Studies On Some Heavy Metals and Ivermectin Residues in Raw Milk

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Abstract

Milk and milk products are a significant part of a healthy diet. However, contamination of milk can be dangerous and detrimental for the health of consumers. As levels of harmful substances and environmental contaminants rise, milk and milk products become less safe. Heavy metals contamination of milk and dairy products can occur from food and water, as well as from manufacturing, packing, and dairy equipments. In this study, 60 samples of fresh milk were collected from various areas in the Damietta Governorate, Egypt, in order to determine the residual levels of three metals (lead, cadmium, and zinc) as well as the antiparasitic (ivermectin). Lead and cadmium were not found in any of the milk samples that were analyzed; nevertheless, zinc levels varied from 1.72 to 4.46, with an average of 2.422 ± 0.1 ng/liter. On the other hand, ivermectin ranged from 1.47 to 1.5 ug/kg with mean value of 1.1 ± 0.057. The results indicate that none of the examined milk samples exceeded the acceptable limit suggested by the Codex Alimentarius Commission and Egyptian Standards 0.02

Keywords: Milk. Heavy metals. Ivermectin. Contaminants, Residues

Introduction

One of the most popular foods consumed worldwide is milk. It is crucial for nutrition and also has a significant impact on the economy ***(FAO/WHO, 2017).*** Milk contains protein, fats, minerals, carbohydrates, and vitamins that

serve vital nutritional and biochemical functions, especially for young children and elderly. Casein represents 78.3% of milk's basic protein, followed by whey protein (19%) and other proteins (2.7%) ***(Walstra et al.,2006).*** The primary carbohydrate in milk is lactose, which is made up of the monosaccharides galactose and glucose and gives humans 16.8 kj/g of energy. ***(FAO, 2003).***

Heavy metals are poisonous compounds that build up in the body over time as a result of consuming repeated trace amounts of them. Heavy metals represent the group of metals and metalloids that having an atomic density more than 6 g/km including the metals that are frequently linked to toxicity and pollution issues, such as cadmium, lead, mercury, and zinc ***(Alloway and Ayres, 1993*)**. The contamination of milk and dairy products by heavy metals can occur from food and water, as well as from manufacturing, packing, and dairy equipment***s (Ayar et al., 2009).*** Furthermore, rapid industrial development and the rise in heavy metals and emissions from various technical processes, such as fertilizers, pesticides, and field irrigation, may seriously contaminate our environment ***(Tajkarimi et al., 2008).***

Because of the creation of a lipophilic complex, milk has the ability to retain metals ***(Buechler et al., 2002).*** Cadmium is a cumulative toxic metal with biological half-life ranging from 20 to 40 years **(*Shibamoto and Bjeldanes, 1993)***. Cadmium mostly builds up in the kidney and liver **(*Goyer 1989).*** Zinc is considered an essential element as it is necessary to produce hundreds of enzymes throughout the body. ***(Agnew and Slesinger, 2022)***. The harmful toxic effects of lead and cadmium are well known, and lead is readily excreted via milk. Leads have an impact on the gastrointestinal, renal, hematopoietic, and hematic systems ***(Correia et al., 2000).*** High blood pressure, prostate cancer, mutations, and embryonic mortality are all related to cadmium exposure ***(Pitot and Dragan, 1996).***

The most frequent reasons why milk has residues beyond the permissible Maximum Residual Limits (MRL) include the widespread use of these drugs, inadequate withdrawal periods, and a lack of documentation ***(Kaneen and Ahl, 1987).*** The lack of good veterinary practice and illegal use of veterinary drugs by farmers exacerbate the problem ***(Oliver et al., 1990*** and ***Mc Ewen et al., 1991).*** Ivermectin is used in dosages of 0.2 and 0.5 mg/kg body weight and comes in topical and subcutaneous forms. Ivermectin's long-term persistence in the body is facilitated by its high lipophilia and propensity to accumulate in fat tissues, which serve as a reservoir ***(Schenck and Lagman, 1999).*** This drug accounts for its long resistance in plasma and excretion in milk ***(Aniello et al., 2002).*** Ivermectin was found in the

milk of lactating cows many weeks after dosing ***(Alvinerie et al., 1996).*** Ivermectin has been shown to produce severe diarrhea, functional impairment, and major adverse neurological effects in human; including headache and dizziness ***(Chandler, 2018).***

Materials and Methods

Sample collection

A total of 60 raw cow's milk samples were collected from various farms in Damietta Governorate between October and December 2022. Each 500 mL sample was stored in polyethylene bags and kept frozen until analysis.

Experiment (I): Determination of heavy metals contaminants in examined raw milk samples

Method of analysis:

Flame atomic Absorption Spectrophotometry (AAS) Perkin Elmer 2380 was used to measure the amounts of lead (Pb), cadmium (Cd), and zinc (Zn) A combination of nitric and perchloric acids was used to digest the samples, and each metal was examined at a different wavelength.

Experiment (II): Determination of ivermectin residues in examined raw milk samples

Methods of analysis:

The samples of raw milk were analyzed as procedures which described by ***Schenck* and *lagman, (1999)*** using liquid chromatography fluorescence spectrophotometer (LC) Model 2210.

Results

Pb and Cd were not detected in any of the examined raw milk samples, the detection limit of Pb is 0.004 and for Cd is 0.002. Zn levels ranged from 1.72 – 4.46 with a mean value of 2.422 ± 0.135 mg/kg, which is below the permissible limit of 5 mg/kg in **Table (1).**

Results are presented as Mean ± S.E in **Figure (1)** and show the mean values of zinc 2.422 ± 0.135 mg/kg.

2- Ivermectin concentrations ranged from 1.47 to 1.5 µg/L, with none exceeding the MRL of 10 µg/L in **Table (2).** The drug's lipophilic nature leads to prolonged presence in milk, although all samples remained within acceptable limits.

The results are displayed as Mean ± S.E in **Figure (2),** which displays the ivermectin mean values of 1.1 ± 0.057.

Table (1): Heavy metals residues in raw milk samples (n=60)

Metal	Number of examined samples (n=60)		Incidence%	Min	Max	Mean ± S.E	MRL (mg/kg)	Within (MRL) and %	Over (MRL) and %
	Number of +ve samples	Number of -ve samples							
Lead	Zero	60	0 %	(<0.004)	(<0.004)	0.004 ±0	0.02^{a+b}	60(100%)	(zero%)
Cadmium	Zero	60	0 %	(<0.002)	(<0.002)	0.002 ±0	$0.05^{c}0.03^{d}$	60(100%)	(zero %)
Zinc	60	Zero	100 %	1.72	4.46	2.422 ± 0.135	5^{e}	60(100%)	(zero%)

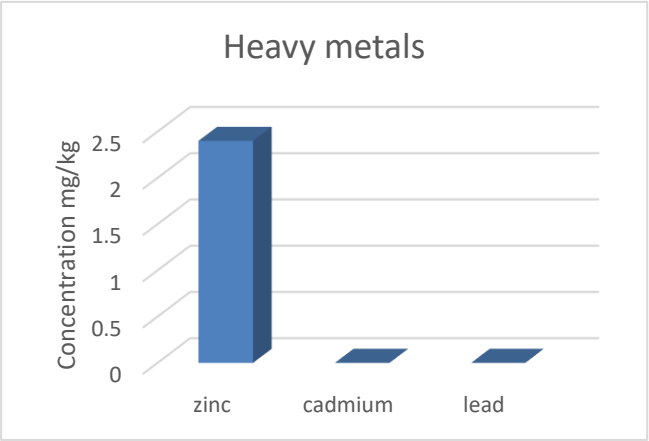

Figure (1): means value of heavy metals in examined raw milk samples:

Table (2): Ivermectin residues (ppb) in examined raw milk samples (n=60)

Antiparasitic	Number of examined samples (n=60)		Incidence %	Min	Max	Mean± S.E	MRL (ppb)	Within (MRL) and %	Over (MRL) and %
	Number of +ve samples	Number of -ve samples							
Ivermectin (ug/kg)	8	52	13 %	1.47	1.5	1.1± 0.057	10	60 (100%)	- (zero %)

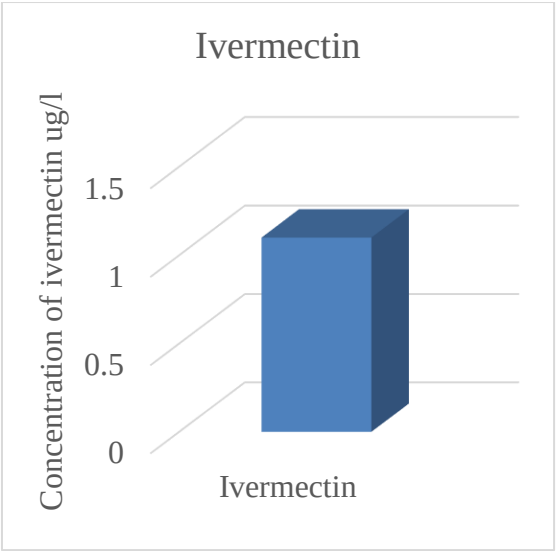

Figure (2): means value of ivermectin residues in examined raw milk samples

Discussion

Because heavy metals are persistent and non-biodegradable, they accumulate and are eliminated through milk **(*Burger and Elbin, 2015).*** A vital part of the human diet is milk and its derivatives. Even at low concentrations, lead (Pb) and cadmium (Cd) can cause metabolic abnormalities, are thought to be carcinogenic, and are linked to the development of diseases of the kidney, neurological system, cardiovascular system, and skeletal system (***Zhunang et al., 2009).***

Table (1) and Figure (1) revealed that lead (Pb) residues were not detected from any examined samples. Similar findings were reported by ***Benemariya et al., (1993) and Jochum et al., (1995).*** Higher lead concentration in raw milk was reported by ***Anastasio et al., (2006), Ayar et al., (2009), Ahmad et al., (2016***) and in recent study in India **(*Singh et al., 2020)*** reported the mean value of lead (Pb) in raw milk was 6.1 ± 1.5 ppb. In Egypt higher values of lead (Pb) contamination in raw milk were reported by ***Malhat et al., (2012), Meshref et al., (2014), and Khalil (2018)***, ***Saleh et al., (2019)*** found that raw cow's milk and buffalo's milk contain lead (Pb) residues 110 ppb and 140 ppb, respectively. Also, they recorded 26.66% and 33.33% of cow and buffalo milk exceeded the maximum residual limit. Also, higher value was reported by ***Amer et al., (2021),*** they found that the mean value of lead (Pb) in raw milk was 45.06 ppb and 13.33% of the examined samples more than the MRL. Lead alkyl additives, contaminated feed, soil, and water,

and excessive exposure of nursing cows to ambient lead released by heavy traffic can all contribute to the greater lead concentration in raw milk. None of examined milk samples above the acceptable limit according to ***Egyptian Organization for Standardization and Quality Control 7136/2010*** and ***Codex Alimentarius Commission (2012).*** Consuming tainted grass and water has raised the amounts of lead in cow's milk **(*Okada et al., 1997).*** Additionally, raw milk samples from cows raised close to busy major roads which are thought to be a major source of lead in the environment as well as coal burning showed elevated levels of lead ***(Juretric et al., 1997).*** In addition, it might be the result of raw milk becoming contaminated by metallic dairy equipment and utensils. Results recorded in Table (1) and Figure (1) showed that cadmium was not detected from any examined raw milk samples. These results were in agreement with those obtained by ***Lante et al., (2006), Ataro et al., (2008) and Gonzalez-Montana*** **(2012).** On the other aspect higher levels of cadmium were reported by ***Cerkvenik et al., (2000), Islam et al., (2009)*** *and* ***singh et al., (2020).*** In Egypt high levels of cadmium (Cd) residues in raw milk were reported by ***Malhat et al., (2012), Meshref et al., (2014).*** Also, ***Khalil (2018), Saleh et al., (2019) and Amer et al., (2021*****)** where they reported different levels of cadmium in examined raw milk samples. All examined raw milk samples lie within the permissible limit and none of the examined samples exceeded the MRL according to ***Egyptian Standards (1993)*** 0.05 mg/kg and according to ***European Commission (1997)*** 0.03 mg/kg Also, all examined raw milk samples are accepted. Data in Table (1) and Figure (1) revealed that the mean zinc (Zn) value in all examined raw milk samples (mg/kg) was 2.422 ± 0.135 with a maximum of 4.46 and a minimum of 1.72. These results were nearly similar to those recorded by ***Nasif (2002)***. Lower Zn concentrations than those obtained in the present investigation were reported by ***Khalil (2018)*** who reported the zinc concentration from three locations in Aswan, Province Egypt were ranged from 0.417 - 1.420, 0.737 - 1.135 and 0.649 - 1.173(mg/kg). Zinc concentrations in all examined raw milk samples were below the permissible limit (5 ppm) reported **by *Citek et al., (1996)***, Also, all examined raw milk samples (100%) were accepted. More than 200 metalloenzymes, proteins, hormones, and neuropeptides require zinc as a cofactor **(*Lee 1998)***.

Although it is not licensed for use in nursing animals, ivermectin (IVM), a broad-spectrum antiparasitic medication, is widely used to treat diseases in animals caused by endo- and ectoparasites. However, it is still used in many parts of the world for dairy cattle, goats, and sheep **(*Imperiale et al., 2002).*** Regarding

ivermectin residues in examined raw milk samples Table (3) and Figure (3) showed that ivermectin in the examined raw milk samples ranged from 1.47 to 1.5 with mean value 1.1 ± 0.52 ug/L. None of any examined raw milk exceeds the (MRL) of ivermectin in milk 10 ug/L set by ***Codex Alimentarius Commission (2002)*** and ***WHO (2018).***

IVM's (ivermectin) high liposolubility causes undesirable drug residues to persist in animal bodies and fluids for an extended period of time, which is problematic because of their lengthy excretion time and the resulting contamination of milk and dairy products ***(Crooks et al., 2000).*** Higher results of ivermectin concentration detected in this investigation were reported by ***Lobato et al., (2006)*** who detected ivermectin ranged from 2-10 ppb. On the other hand, ***Zakaria et al., (2019)*** in recent study found that ivermectin (IVM) ranged from 2.7 to 11.5 with a mean value 5.84 ± 0.6 and the samples exceeded MRL (10 ppb) according to ***WHO (2018)*** was 2 samples (3. 3%).In Egypt, there is no established maximum residual limit (MRL) for IVM in milk; therefore, any amount detected in milk constitutes a residue violation ***Zakaria et al., (2019).***

Conclusion

The current study concludes that the level of heavy metal contamination in raw milk samples was significantly below the Maximum Residual Limit (MRL) based on the examination of milk samples. Therefore, milk is safe for human consumption. The study's findings indicate that ivermectin (IVM) is being used legally in Egypt to treat nursing cows, which causes measurable residues in milk and should be restricted. Since IVM-contaminated discharge milk can be utilized to make skimmed milk and low-fat dairy products, it is advantageous to apply technical processes such milk skimming to lower the amount of ivermectin residues in milk and lessen the financial loss that results from such discharge.

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دراسات عن بقايا بعض المعادن الثقيلة وايفرميكتين في اللبن الخام

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الملخص

يعد التلوث من المشكلات المعاصرة والهامة التي تهدد صحة الإنسان في كل بقاع العالم ومن بين هذه الملوثات وأكثرها خطورة علي صحة الانسان هي تلوث البيئة بالمعادن الثقيلة وبقايا المضادات الحيوية والتي أمتدت إلى الغذاء اليومى الذى يتناوله الانسان.

وقد أجريت هذه الدراسة على ٦٠ عينة من اللبن الخام وتم تجميع العينات من مختلف محلات بيع الألبان بمحافظة دمياط لاستبيان تركيز المعادن الثقيلة (الرصاص - الكادميوم - الزنك) وبعض بقايا الأدوية البيطرية مثل ايفرميكتين ومقارنة هذه التركيزات بالحدود القصوي المسموح بها عالميا ومحلياً.

بقايا الرصاص فى عينات اللبن الخام: أوضحت النتائج أنه لم يستدل على وجود بقايا للرصاص في جميع عينات اللبن الخام المفحوصة.

بقايا الكادميوم فى عنيات اللبن الخام : لم يستدل على وجود عنصر الكادميوم فى أى من عينات اللبن الخام اللي تم فحصها.

بقايا الزنك فى عينات اللبن الخام : أوضحت الدراسة تواجد عنصر الزنك في عينيات اللبن الخام بنسبة تراوحت١,٧٢ - ٤,٤٦ مللي جرام / لترمن اللبن بمتوسط١٣٥,± ٢,٤٢٢ وثبت أن جميع العينات تحت الفحص لم تتجاوز الحدود القصوى المسموح بها عالميا.

بقايا أيفرمكتين فى عنيات اللبن الخام: أشارت الدراسة أن تركيز ايفرمكتين فى عينات اللبن الخام تراوح من٤٧، ١إلى ١,٥٠ متوسط ٠،٥٧ ± ١، ١جزء فى البليون وأن جميع العينات المفحوصة لم تتجاوز الحدود القصوى المسموح بها عالميا.

ومما سبق يتضح أن عينات اللبن الخام التي تم فحصها لم تتجاوز الحدود القصوي لبقايا المعادن الثقيلة و أيفرميكتين و لكن احتواءاها علي بعض النسب البسيطة من هذه البقايا الضارة مما يجعل الاثر التراكمي علي المدي الطويل ذو تأثير علي الصحة العامة للمستهلك. هذا و قد تم استعراض الخطورة الصحية لبعض الملوثات الكيميائية في الحليب ومنتجاته وكذلك اقتراح الطرق الصحية للوقاية منها.