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Multi-residue determination of pesticides in the meat of cattle in Faisalabad-Pakistan

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ABSTRACT

The aim of study was to determine the contamination in the meat and organs of cattle reared in pesticide spraying areas of Faisalabad, Pakistan. Because no such published information is available in this region. The meat and organs such as liver, lung and kidney were collected from villages situated within the radius of 25-35 Km on four different localities (Pensara, Aminpur, Jaranwala, and Sheikhupura roads) in the Northeast and Southwest of city during winter and spring seasons of 2009. Five pesticides (cyhalothrin, endosulfan, chlorpyrifos, cypermethrin and methyl parathion) were analyzed in the collected meat and organs (n=600) with solid phase microextraction and high performance liquid chromatography techniques. The residue analysis revealed that about 13, 21, 4, and 2 % muscle samples were contaminated with chlorpyrifos, cyhalothrin, cypermethrin and endosulfan, respectively. The concentration (ppm) of Chlorpyrifos (0.373 ± 0.001 vs. 0.297 ± 0.006), cypermethrin (2.962 ± 0.003 vs. 1.789 ± 0.228), endosulfan (12.938 ±0.007 vs. 14.487 ± 4.497) and cyhalothrin residues (4.521 ± 1.143 vs. 4.790 ± 0.933) were non-significantly different ($p>0.05$) in north east and southwest direction, respectively. Similarly, the levels of these pesticides were non significantly different in spring and winter seasons. Parathion-methyl was not detected in muscle samples. The same trend of pesticide contamination was observed in the kidney samples. Three pesticides (chlorpyrifos, cyhalothrin, cypermethrin were detected in liver and lung samples while endosulfan and parathion methyl were only detected in traces. Pesticides residues in muscle and organs were found higher than the Maximum Residual Limit (MRL) established by the international health regulatory agencies. Comparative results have indicated that chlorpyrifos, lambda-cyhalothrin, cypermethrin and endosulfan residues in muscles were about 34 times, 23 times, 47 times, and 27 times, respectively, higher than the MRL. These findings alarm a threat to the public health and suggest the need to create awareness in dairy farmers regarding the avoidance of pesticide residues in meat. (This work was supported by the Higher Education Commission, Islamabad, Pakistan.)

Key words: pesticides, residues, meat, cattle, organs

INTRODUCTION

The growing demand for enhanced food productivity to meet the needs of the global population has led to use sophisticated agriculture technology in which pesticides play a crucial role. Pesticides are extensively used to increase agricultural products by preventing, controlling, or lessening the damage caused by a pest (John *et*

al., 2001). Pesticides have been widely used througout the world since the middle of the last century. They are mainly used in agriculture and animal production, both including substances with high toxic effects and persistance in the environment (Beyer and Biziuk, 2008).

Pesticide residues in livestock generally accumulate by two ways. Either pesticides are applied to animals through insecticide-impregnated ear tag, spray, self treatment back rubber, dust bags, injectable or through pesticides spray on agricultural crops and fodder (Poppenga, 1999). Pesticide use has a positive and dramatic effect on agricultural production through protection of crops against insects, pests and diseases (Kaeew *et al*., 1996). Also for all pesticides to be effective against the pests, they must be biologically active, or toxic. Because pesticides are toxic, they are also potentially hazardous to humans, fauna, animals and environment in general (Vega *et al.*, 2005). At the same time, the livestock reared on pesticides contaminated soils, crops, and fodders may accumulate considerable residues in edible tissues. For example the accumulation of dieldrin residues in sheep from ingestion of contaminated soils was studied and it was concluded that dieldrin concentration in the fats of sheep that consume dieldrin contaminated soil fall within 10 days of removal from the source of contamination. However, dieldrin accumulates in the wool of sheep that consume dieldrin contaminated soils (Paton and Petterson, 1997).

In Pakistan, pesticides are pre-dominantly used in the provinces of Punjab and Sindh. Some pesticides are biodegradable while others persist in the soils for longer times (Tariq *et al*., 2006). Pesticide residues have been detected in the vegetables in Karachi (Perveen *et al*., 2005), in fruits and vegetable in Islamabad (Tahir *et al*., 2001) and in various tissues of fish in the local lakes (Saqib *et al*., 2005). Futhermore, pesticide residues also accumulate on cropland soil (Jabbar *et al*., 1993). Animals can accumulate these substances from contaminated feed and water. Also, due to the lipophilic nature of these pesticides, milk and other fat-rich substances are the key items for their accumulation (John *et al*., 2001).Therefore an indirect source of pesticides accumulation can be represented by animal-drived products. Such pesticides contaminated animal foods are ultimately consumed by humans and therefore these toxicants represent a serious risk for human health (Pagliuca *et al*., 2005).

In order to avoid the toxic health hazards, it is necessary to determine the levels of pesticides in edible tissues like meat, liver, lung and kidney of common food animals (cattle) which are reared on pesticides spraying area.

MATERIAL AND METHODS

Pesticides Standards:

Five pure pesticides (chlorpyrifos, cypermethrin, endosulfan, cyhalothrin and parathion-methyl) were purchased from the reagent trade chemical provider. Standard solution of 1 ppm, 5 ppm, 10 ppm, 20 ppm, 50 ppm, and 100 ppm were prepared in methanol and analysed by HPLC. Their respective peaks and areas under curve were obtained.

Area selection for meat collection

Four different direction of Faisalabad City South, West, North and East i.e., Pensara road, Ameenpur road, Shaikhupura road and Jaranwala road respectively, were selected for meat collection of cattle and goat with the radius of 25-30 Km. On each road,

five different butchers were selected, and from each butcher five meat samples were purchased.

Meat and organs collection

Meat samples were collected once in a month for six months from July, 2009 to December, 2009 and from four main roads i.e., Ameenpur road, Jaranwala road, Shaikhupura road and Jhang/Pensara road of Faisalabad city. Along each road five different spot in the radius of 25-30 km were identified for sample collection. From each spot/butcher two muscle samples (one from thigh and one of shoulder), one of liver, one of kidney and one sample of lung were purchased. During one month, 25 meat samples, of cattle were collected from one main road. And from four roads 25 x 4 (100) samples, and for duration of six months 100 x 6 (600) samples were purchased/collected.

These samples were collected in polythene zipper bag, and placed in ice-cooler during transportation. The samples were kept frozen at -4 ^{0}C until analysis.

Extraction of pesticides from meat/organs

Frozen meat/organs was thawed in water bath for half and hour, then meat was chopped in electric chopper and five grams of chopped meat was drenched with 14 ml ethyl acetate, and thoroughly mixed for 3 minute in ice cooled water bath maintaining below 10 ^{0}C. Then 2.8 g anhydrous sodium sulphate was added to this mixture, and extraction centrifuged for 2 min at 3000g. After centrifugation, the supernatant was decanted and filtered, and that filtrate was made evaporated in Petri plate. Subsequently 20 ml ethyl acetate: methanol (3:7v/v) was added and this extract was transferred to screw cap bottle (Juhler, 1997). Further extraction was done by a manual solid phase micro extraction (SPME) holder and 100 micrometer thickness dimethyl siloxane (PDMS) fiber assembly purchased from Supelco (Bellefonte, PA, USA). The fiber was immersed directly into glass vial containing the extraction for half an hour with continuous stirring arranged on magnetic starrier. Than fiber was retracted and desolated by 1 ml methanol for 15 min. The fiber was cleaned by keeping in methanol for 10, 5 and 5 mintues in separate vials before the second extraction with fiber (Gonzlez-Rodguez *et al*., 2005; Cardeal *et al*., 2006).

Analytical procedures

High-performance liquid chromatography (HPLC)

Pesticides (chlorpyrifos, cypermethrin, endosulfan, cyhalothrin and parathion-methyl) residues concentration in meat was determined by analyzing the eluted sample by High-performance liquid chromatography (Lacorte and Barcelo, 1995; Lacassie *et al*., 1998; Oliva *et al*., 1999), system consisted of a model DGU-12A, Flow controller FCV-10AL, equipped with pump LC-10AT and UV detector (SOD-10AV). The software CSW 32 was used to obtain peak of height and area under curve.

.**Statistical analysis**

After the analysis of all the meat samples, mean ± SE values were calculated and the data was subjected to ANOVA and Student's t-Test in Microsoft Excel version 2003 to see the significance ($P<0.05$) of the results.

RESULTS

Coparison between directions Pesticides residues in muscle samples of cattle collected from South-West and North-East

The levels of pesticide residues were determined in the muscle samples of cattle collected from North-East (Jaranwala and Shaikhupura roads) and from South-West (Pensara and

Ameenpur roads) of Faisalabad city. Mean ± SE values (ppm) of pesticides residues during the months of July, 2009 to December, 2009, have been presented in Table 1. It can be seen that the concentration of Chlorpyrifos (0.373 ± 0.001 vs. 0.297 ± 0.006), cypermethrin (2.962 ± 0.003 vs. 1.789 ± 0.228), endosulfan (12.938 ±0.007 vs. 14.487 ± 4.497) and cyhalothrin residues (4.521 ± 1.143 vs. 4.790 ± 0.933) are non-significant, while parathion-methyl was below the detection limit.

Table 1: Mean ± SEM values (ppm) of pesticides in the muscles of cattle collected around Faisalabad in different directions during the months of July, 2009 to December, 2009.

Location / pesticides	South-West	North-East
Chlorpyrifos	0.373 ± 0.001[a]	0.297 ± 0.006[a]
Lambda-Cyhalothrin	4.521 ± 1.143[a]	4.790 ± 0.933[a]
Cypermethrin	2.962 ± 0.003[a]	1.789 ± 0.228[a]
Endosulfan	12.938 ±0.007[a]	14.487 ± 4.497[a]
Methyl-Parathion	N.D.	N.D.

Note: Similar letters in superscript are non significantly different (p>0.05)

Comparison between seasons

Pesticides residues in muscle samples of cattle in summer and in winter

The concentration of pesticides residues were determined in the muscles samples of cattle collected around Faisalabad city during summer (July, August, and September) and winter (October, November and December) seasons. Mean ± SE values (ppm) of pesticides residues are given in Table 2. The concentration of Chlorpyrifos (0.354 ± 0.003 vs. 0.342 ± 0.009), cypermethrin (2.264 ± 0.839 vs. 2.711± 0.229), endosulfan (12.933 ±0.006 vs. 14.022 ± 7.718) and cyhalothrin (4.476 ± 0.907 vs. 4.840 ± 0.997) is non-significantly (p>0.05) different from each other, while parathion-methyl was not detected.

Table 2: Mean ± SE values (ppm) of pesticides in the muscles of cattle collected around Faisalabad in different seasons during the months of July, 2009 to December, 2009.

season / pesticides	**Summer**	**Winter**
Chlorpyrifos	0.354 ± 0.003[a]	0.342 ± 0.009[a]
Lambda-Cyhalothrin	4.476 ± 0.907[a]	4.840 ± 0.997[a]
Cypermethrin	2.264 ± 0.839[a]	2.711± 0.229[a]
Endosulfan	12.933 ±0.006[a]	14.022 ± 7.718[a]
Methyl-Parathion	N.D.	N.D.

Note: Similar letters in superscript are non significantly different (p>0.05)

Pesticides residues in kidney samples of cattle in summer and in winter

The concentration of pesticides residues were determined in the kidney samples of cattle collected around Faisalabad city during summer (July, August, and September) and winter (October, November and December). Mean ± SE values (ppm) of pesticides residues are given in Table 3. Comparative values of Chlorpyrifos (0.342 ± 0.001 vs. 0.328 ± 0.013), cypermethrin (2.025 ± 0.001 vs. 2.554 ± 0.401), endosulfan (14.982 ± 1.002 vs. 14.774 ± 0.911) and cyhalothrin (4.517 ± 0.659 vs. 4.551 ± 0.323) are non-significantly (p>0.05) different from each other, while parathion-methyl was below the detection limit.

Table 3: Mean ± SE values (ppm) of pesticides in the kidney of cattle collected around Faisalabad during the months of July, 2009 to December, 2009.

season / pesticides	**Summer**	**Winter**
Chlorpyrifos	0.342 ± 0.001[a]	0.328 ± 0.013[a]
Lambda-Cyhalothrin	4.517 ± 0.659[a]	4.551 ± 0.323[a]
Cypermethrin	2.025 ± 0.001[a]	2.554 ± 0.401[a]
Endosulfan	14.982 ± 1.002[a]	14.774 ± 0.911[a]
Methyl-Parathion	N.D.	N.D.

Note: Similar letters in superscript are non significantly different (p>0.05).

Pesticides residues in liver samples of cattle in summer and in winter

The concentration of pesticides residues were determined in the liver samples of cattle collected around Faisalabad city during summer (July, August, and September) and winter (October, November and December). Mean ± SE values (ppm) of pesticides residues are given in Table 4. Comparative values of Chlorpyrifos (0.344 ± 0.010 vs. 0.379 ± 0.001), cypermethrin (2.482 ± 0.496 vs. 2.48 ± 0.514) and cyhalothrin (4.389 ± 0.729 vs. 4.144 ± 0.481) are non-significantly (p>0.05) different from each other while endosulfan and parathion-methyl were not detected.

Table 4: Mean ± SE values (ppm) of pesticides in the liver of cattle collected around Faisalabad during the months of July, 2009 to December, 2009.

season / pesticides	Summer	Winter
Chlorpyrifos	0.344 ± 0.010[a]	0.379 ± 0.001[a]
Lambda-Cyhalothrin	4.389 ± 0.729[a]	4.144 ± 0.481[a]
Cypermethrin	2.482 ± 0.496[a]	2.48 ± 0.514[a]
Endosulfan	Traces	Traces
Methyl-Parathion	N.D.	N.D.

Note: Similar letters in superscript are non significantly different (p>0.05)

Pesticides residues in lung samples of cattle in summer and in winter

The concentration of pesticides residues were determined in the lung samples of cattle collected around Faisalabad city during summer (July, August, and September) and winter (October, November and December). Mean ± SE values (ppm) of pesticides residues are given in Table 5. Comparative values of Chlorpyrifos (0.353 ± 0.002 vs. 0.354 ± 0.008), cypermethrin (1.999 ± 0.018 vs. 2.039 ± 1.772) and cyhalothrin (4.643 ± 1.388 vs. 4.583 ± 0.632[a]) are non-significantly (p>0.05) different from each other while endosulfan and parathion-methyl were not detected.

Table 5: Mean ± SE values (ppm) of pesticides in the lung of cattle collected around Faisalabad during the months of July, 2009 to December, 2009.

season / pesticides	Summer	Winter
Chlorpyrifos	0.353 ± 0.002[a]	0.354 ± 0.008[a]
Lambda-Cyhalothrin	4.643 ± 1.388[a]	4.583 ± 0.632[a]
Cypermethrin	1.999 ± 0.018[a]	2.039 ± 1.772[a]
Endosulfan	Traces	Traces
Methyl-Parathion	N.D.	N.D.

Note: Similar letters in superscript are non significantly different (p>0.05)

Percentage of contaminated samples.

All the samples of muscles and organs of cattle were not contaminated but a few samples were found to be contaminated with pesticides residues which are given in Tables 6. About 13%, 25%, 4% and 2% muscle samples of cattle and goat were contaminated with chlorpyrifos, lambda-cyhalothrin, cypermethrin and endosulfan, respectively.

Table 6: The percentage of muscles and organs samples of cattle found to be contaminated with pesticides

pesticides / sample	Chlorpyrifos (%)	Lambda-Cyhalothrin (%)	Cypermethrin (%)	Endosulfan (%)	Methyl-Parathion (%)
Muscles	12.91	20.83	4.16	2.08	Traces
Kidney	18.33	24.16	5	5.83	N.D.
Liver	14.16	21.66	4.16	1.66	N.D.
Lung	15	25	4.16	2.5	N.D.

Level of pesticides residues.

Muscles and organs samples of cattle and goat contaminated with pesticides residues contain different concentration of residues. However, these samples contain pesticides residues higher than the Maximum Residual Limit (MRL) established by United States Food and Drug Administration (USFDA). Chlorpyrifos, lambda-cyhalothrin, cypermethrin and endosulfan are about 34 times, 23 times, 47 times, and 27 times respectively, increased than the MRL. The Maximum Residual Limit (ppm), level of pesticides residues detected in samples and the difference that how much they are increased are given in Tables 7.

Table 7: Increased concentration of pesticides than the Maximal residual limit in meat sample of cattle

Pesticides	Maximal residual limit (ppm)*	Detected average concentration (ppm)
Chlorpyrifos	0.01	0.341
Lambda-Cyhalothrin	0.2	4.655
Cypermethrin	0.05	2.397
Endosulfan	0.5	13.381
Methyl-Parathion		

* Values obtained from USFDA

DISCUSSION

Chlorpyrifos in cattle

The mean levels of Chlorpyrifos residues in meat samples of cattle collected from north-east were non-significantly different than those collected from the south-west of Faisalabad city, 0.297 ± 0.006 ppm, and 0.373 ± 0.001 ppm, during the months of July, 2009 to December, 2009 (Table 1). These values of present study are higher than their corresponding values in literature. No difference in concentration of chlorpyrifos was observed in muscle, kidney, liver and lung of cattle during summer and winter, while Pagliuca *et al*. (2006) reported the progressive decrease of positive samples from Autumn to Spring and their results was confirmed by Gazzotti *et al*. (2008). Chlorpyrifos was found almost 15% of the analyzed samples and its detected value was many times more than the MRL value established by USFDA. In some studies conducted in milk the

main pesticide pollutant was chlorpyrifos (Gazzotti *et al*., 2008) and its contamination ranged from 5 to 18 mg/kg. In a bioaccumulation experiments, fish meat was detected to accumulate chlorpyrifos up to 5.9 ng/g (Varo *et al*., 2002).

Lembda-cyhalothrin in cattle

The values of Lembda-cyhalothrin residues in cattle meat from north-east are higher than those in the meat from south-west of Faisalabad city, 4.790 ppm and 4.521 ppm, respectively (Table 1). Lembda-cyhalothrin concentration remained same during summer and winter seasons in muscle, kidney, liver and lung samples of cattle. Lembda-cyhalothrin was proportionally highest in frequency in meat as compared to other pesticides and was detected almost 25% of samples, this high frequency could be explained by its wide use as an insecticide and ectoparasite on livestock (Gazzotti *et al*., 2008), contrary to a study conducted in porcine muscle and pasteurized milk samples collected from major cities in the Republic of Korea where no residues of the selected pesticides were detected in any of the samples whereas limit of detection was ranged from 3.3 to 9 and 3 to 8.1 ppm, in porcine muscle and pasteurized milk respectively (Khay *et al*., 2009). Its detected level was many folds high than the MRL established by USFDA.

Cypermethrin in cattle

The values of Cypermethrin residues in cattle meat from north-east are lesser than those in the meat from south-west of Faisalabad city, 1.789 ppm and 2.962 ppm, respectively (Table 1). Khay *et al*., (2009) analyzed muscle samples and could not find cypermethrin in detected amount. While in our study, cypermethrin concentration was high in winter season than in summer season in muscle, kidney and lung samples of cattle. It could be due to evaporation and degradation of cypermethrin from fodder and field in summer season under high temperature. Cypermethrin was found in 5% of samples and their value was also higher than the MRL.

Endosulfan in cattle

The levels of endosulfan in the meat from north-east were higher than the corresponding value in the south-west of Faisalabad city, 14.487 ppm and 12.938 ppm, respectively (Table 1). Mean endosulfan concentration in the meat of cattle was 14.764 ppm ranging from 12.938 ppm – 15.147 ppm. The fodder might have heavy concentration of endosulfan and it has high capacity to deposit in edible tissues. These values of present study are lower than their corresponding values in literature. Darko and Acquaah, (2007) reported the levels of endosulfan in the beef as 2.28 ppm and 0.59 ppm in fat and in lean beef, respectively (Table 2.1). Endosulfan was detected in 18 out of 20 (90 %) of the beef samples (Darko and Acquaah, 2007) but in current study the frequency was very low 2.08 % in the tissues but endosulfan concentration was very high almost 15ppm as compared to MRL of endosulfan which is 0.5 ppm (USFDA) in cattle meat and it was surpassed by many times in all meat samples analyzed in the current study. However, the highest level of endosulfan detected was 105.58 µg/kg (Darko and Acquaah, 2007).

Methyl-Parathion in cattle

In meat samples of cattle methyl-parathion were below the detection limit or detected in traces. This is because methyl-parathion is banned throughout Pakistan due to its hazardous effect on human health. However it was found in only 2 milk samples out of 298 samples analysed for methyl-paration contamination and

detected level was 5mg/kg (Gazzotti *et al*., 2008).

In present study, the higher meat concentration of pesticides residues can be attributed to the use of contaminated fodder with pesticides. It has also been observed that the animals have direct access to this contaminated fodder. The uptake of pesticides residues in the soil, vegetables, fodder and other herbage produced in the north-east and south-west of Faisalabad city may have a definite role in the contamination of the meat composition. The standard residual risk in the meat of cattle and goats with reference to the concentration of pesticides has not yet been established at the national level. However, it is certain that meat from various species might have a certain acceptable range of concentration for pesticides. The current data suggests that the meat contamination have its subsequent effect on the human and animal body. The findings of the present study show that the chlorpyrifos, cyhalothrin and cypermethrin residues in the meat of cattle and goats collected form south-west were higher as compared north-east to their related values in of Faisalabad city. The difference may be attributed to the presence of slaughter house on this direction as farmer has access to slaughter house so they are rearing more animal and spraying more pesticides to fetch the need of fodder for animal.

CONCLUSIONS

The residues of chlorpyrifos, cypermethrin, lambda-cyhalothrin and endosulfan in the meat of cattle in present study are higher than the available MRL in the literature and the most reported values.

The meat samples of cattle from three sites in south-west are having slightly higher levels of residues of chlorpyrifos, cypermethrin, lambda-cyhalothrin and endosulfan than those collected from three sites in north-east of Faisalabad city.

The residues of chlorpyrifos, cypermethrin, lambda-cyhalothrin and endosulfan in the meat of cattle remained same in summer and in winter seasons.

Recommendations

The concentration of all pesticides residues found in the current study is alarming. The higher concentration of pesticides residues poses a potential threat to both livestock and human beings. Our data deserves a particular attention. All those products which surpass the permissible limits may be declared unhygienic for their associated toxicological risks. Both the public and private sectors must cooperate in tackling the problem jointly.

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