue (bovine kidney, beef, mutton, poultry and fish meat), were screened for CAP residues and confirmed as negative control for the calculation of CCα and CCβ. The CCα was estimated by adding 2.33 times SD to mean concentration (calculated from standards curve). Similarly, to measure the CCβ, 20 samples of milk (negative) were fortified with CAP standard at the level of interest *i.e.*, half of the MRPL (0.15 n*g* mL^{-1}). The recovered concentrations were further used for calculation of CCβ by adding 1.64 times SD in CCα value. For milk samples, CCα and CCβ were 0.10 and 0.12 n*g* mL^{-1}, respectively. Similarly, for tissue samples, CCα was 0.09 n*g* g^{-1} and CCβ was 0.12 n*g* g^{-1}.

Recovery (%)

For recovery calculations, the known negative bovine milk (n=5) and tissue samples (n=25 including 5 of each kidney, beef, mutton, poultry and fish meat) were fortified with concentrations above and below the MRPL *i.e.*, 0.2, 0.3, 0.5, 1.0 & 1.5 n*g* mL^{-1} and extracted with methanol and ethyl acetate. Results showed that the recovery calculated from 73 to 100% in milk samples and 80 to 94% in tissue samples. The overall results indicated that the recovery (%) in milk samples was found better than the tissue samples. In milk samples, recovery decreased with the increase in spiking concentrations except the highest one while in tissue samples, recovery showed arbitrary trend with different spiking concentrations. The coefficient of variation (CV) was ranged 9 to 15% in milk samples while 13 to 19% in tissue samples (Table 2).

Monitoring of CAP residues in samples

After standardization and validation, the CAP residues was determined in bovine milk and tissue samples, collected from different locations of District Faisalabad, Pakistan. Results indicated that out of total 165 analysed samples, 140 samples were found free from CAP residues. Among 51 samples containing CAP residues, 25 samples were found positive, having concentration from 0.35 to 1.57 n*g* g^{-1}. Out of 40 bovine milk samples, 16 were found positive with maximum CAP residues 0.0.81 n*g* mL^{-1}. In bovine kidney (n=25), 4 samples found positive with CAP concentration 1.57 n*g* g^{-1}.

Table 2: Relative absorbance (RA) and recovery (%) of CAP residues in milk and meat using ELISA

Spiking conc. (n*g* mL^{-1})	No. of samples spiked (n)	Mean OD	RA (%)	Measured conc. (n*g* mL^{-1})	Recovery (%)	CV (%)
Bovine milk samples by using in-house ELISA						
0.2	5	1.090	79.52	0.20 ± 0.02	100	9
0.3	5	1.024	74.58	0.27 ± 0.03	89	11
0.5	5	0.848	61.58	0.44 ± 0.05	88	10
1.0	5	0.716	52.78	0.73 ± 0.11	73	15
1.5	5	0.642	47.52	1.19 ± 0.17	80	14
*Tissue samples by using commercial ELISA kits						
0.2	25	1.005	81.11	0.17 ± 0.03	85	17
0.3	25	0.909	73.37	0.24 ± 0.04	80	16
0.5	25	0.751	60.61	0.45 ± 0.06	90	13
1.0	25	0.615	49.63	0.81 ± 0.15	81	19
1.5	25	0.486	39.23	1.41 ± 0.25	94	18

*included 5 of each kidney, beef, mutton, poultry and fish meat

Table 3: Chloramphenicol residues in milk and meat by ELISA

Type of samples	No. of samples	Samples containing CAP (%)	Samples containing above MRPL (%) CAP	Negative samples (%)	Max. concentration o CAP (n*g g*$^{-1}$)
Bovine milk	40	16 (40)	9 (22.5)	31 (77.5)	0.81±0.04
Bovine kidney	25	9 (36)	4 (16)	21 (84)	1.57±0.09
Bovine meat (beef)	25	8 (32)	5 (20)	20 (80)	0.51±0.04
Ovine meat (mutton)	25	4 (16)	1(4)	24 (96)	0.35±0.01
Poultry meat	25	10 (40)	6 (24)	19 (76)	0.88±0.06
Fish meat	25	4(16)	Nil	25 (100)	0.12±0.03
Total (all matrices)	165	51 (30.9)	25 (15.2)	140 (84.8)	0.12-1.57

*Values are mean ± standard deviation

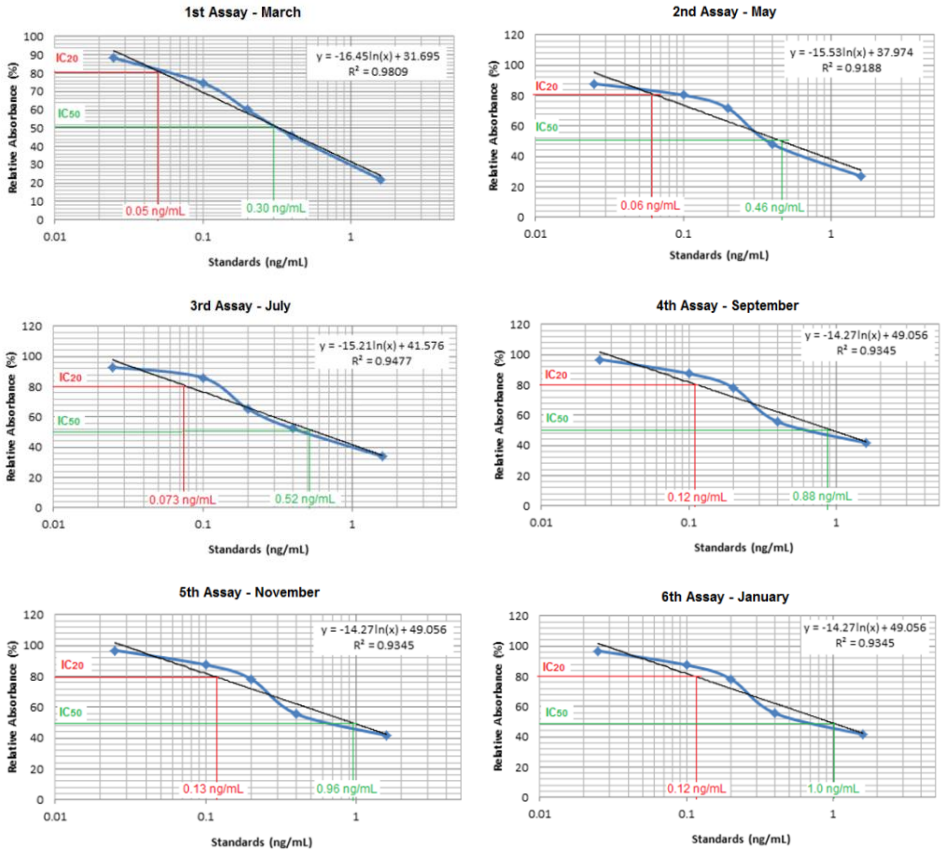

Fig. 2: Inhibition concentration (IC_{20} & IC_{50}) in different assays for test performance

Among beef samples (n=25), 5 were found positive with highest concentration 0.51 n*g* g^{-1} while only one mutton sample found positive with CAP residues 0.35 n*g* g^{-1} and all fish meat samples were found negative with highest CAP

Table 4: Detected concentration of CAP residues and HRI of milk and meat

Sample identification code	CAP conc. (μg kg^{-1})	HRI	Health risk	Sample identification code	CAP conc. (μg kg^{-1})	HRI	Health risk
Bovine milk: High risk = 22.5%, Low risk = 17.5%, Safe = 60%							
BMI-17-001	0.07	0.233	Low	BMI-18-007	0.68	2.267	High
BMI-17-002	0.39	1.300	High	BMI-18-010	0.05	0.167	Low
BMI-17-004	0.07	0.233	Low	BMI-18-011	0.09	0.300	Low
BMI-17-008	0.81	2.700	High	BMI-18-014	0.59	1.967	High
BMI-17-012	0.41	1.367	High	BMI-18-016	0.08	0.267	Low
BMI-17-013	0.35	1.167	High	BMI-18-018	0.52	1.733	High
BMI-18-001	0.12	0.400	Low	BMI-18-022	0.78	2.600	High
BMI-18-005	0.42	1.400	High	BMI-18-024	0.11	0.367	Low
Beef: High risk = 20%, Low risk = 12%, Safe = 68%							
BMT-18-002	0.04	0.134	Low	BMT-18-015	0.39	1.302	High
BMT-18-003	0.42	1.402	High	BMT-18-018	0.06	0.200	Low
BMT-18-006	0.11	0.367	Low	BMT-18-021	0.38	1.268	High
BMT-18-010	0.51	1.702	High	BMT-18-023	0.47	1.569	High
Poultry meat: High risk = 24%, Low risk = 16%, Safe = 60%							
PMT-18-001	0.68	2.267	High	PMT-18-016	0.08	0.267	Low
PMT-18-002	0.43	1.433	High	PMT-18-020	0.51	1.700	High
PMT-18-005	0.14	0.467	Low	PMT-18-022	0.19	0.633	Low
PMT-18-010	0.52	1.733	High	PMT-18-023	0.88	2.933	High
PMT-18-012	0.76	2.533	High	PMT-18-025	0.12	0.400	Low
Mutton: High risk =4%, Low risk =12%, Safe =84%				Fish meat: High =0%, Low = 16%, Safe 84%			
OMT-18-02	0.1	0.333	Low	FMT-19-005	0.08	0.267	Low
OMT-18-10	0.08	0.267	Low	FMT-19-013	0.15	0.500	Low
OMT-18-18	0.07	0.233	Low	FMT-19-016	0.11	0.367	Low
OMT-18-23	0.35	1.167	High	FMT-19-022	0.19	0.633	Low

BMI: Bovine milk; BMT: Bovine meat (Beef); PMT: Poultry meat; OMT: Ovine meat (Mutton); FMT: Fish meat
HRI: Health Risk Index; Cut-off value for HRI = 1 set at MRPL 0.3 μg kg^{-1}

concentration 0.12 ng g^{-1}. Similarly, 25 poultry meat samples were analysed and out of 10 CAP containing samples, 6 were found positive with highest CAP residues 0.88 ng g^{-1}. So, overall results showed that 84.8% samples were found negative (Table 3).

Health risk assessment

The health risk assessment associated with CAP residues in animal-derived food was done by measuring health risk index (HRI). The cut off value for HRI was set at 1 (equivalent to the MRPL). Due to zero tolerance of CAP residues, the HRI values > 1 were considered as high risk, between 0 to 1 as low risk and the samples without CAP residues were considered safe for health. Results indicated that the value of HRI surpass 1 in 25 meat and milk samples indicating potential of health risk in connection with intake of CAP residues through milk and meat consumption. Detection rate of CAP was calculated 40% in milk samples, 32% in beef samples, 16% in mutton & fish meat and 40% in poultry meat samples (Fig. 3). Among bovine milk samples, 22.5% were of high risk, 17.5% low risk and 60% safe. In beef samples, 20% were of high risk, 12% low risk and 68% safe. In mutton samples, 4% were of high risk, 12% low risk and 84% safe. Similarly, in poultry meat, 24% were of high risk, 16% low risk and 60% safe while there is no high risk involve in fish consumption as 84% safe samples (Table 4).

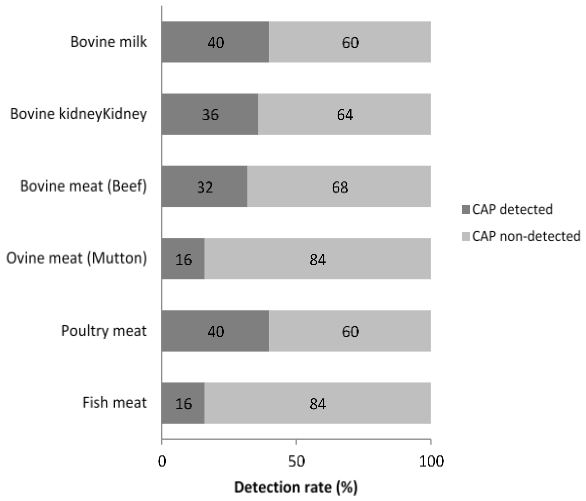

Fig. 3: Detection rate of CAP residues in milk and meats in Faisalabad, Pakistan

Discussion

The food safety concerns regarding public health issues enhanced the importance of health risk assessment for better monitoring and regulatory system. For disease and insect-pests management, a variety of veterinary medicines are extensively used in livestock and poultry sectors. So, the overuse of drugs can contaminate food products that can pose serious health issues in humans (Zhang *et al*. 2020). The CAP residues intake through food and feed not only induce aplastic anaemia in humans but also cause hepatotoxic and reproductive issues in animals, respectively (Mhungu *et al*. 2020). Therefore, the use of CAP is prohibited in food-producing animals since last 20 years in Europe and some

advanced countries (Sathya *et al.* 2020). Previous studies reported the presence of CAP residues in different food matrices like milk, meat, egg and honey, probably indicated its illegal use in farm animals. The European Food Safety Authority (EFSA) also reported that the prolonged intake of CAP residues at or above 0.3 mg kg^{-1} through contaminated food is associated with major health concerns (EFSA 2014).

The safe level for intake of CAP metabolites in food is yet not established, so it has a zero tolerance level. The EU Commission defined the MRPL as "minimum content of an analyte in a sample which at least has to be detected and confirmed" (Byzova *et al.* 2010). Various analytical methods have been developed to monitor CAP residues in different food matrices including immunochemical and chromatographic techniques like HPLC with diode array detector and GC with electron captured detector. In present study, attempts were made to analyse CAP residues in milk and tissue samples by using in-house and commercial ELISA.

Different studies indicated the use of commercial ELISA kits for the detection of CAP residues in meat and milk (Impens *et al.* 2003; Samsonova *et al.* 2010). For detection of CAP residues in tissue, ELISA protocol was developed by Bilandzic *et al.* (2011a), having detection capability and detection limit 0.23 and 0.0008 ng g^{-1} respectively with 20% CV. Similarly, Murilla *et al.* (2010) established ELISA method to detect CAP residues in ovine meat, with 70 to 92% recovery, 0.6 n*g* g^{-1} limit of detection and 1.0 ng g^{-1} detection capability. In present study, the recovery was calculated from 73 to 100% in milk samples and 80 to 94% in tissue samples. Similarly, the CCα and CCβ for milk samples were 0.10 and 0.12 n*g* mL^{-1} while for tissue 0.09 and 0.12 n*g* g^{-1}, respectively. Wang *et al.* (2010) improved 8-folds the sensitivity of ELISA method to detect CAP residues up to 0.042 n*g* mL^{-1} in milk by using a biotin-streptavidin while Zhang *et al.* (2006) achieved highest sensitivity 0.06 n*g* mL^{-1}.

Globally, the false negative (compliant) results were reported as 2.2% for milk and 0% for muscles that clearly followed the EU criteria (must be less than 5%). However, Gaudin *et al.* (2003) reported satisfactory results with 16.7% false positive samples (non-compliant) in milk and 10% in muscles. Similarly, in present study, the false complaint rate (β-error) was less that 5% as in all spiking cases.

The maximum CAP residues in present study ranged from 0.12 to 1.57 n*g* g^{-1} that were in accordance with the results reported by Bilandzic *et al.* (2011b) as the CAP concentration in milk and dairy products ranged from 0.3 to 1.27 m*g* kg^{-1} in 39 samples of Eastern Europe. Later, Ebrahimzadeh *et al.* (2014) also analysed 91 samples of chicken muscle for CAP residues, collected from local markets of Tabriz, Iran and found 28 (31%) samples with detectable concentration. Similarly, in 31 broiler chicken samples including kidney, liver and thigh muscles, collected from poultry abattoir in Mashhad (Iran), 13 samples were found positive out of 55% samples showed detectable concentration of CAP residues by using ELISA (Mehdizadeh *et al.* 2010). Yibar *et al.* (2011) also reported 15 positive samples out of 180 chicken tissue samples collected from Bursa province, Turkey with CAP concentration from 57 to 256 n*g* kg^{-1}. However, in our study, 28% chicken muscles samples containing detectable concentration of CAP residues with 8% positive samples having highest concentration 0.88 n*g* g^{-1}. The detection of CAP residues in food items in above studies indicated the alarming situation of public health due to illegal or overuse of CAP in farm animals in respective countries.

Recent studies depicted that 98% surveyed population is unaware of meat contamination through drug administration in food-producing animals (Vougat-Ngom *et al.* 2020). Due to which the probability of drug residues intake can be increased by using contaminated food products as evident in present study. The food processing techniques like cooking has significant potential to reduce the harmful effects of CAP residues in association with health risks as reported by Sensoy (2014) and Boobis *et al.* (2017). Tsai *et al.* (2019) reported the detection of CAP residues in only one shrimp tissue out of 51 samples with concentration 0.31 n*g* g^{-1}. Wang *et al.* (2021) reported CAP residues in 248 samples out of 1454 (17%) with mean concentration 19.1 μg kg^{-1}. In Chinese markets, the frequency of CAP positive samples was found high in shellfish samples with concentration above the national safety limit (Yang *et al.* 2019). Likewise in China, CAP is banned but widely used in livestock due to low-cost broad-spectrum antibiotic, easily available and weak law & enforcement to control their illegal use (Gao *et al.* 2016).

Conclusion

The data generated in present study shown the presence of CAP residues in commercial milk and edible tissues (bovine milk, bovine kidney, beef, mutton, poultry and fish meat) in concentration exceeding MRPL (0.3 μg kg^{-1}). Out of total 165 samples, 22.5% bovine milk, 16% bovine kidney, 20% beef, 24% poultry meat and 4% mutton samples found positive while all fish meat samples found negative. Health risk indices depicted that health risk surpassed 1 (which is the cut off value as equivalent to MRPL) in 25 samples, indicating the possibility of potential health risk associated with exposure to detected CAP residues through milk and tissues consumption in human beings. Regular monitoring and best management practices in the poultry and dairy farms may help to avoid or reduces the chances of contamination.

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Author Contributions

MIC planned experiments and conducted sampling, validation studies, analysis and write up. UM contributed in interpretation of results. MM and MY statistically analyzed the data and made illustrations.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable

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