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Determination of Pyrothyroid (Deltamethrin) Pesticides and Ivermectine Residues in Local Sheep Milk and Mutton by High Performance Liquid Chromatography (HPLC)

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Abstract

Food safety remains a major concern for society, applications to protect milk and animal productions from chemical residues by using precise methods to detect the smaller levels of these residues. The identification of DLM and IVM residues in 80 samples (40 each sheep milk and mutton) collected from different area and slaughterhouses shop of wasit was performed. Production was monitored by using HPLC technique to determine the degree of DLM and IVE residues contamination in milk and mutton from April to July of 2018. The analysis showed that 92.5 % of the milk and 90% of mutton samples were contaminated above MRLs with DLM residues ,but all samples 100% (milk and mutton) were positive for IVM residues at the period of study .The results of milk and mutton revealed were 100% violation of the MRLs (above 0.026) according to MRLs Permissible by the WHO and FAO from DLM and show a significant difference ($P<0.05$) between July and April ,May and June in sheep milk and mutton (0.95 ± 0.04,0.83 ± 0.10,0.62 ± 0.08, and0.82 ± 0.16), (1.34 ± 0.11,0.36 ± 0.06, 0.62 ± 0.12 and 0.86 ± 0.10) respectively , but in IVM residues (ppm) result show found significant difference ($P<0.05$) in sheep milk between June and April ,May and July (0.83+0.12, 0.45+0.08, 0.69+0.12 and 0.73 + 0.10) ,and between July and April ,May and June in mutton (1.2 ± 0.08, 0.84± 0.09, 0.78±0.08 and 0.99±0.09). The results of this study confirm the need for monitoring programs for the residues of pesticides and ecto-parasites in animal products to protect the consumer health from the dangers of exposure to these remains.

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Introduction

Currently, Increased concerned of consumer about the present of chemical substances used in animal husbandry in animal's production (milk, egg and meat), these residues represent challenge to the animals industry. Alarge number of drugs and other chemical substance used in animals sector directly administration or indirectly. Veterinarians and producers have used animal medications approximately, 42% as growth promoters to improve feed utilization and production, 19% as anti-infectives, 13% as parasiticides, 11% as biologicals and 15% represent other pharmaceuticals [1].

Some studies report that animal origin food is responsible for 90% pesticides entry in the human body [2]. The cause of food contaminated can come from produce at the ranch level, animal's production transportation, storage and manufacturing for consumer [3].

The outcome of random use of pesticides and veterinary drugs in animals resulted in drug residues that could be found at different concentration levels in products from animal origin, [1], especially in milk when the farmers not respected withdrawal [4].

Also, animals fed and water can be contaminated by exposed to pesticide residues indirectly by spray animals year and the ingestion pesticide [5]. Pesticides after ingested, are metabolized and bio-accumulation in fat and muscles tissues. They might be also secreted into the milk depend lipophilicity character [6].

Ivermectin (IVM), a macrocylic lactone belonging to the Ivermectin group of chemicals, a class of ecto-parasitic drugs derivative from the nonpathogenic microorganism live in soil Streptomyces avermitilis. It is commonly used for the treatment of diseases caused by ecto-endo parasites in various species (sheep, cattle and swine, equine) [7]. It is a mixture of the two homologous compounds 22, 23- dihydroavermectin (H2B1a, at least 80%) and 22,23- dihydroavermectin (H2B1b, not more than 20%) [8]. The IVM molecule has high liposolubility, this fact is important because their excretion could be by milk [9].

Deltamethrin (DEL) is a common name for the synthetic pyrethroid insecticide S-cyano-3-phenoxybenzyl-cis-(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate,) derived from natural pyrethrins (esters of chrysanthemic and pyrethric acid extracted from chrysanthemum flowers, Chrysanthemum cinerariaefolium, and related species) [10]. Deltamethrin largely used in Iraqi provinces for sheep, goat, buffalo and cattle dipping or spray as well as agriculture formulations to control numerous insect pests on fruits, vegetables, and field crops by the veterinarians and farmers [11]. Pesticide residues have greater impact on human diet so contamination with these residues checked with greater concern in meat, milk and milk products [12].

Countries have enacted regulations to set the Maximum Residue Level (MRL) in milk and animal products to protect the consumers' health [5]. The National Residue Program (NRP) is an important part of the national system responsible for managing the risk of residual veterinary drugs and environmental contaminants in animal-derived food products [13]. Several NRPs have been carried out worldwide and their results have led to improvements in food safety [14].

Some countries developed monitoring programs in order to detect the presence of IVM residues in foods of animal origin. In that way, increased Monitoring the presence of these compounds has an important role in quality control of food, and demands a method capable of detecting these compounds in smaller levels. For quantitative analysis it is necessary to use instrumental techniques such as thin-layer chromatography, liquid chromatography, and immunochemistry However, the most important approach is based on High-Performance Liquid Chromatography Method (HPLC) [15].

During the recent years, chromatography technique has undergone a rapid development and high performance liquid chromatography (HPLC) is one the achievement. The detection by these techniques is simple, rapid and sensitive. HPLC with a high sensitivity is widely used in the quantification of pyrethroid pesticides and drugs.

Material and methods

Sampling

A study was done to check Deltamethrin pesticides (DLM) and Ivermectin (IVM) residues in different sheep milk and mutton samples will obtained from different small farms and shops of Wasit province. A total of 80 sheep milk and mutton samples (40 of each) were obtained. Raw milk samples (500 mL) were collected directly from udder ,all samples came from declared healthy animals by the owner and this milk is intended for human consumption or is delivered to different dairies as well as from mutton samples(weighing 500 g each) including muscle and fat from different parts of carcass were obtained from slaughterhouses and butcher shops in the different location. All samples were analyzed to determined Daltamethrine (DM) and Ivermectine (IVM) residues between April and July 2018. Samples were recognized, put up in polypropylenes bags, and sent to the laboratory immediately to analysis. All Samples were processed and analyzed for the determination of concentration of (DLM) and (IVM) residues by using High Perfeormancei Liquid Chromatography (HPLC) apparatus made of Shimadzu Corporation, Japan.

Preparation of standard curves

Stock standards solution of (DLM) and (IVM) were making by dissolve 10 mg of (DLM) and (IVM) in 0.1L of methanol to get a concentration 1 $mg.ml^{-1}$. Stocker standard solution were storage at refrigerator (-20°C) for three month.

Sample preparation and separation

Take 10ml of acetonitrile and 10ml of raw mill were put into a 50 ml centrifuge tube and then mixed was shaken for 1 min manually. Vortex mixing for 2 min, than added sodium chloride (1 gm) and magnesium sulphate (4gm). Samples were centrifugation at 4000 rpmi for 15 min after shaking for 2 min to prevented agglutination forming during magnesium sulfate hydration. The supernatant was transferred and filtrated by syringe filter to HPLC vials for analysis.

Ten gram of minced mutton sample were mixed for 5 min in a homogenizer device after addition of 20 ml acetonitrile in a 50 ml test tube for half an hour to shake well. Centrifuged the homogenized mixture at 4000 r/min for 10 minutes. Then put in ultrasonically for 12 minutes to extracted and then put centrifugation for 5 minutes, the supernatant remove and were saved in a clean glass container (250 ml) and evaporated till drying The dried residue was reconstituted with 1 ml acetonitrile and filtered through 0.22 µm nylon syringe filter. Then 20 µl were knuckle under to HPLC analysis.

Statistical analyses

Data were analyzed of each materials in each period using SPSS 23.0 for Windows. Least Significant Difference (LSD) among different group means at 5% level was applied.

Results

Determination of (DLM) residue in sheep milk and mutton

The distribution level of DLM residues determined in the analyzed sheep raw milk and mutton samples obtained during period of study is illustrative in (Table 1). April, May and July were detected in 100% above the MRLs of the analyzed sheep raw milk with range (0.45-1.17, 0.50-1.10 and 0.67-1.18) (ppm) respectively, but June were detected 70% above the MRLs with range (0.02-1.21 ppm). April and May were detected in 80% above the MRLs of the analyzed mutton sample with rang (0.01-0.74 and 0.01 -1.01 ppm), but June and July were detected 100% above the MRLs with range (0.29-1.20 and 0.29-1.74 ppm).

The maximum level of DLM in sheep milk was record 1.18 ppm, but mutton was record 1.74 ppm in July.

In total, 40 sample (for each milk and mutton) were randomly collected during the study period of April to July, 2017. Most samples (n = 37; 92.5%) (n = 36; 90%) contained detectable residue levels of DLM exceeded the permissible limit in samples.

Table 1: The residual levels (ppm) of Deltamethrin detected in sheep raw milk and mutton.

	Fresh raw milk				
Periods	**No. sample**	**+ve**	**-ve**	**Range**	**%violation MRLs=0.05**
April	10	10	0	0.45-1.17	100%
May	10	10	0	0.50-1.10	100%
June	10	7	3	0.02-1.21	70%
July	10	10	0	0.67-1.18	100%
Total	40	37	3		92.50%
Mutton					
Periods	**No. sample**	**+ve**	**-ve**	**Range**	**%violation MRLs=0.026**
April	10	8	2	0.01-0.74	80%
May	10	8	2	0.01 -1.01	80%
June	10	10	0	0.29-1.20	100%
July	10	10	0	0.29-1.74	100%
Total	40	36	4		90%

Table 2: The residual levels (ppm) of Ivermectine detected in sheep raw milk and mutton.

	Fresh raw milk				
Periods	**No. sample**	**+ve**	**-ve**	**Range**	**%violation MRLs=0.01**
April	**10**	10	0	0.5-1.13	100%
May	**10**	10	0	0.3-0.72	100%
June	**10**	10	0	0.21-0.85	100%
July	**10**	10	0	0.50-1.18	100%
Total	40	40	0		100%
Mutton					
Periods	**No. sample**	**+ve**	**-ve**	**Range**	**violation % MRLs =0.02**
April	**10**	10	0	0.040-0.60	100%
May	**10**	10	0	0.51-0.78	100%
June	**10**	10	0	0.75-0.97	100%
July	**10**	10	0	0.90-1.4	100%
Total	40	40	0		100%

Determination of (IVM) residue in sheep milk and mutton

The results showed that all the sheep raw milk samples which 100% exceeded the maximal limits residues of WHO and FAO for IVM residues in sheep milk (MRLs> 0.01ppm), (Table 2).

The results of HPLC revealed all regions were exceeded the normal levels MRLs (0.02), which violate the legal levels of WHO/FAO in mutton (Table2).

The highest concentration levels (ppm) of IVM residues in the sheep raw milk and mutton samples were recorded during the July month.

Concentration levels of DMT and IVM residues in sheep milk and mutton during months

The mean residual levels of DLM and IVM detected by HPLC in sheep raw milk and mutton during the months of the study (April, May, June and July) is shown in (Table 3).

The results of HPLC analysis revealed the mean concentration levels of DLM and IVM from all months of study were above the allowed levels as it was stander by the WHO and FAO (MRLs > 0.026.ppm) (MRLs > 0.01.ppm in milk ,0.02 ppm in mutton) respectively , IVM residues was highest followed by DLM in sheep raw milk and mutton

Table 3: Concentration levels of DMT and IVM residues (ppm) (Mean ±SE) in sheep milk and mutton samples during the study periods (months).

Residues		**April**	**May**	**June**	**July**	**LSD**
DLM	Sheep Raw Milk	0.83 ± 0.10 b	0.62 ± 0.08 c	0.82 ± 0.16 b	0.95 ± 0.04 a	0.11
	Mutton	0.36 ± 0.06 d	0.62 ± 0.12 c	0.86 ± 0.10 b	1.34 ± .11 a	0.10
IVM	Sheep Raw Milk	0.45 + 0.08 c	0.69+0.12 b	0.83 + 0.12 a	0.73 + 0.10 b	0.09
	Mutton	0.84 ± 0.09 c	0.78 ± 0.08 d	0.99 ± 0.09 b	1.2 ± 0.08 a	0.12

Deltamethrin residues in raw milk samples and mutton of different months recorded a significant (P<0.05) monthly variation values , July recorded higher value than April ,May and June in milk samples and mutton (0.95±0.04, 0.83± 0.10 , 0.62±0.08 and 0.82±0.16 ppm respectively) (1.34±.11 , 0.36±0.06 , 0.62±0.12 ,and 0.86±0.10 ppm respectively).

From obtained results, Ivermectin residues show that there was a significant (P<0.05) monthly differences in milk samples and mutton, June had showed higher value significantly (P<0.05) than April ,May and July from milk (0.83+0.12, 0.45+0.08, 0.69+0.12 and 0.73+0.10 ppm respectively). But July had showed higher value significantly (P<0.05) than April, May

and June from mutton (1.2±0.08, 0.84± 0.09, 0.78±0.08 and 0.99±0.09 ppm respectively).

Discussion

The HPLC method was used to separate and detection the residual DLM from raw milk and mutton. A total of 40 sample of sheep (each milk and mutton) examined show the presence of DLM residues (Table 1). The proportion of positive samples exceeds the stander levels of MRLs define by WHO and FAO was 100%, 100%, 70% and 100% in the raw milk during study period. This finding has similarities with the report of occurrence of DLM residues may be attributed to the overdose of DMT used by the farmer .Generally, farmers believe that using high concentration of drug is better to killed ticks and other parasite. Secondary, farmer have receive were less educated and did not abide by veterinarians instruction, often likewise, the notification by National Pesticide Information Center [16] has reported the same data ,where they have illustrated that the quantities of the residues of pesticide may stay in the animal production more than the half -life depend on doses applied pesticides.

Furthermore, farmers can prevent infestation in the animal's yard using pesticide spray. Consequently, using pesticide directly on animals feed and spraying cause to bio-assemblage of pesticides in animal tissue and products [17].

Ul-Hassan etal., [18] used HPLC technique to detect pesticide residue in milk sample was showed that 70% milk sample excessed level of pesticides residuce. Overall results indicated that the percentage of contaminated milk sample with Deltamethrin were 7%.

Shahzadi [19] reported that the percentage of contaminated milk sample with pyrethroid residues present in 50% of samples (milk). Most significantly present pesticides were Deltamethrin and maximum contamination was found in sheep milk was 0.02-0.80 mg/kg.

Ombui [20] study 231 milk sample, found 73% deltamethrin residue contamination.

Abd Al-Zahra and Najim [21] revealed that milk samples that were collected from buffaloes, ewes recorded significantly differences ($P<0.05$) the deltametrin residues compared cow and camel such results could be attributed to the higher fat content of buffalos and ewes milk than the other animals as well as the lipophilic nature of the deltamethrin.

The proportion of positive samples exceeded the permissible MRLs that recommended by two organization (WHO and FAO) was 80%, 80%, 100% and 100% in the mutton during study period.

These results agreed with Sallam and Morshedy [22] whom found in Egypt meat of sheep cattle and camel highest the MRLs of Daltamethrine.

Also, the DMT residues in meat of sheep exceeded the allowed level as it was stander by Heitzman [23]. Furthermore, the above mentioned findings in consistent with Abdurrahman [11] result who reported the violation of DMT allowed levels in sheep and goat meat in Iraq. The same was found the experimented of Muhammad etal. [24] which conducted in Pakistan on cattle meat.

Hawazin [25] experimented in beef meat sample, who found 100% positive of DMT residues of and there was 2253% exceeded of the maximum levels (0.026) recommended by the WHO and FAO.

In contrary, our result disagree with Misra etal. [26] findings regarding the cow and poultry meat alow levels of DMT under the threshold levels of by WHO/FAO, and [27] for beef and mutton .

In addition, no pyrothyroid remains were found the cattle of Uganda, it is show by Turyahikayo [28].

The HPLC analysis shown that all the 40 samples(each milk and mutton/monthly) were positive for IVM residues 100% above the permissible MRLs that established by the WHO and FAO (Table 3(.This indicates that non observance of withdrawal requirements following drug therapy the main factor considered to underlie the presence of the residues.

Using Ivermectin to treat cattle is seasonal depending the perception of both farmers and veterinarians. The excretion [29].

The excretion of IVM in ewes milk depend on its character of lipophilicity during subcutaneously treatment, the residence time of IVM is long indicating the long shelf preservation after administration .the finding are caused by the economic cost ofIVM and illegal usage of the drugs without restrictions, and farmers buy drugs and treated their animals themselves.

One study found that usage of 0.2 mg/kg BW ivermectin subcutaneous produced milk residues above 5 ppb for 16 day, with a milk elimination half-life of 4.7 day [30].

Milk samples contained detectable Ivermectin residues for 10 day, with maximum residues occurring 3-4 d post-treatment [31]. Similar data were found from earlier trials in which the same dose was used with milk samples from 6 Holstein and 6 Jersey cows, all of which contained residues 9 d post-treatment [31].

In 2005/2006, violative endectocide residues were found in only 2.5% of random raw milk samples (n = 400) tested by the CFIA [32] 5 samples contained ivermectin (range 0.2 to 6 ppb).

Anastasio and coworkers [33] studied the ivermectin in buffalo milk, where they gave (0.2 mg kg-1 b.w.) to buffalo cattle and show that the IVM levels is reach to (23.6 ± 2.6 ng mL-1) after 2.8 ± 0.44 in milk.

The accurate detection of small levels of chemical residues in livestock production is not only of benefit for governmental control, animal industry and laboratories but also for owners to enable them to ensure that individual animals contaminated with residues is not used to consumer [34].

However, all raw milk samples showed a 100% contamination with ivermectin in raw milk from and mutton above the maximum residue limit. The contaminated levels of drugs in each country, depending on the law of legislation and methods applied in different countries [35].

Many studies, conducted to detected attributed anthelmintic residues in the milk and livestock production in Europe. The study found (0.8 -3%) contaminated with these drugs, they correlated with governmental control in Europe.

In most cases, the carcasses slaughter the livestock without taking into consideration the withholding periods of drugs.

Ivermectin application at the label dose for mutton will cause detectable milk residues that may persist for an extended period.

In sheep meat, HPLC analysis show differ between July and other months of study (0.99 ± 0.18) and (0.50 ± 0.08) respectively. Many reasons correlated with these results included ,sheep shearing occur in Iraq during june lead to decrease absorption of ectoparasit drug via skin after dipping and the animals lesser wool need short time from drying. The above mention finding in consistent with David and Greg [36] resulted sheep with longer wool exposure with ectoparasite residues risks.

The hot temperature during the June -July leads to more animal sweating, vasodilatation and higher absorption of pesticides [37]. Revealed the quantities of pesticides residues in soil during summer and spring more than other season these finding related with higher applied pesticides during this season [38].

Stroud [37] who mention livestock dipping July month higher than in June, can cause problem because animals suffer from heat strees and this act as main cause for the absorption of higher levels of DMT in sheep and goat via skin.

Furthermore, in July, represented of wheat harvest and farmers using pesticide to protect crop, animals are usually fed on straw, and residual wheat after harvesting; this may also lead to exposure animals to the high level of pesticide and bio-accumulation in products.

These result agreement with Jermannaud and Pochon [39] whom found pork and poultry meat contaminated with DMT after animals fed on wheat and hay treated with DMT.

Another cause were attributed by Vijaya and Ravindra [40] that the using of ectoparasite drug during control season of insects, fly and external parasites on animal pen. The main sources of contaminated beef meat with DMT mentioned by Aydin and coworkers [41] is that water contaminated with pesticide during spraying animal without any precautions to protect animals water and absence of typical area for pesticides usage .

in Iraq usage DMT from treats of Ommatissus lybicus Deberg (spring generation) that infect palm tree, this may be led to increased accumulation of DMT residues in soil and effect indirectly to animals by fed of. The causes of differences between mount might be due to farmers low education and without acknowledge withdrawal period of pesticide and veterinary drugs.

Anwar etal., [42]. Whom found no differences in year month and season because Use a certain type of pyrethroid and no different between the ambient temperature in Pakistan.

Since the amount of Ivermectin used at different times of the year is expected to be variable in milk and tissues. Dairy producers quite literally make their living milking cow in most cases, their financial well-being relies on robust milk production, and one thing they cannot afford is to stall or halt milk production because they treated their lactating herd with ivermectin.

Chiu etal., [43]. Show the fatty tissue more residue concentrated of Ivermectin than muscle of rats, sheep and cattle.

Ivermectin application at the label dose for mutton will cause detectable milk residues that may persist for an extended period.

Metabolism rates vary from individual to individual, thus withdrawal times can vary according to each individual animal's ability to metabolize (process, detoxify, get rid ofthe drug). An animal with a compromised immune system will take longer to metabolize a toxin thereby resulting in a longer withdrawal time.

Conclusion

To ensuring health quality is a main part of animal production arrives at the consumer after undergoing various process in the industry, Chemical substances applied in animal husbandry and in crop protection represent substantial environmental loads. Instrumental techniques and accurate detection of chemicalization residues at small levels to use of HPLC is not only of great benefit for government controls, but also for laboratories and the animals industry owners to enable them to control contaminated milk and products with chemical residues ,can also be used for screening, and provides much higher sensitivity and greater specificity.

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