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Evaluation of bacterial contaminants and heavy metals in cow and buffalo raw milk sold in Baghdad governorate

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Abstract

The purpose of this study was to investigate bacterial contamination and heavy metal concentrations in 80 samples of raw milk (cow: 40 and buffalo: 40) gathered from local markets in Baghdad, Iraq. The culture results were classified into ten categories: *E. coli* was 100% in each cows and buffaloes, *Enterobacter Spp* 23.75% (25% cow, 22.5% buffalo), *Pseudomonas Spp* 13.75% (15% cow,12.5% buffalo), *Klebsiella Spp* 15% (17.5% cow, 12.5% buffalo), *Staphylococcus aureus* 12.5% (15% cow, 10% buffalo), *Staph. epidermidis* 5% (for each cow and buffalo), *Proteus spp*. 10% (12.5% cows, 7.5% buffaloes), *E. coli* O157 15% (25% cow, 5% buffalo), *Yersinia enterocolitica* 3.75% (5% cow, 2.5% buffalo) and *Salmonella* 13.75% (25% cow 2.5% buffalo). The averages of heavy metals concentrations in cow milk samples were 0.62±0.25, 0.25±0.22, 0.31±0.20 and 21±2 mg/kg and in buffalo milk samples were 0.60±0.3, 0.33±0.15, 0.27±0.11 and 18±2.5 mg/kg for Lead (Pb), Cadmium (Cd), Copper (Cu) and Nickel (Ni) respectively. The high concentrations of pathogenic bacteria and metals found in the milk products is a sign of inadequate hygiene and sanitation during milking and post-milking operations, as well as excessive levels of heavy metal pollution in the environment which will affect meat and milk produced by these animals.

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Introduction

Milk and milk products are complete foods for people from birth to old age because they contain all the nutrients necessary for growth and body health protection. Humans have consumed milk for thousands of years (1). Buffalo's and cow's dairy is the essential dairy species all over the world; in many developing countries, milk plays an essential function in satisfying the nutritional demands of humans, it has a rich, complex nutritional value. Buffalo's and cow's milk receive increasing research attention in various countries according to the charming nutrient contents (1,2). The environment, production, storage parameters, microbial and somatic cell counts influence chemical parameters fat and protein content as well as the absence of inhibitory compounds all impact milk and milk products quality. *Pseudomonas* or *Acinetobacter spp*. are the common spoilage bacteria, can create heat-stable proteases and lipases, which remain active after pasteurization spoiling milk during long-term storage (3). Milk contains both macroelements such as Ca, K and P, also microelements such as Cu, Fe, Zn, Se, also may contain heavy metals which come from different sources, including food, water, during manufacturing and packaging, they may reach toxic levels in humans (4). Milk contamination with toxic metals is a health threatening danger to humans, particularly in infants and children because they largely consumed milk (5). Many toxic compounds in animal’s milk and meat are of food origin due to contamination of the environment with heavy metals. In food chains and food webs, heavy metals are biotransferred, bioaccumulated, and biomagnified in the animals’ bodies and either accumulate or cause injury to them (6). As a non-essential element, lead can be readily absorbed through milk, yet it can cause severe diseases like Alzheimer's, kidney, reproductive and endocrine problems and lower children's intelligence quotient (IQ) (7). Cadmium causes antiperistalsis, vomiting, and diarrhea. It may also play a role in developing other health problems, such as high blood pressure, prostate cancer, and even mortality (8). Hepatic Copper II Overload Illness, tissue injury, lung irritation, and hepatic cancer can all be caused by copper overexposure (9).

As a result, the study's goals were to assess the microbiological status of cow and buffalo milk sold in the Iraqi market and to estimate the presence of heavy metals in the milk.

Materials and methods

Sample collection

Eighty samples of the raw milk Cow: 40 and buffalo: 40, 250 ml for each were collected from markets in Baghdad then were sent in a cool box to the microbiology laboratory in the Department of Public Health, College of Veterinary Medicine, University of Baghdad for isolation and identification of bacterial contaminants and market research and consumer protection center/university of Baghdad to for detection of heavy metals: Lead (Pb), Nickel (Ni), Cadmium (Cd), and Copper (Cu).

Isolation and identification of bacterial contamination

One ml of each milk sample was inoculated into 99 ml of peptone broth and incubated at 37°C for 24-48 hours. After incubation, about 0.1 ml of inoculated broth were subcultured on Nutrient agar plates, Blood agar, Eosin methylene blue EMB, MacConky agar, *Salmonella Shigella* Agar SS Agar, sorbitol-MacConky agar with cefixime tellurite and CIN agar. Biochemical is performed using Epi 20 system (10,11).

Heavy metals analysis

Determination of heavy metal levels in milk samples was done using Atomic Absorption Spectrophotometer (AAS) according to the dry-ashing method that was described by (12) which is done by three steps: [1] Preparation the sample which include: drying stage, incineration stage, winnowing stage and cleaning stage. [2] preparation of standard solutions 0.01, 0.02 and 0.04 and measure them to compare with samples measures. [3] Inoculation of samples in spectrophotometer and taking measures.

One hundred ml of each milk sample were kept in the oven at 80°C for 48 h or until dried. The heavy metals analysis was performed by adding con. HNO_3 in to the dried milk powder, 0.5 g of dried milk with 5ml of con. HNO_3 were taken in to the digestion flask then heated at 80-90°C for 10 min by placing on hot plat for digestion then raised to 100°C, more acid was added up to 3-5 ml until clear solution was obtained. The samples were cooled at room temperature and filtered through filter paper and the sample volume was completed with deionized water to 25 ml in volumetric flask (12,13). Statistical analysis of samples was done using SAS 2010.

Results

The result revealed from 80 milk samples submitted from buffaloes 40, cows 40. The culture results were classified into ten categories: *E. coli* was 100% in each cows and buffaloes, *Enterobacter Spp* 23.75% :25% cow, 22.5% buffalo, *Pseudomonas Spp* 13.75% :15% cow,12.5% buffalo, *Klebsiella Spp* 15% :17.5% cow, 12.5% buffalo, *Staphylococcus aureus* 12.5% :15% cow, 10% buffalo, *Staph. epidermidis* 5% for each cow and buffalo, *Proteus spp*. 10% :12.5% cows, 7.5% buffaloes, *E. coli* O157 15% :25% cow, 5% buffalo, *Yersinia enterocolitica* 3.75%: 5% cow, 2.5% buffalo and *Salmonella* 13.75%: 25% cow 2.5% buffalo as shown in table 1. The mean of Pb, Cd, Cu and Ni concentrations in examined milk samples were 0.62±0.25, 0.25±0.22, 0.31±0.20 and 21±2 mg/kg in cow milk samples and 0.60±0.3, 0.33±0.15, 0.27±0.11 and 18±2.5 mg/kg in buffalo milk samples respectively (Table 2). There are no statistically significant differences between results of cow and buffalo milk samples in both bacterial and heavy metals contaminants at $P<0.05$.

Table 1: percentage of isolated bacteria in cow and Buffalo milk samples

Isolated bacteria	No. of isolate (cow)	No. of isolate (Buffalo)	Total No. of isolates
E. coli	40 (100%)	40 (100%)	80 (100%)
Enterobacter Spp	10 (25%)	9 (22.5%)	19 (23.75%)
Pseudomonas Spp	6 (15%)	5 (12.5%)	11 (13.75%)
Klebsiella Spp	7 (17.5%)	5 (12.5%)	12 (15%)
Staph aureus	6 (15%)	4 (10%)	10 (12.5%)
Staph epidermidis	2 (5%)	2 (5%)	4 (5%)
Proteus spp.	5 (12.5%)	3 (7.5%)	8 (10%)
E. coli O157	10 (25%)	2 (5%)	12 (15%)
Yersinia enterocolitica	2 (5%)	1 (2.5%)	3 (3.75%)
Salmonella	10 (25%)	1 (2.5%)	11 (13.75%)

Table 2: percentage of heavy metals mg/kg in cow and Buffalo milk samples

Heavy Metal	mean±SE	
	Cow milk	Buffalo milk
Pb	0.62±0.25	0.60±0.3
Cd	0.25±0.22	0.33±0.15
Cu	0.31±0.20	0.27±0.11
Ni	21±2	18±2.5

Discussion

Escherichia coli spp. was found in all of our samples at 100%, while *E. coli* O157 was found in just 15% of them (25% cow and 5% Buffalo). The present findings are in agreement with those of (14), who discovered that *E. coli* was the most common bacterial isolate (49.8%) in his study on bacterial pathogens causing subclinical mastitis in three bovine dairy herds in three governorates in north upper Egypt.

The present study showed milk samples contaminated with *Staphylococcus aureus* (15% cow, 10% buffalo) and with *Staphylococcus epidermidis* 5% (for each cow and buffalo), this is lower than (15) who found that the most agents isolated from milk samples at four dairy farms in São Paulo State, Brazil, were *Staphylococcus epidermidis* and *Staph. aureus* 17% and 15% respectively, and also lower than (14) who recorded 44.9% *S. aureus* from three bovine herds in Egypt.

Bhutia *et al.* (16) recorded 28.7% *Staphylococcus* spp. from clinical mastitis in buffaloes, India from 2007 to 2016, due to poor hygienic practices for milking lead to high infection with *Staph.* spp.

Our finding showed that *Pseudomonas* Spp was 15% cow, 12.5% buffalo, *Klebsiella* spp 17.5% cow, 12.5% buffalo and *Enterobacter* spp. 25% cow, 22.5% buffalo, these results were higher than (15) who found *Pseudomonas aeruginosa* 9.5%; *Klebsiella rhinoscleromatis* 0.5%, *Klebsiella ozaenae* 0.5%, *Enterobacter cloacae* 0.5% during their study on bacteria causing subclinical mastitis of Buffalo in Brazil, but our findings were lower than the result of (17) who recorded *Klebsiella* spp 21.4% in Damascus and its countryside. Our result of *Salmonella* 25% cow and 2.5% buffalo was higher than (18) who found 19% of *Salmonella* were isolated from plastic container milk.

High infection rates with pathogens are caused by smallholders' use of their hands and traditional equipment for milking practices, which raises the risk of bacterial transmission into milk and dairy products (19). Milk contamination with bacteria may also result from the unhygienic manner of animals, contamination of milk with feces and infected animal wastes, transmission of pathogenic agents from the infected staff and due to uneffective cooling of raw milk samples at temperatures below 4°C which facilitates the survival and proliferation of bacteria (20).

Our results showed that the mean of Pb 0.62±0.25, 0.60±0.3, Cd 0.25±0.22, 0.33±0.15, Cu 0.31±0.20, 0.27±0.11 and Ni 21±2, 18±2.5 mg/kg for cow and buffalo milk samples respectively, these findings were higher than permissible limits determined by (21,22) which are: 0.05, 0.026, 0.10 and 0.10 mg/kg for Pb, Cd, Cu and Ni respectively.

These results were higher than (23) on his study on 30 milk samples of Buffalo and cow collected from dairy shops and groceries at El-Behera governorate/ Egypt, he recorded Cd and Pb levels in cow milk were 0.0978±0.01948, 0.3425±0.03980 ppm and in Buffalo milk were 0.1892±0.01239, 0.4854±0.05043 ppm respectively, also our findings were higher than (13) who studied the concentrations of heavy metals in milk samples collected from southern China, levels of Pb, Cd, Cu, and Ni were 0.0062±0.0022, 0.0019±0.0011, 0.0439±0.015 and 0.0552±0.0813 mg/kg respectively in sterilized milk samples, 0.0089±0.0048, 0.0034±0.0008, 0.0621±0.0239 and 0.0403±0.0290 mg/kg in Fermented milk, 0.0068±0.0022, 0.0041±0.0047, 0.0499±0.0106 and 0.0423±0.0122 mg/kg in Modified milk.

In this study, the results were also higher than those of (24) who studied the concentration of heavy metals and the risk associated with the consumption of raw fresh milk from Buffalo, cow, sheep, and goats in Aswan Province/Egypt and 16 samples of Kareish cheese, Domiati cheese, Mish, and Samna milk products in Aswan Province/Egypt, he recorded that heavy metals arranged from Pb 0.159- 0.733, Cd 0.013- 0.060, Cu 0.041- 0.079 mg/kg in Buffalo, Pb 0.209- 0.430, Cd 0.027- 0.036, Cu 0.111-0.133 mg/kg in cow, Pb 0.143- 0.254, Cd 000- 0.030, Cu 0.254- 0.336 mg/kg in goat and Pb 0.147- 0.737, Cd 0.004- 0.029, Cu 0.148- 0.315 mg/kg in sheep.

Our findings are in agreement with those of (25) who measure the levels of heavy metals Fe, Cu, Pb, Ni, and Cd in 90 samples of cow and buffalo milk and dairy products (cheese and cream) collected from three regions in Basrah such as Basrah center, Abu Al-Khaseeb, and Al-Zubair, their investigations showed that the levels of Fe, were 2.90±0.01, 6.32±0.02, and 4.39±0.02 respectively, for Cu were 0.37±0.01, 0.35±0.03, and 0.03±0.01, and for Pb 0.00± 0.00, 0.05±0.02 and 0.29±0.01 respectively for the cow's samples, and the Buffalo's samples for Fe 2.63±.01, 1.72±.02, and 2.10±.02, for Cu 1.85±0.01, 0.43±0.01 and 54±0.01 and Pb 0.00±0.00, 0.35±0.01 and 0.67±0.01 respectively, while the levels of Ni and Cd were not detected in all samples in their study.

The sources of contamination of raw milk with heavy metals are numerous, including the air (through inhalation of animals), water (through watering cans and utensils used for drinking water), fodder (as a result of contamination of the feed ingredients through the use of chemical materials by farmers such as fertilizers, insecticides, fungicides, sterilizers and mineral additives to the diet), also through the bad habits of the animal, especially the cows, like licking the poles, steel bars, metal troughs, as well as through solid metal and plastic waste such as lead tables that left in the field (12).

The concentration of heavy metals in milk is not affected by heat treatments during the process of pasteurization, condensation, and drying that is carried out on raw milk from livestock during the production process and may even increase the concentration of these elements as a result of milk contamination through tools, metal machines, covers, bags and metal cans used in manufacturing, packaging of powdered, pasteurized liquid and condensed milk products, the presence of heavy elements is one of the most important health pollution problems for human food in the world recent years (22).

Conclusion

The high concentrations of pathogenic bacteria and metals found in the milk products is a sign of inadequate hygiene and sanitation during milking and post-milking operations, as well as excessive levels of heavy metal pollution in the environment which will affect meat and milk produced by these animals.

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Conflict of interest

No conflict of interest was reported with this study.

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تقييم الملوثات البكتيرية والمعادن الثقيلة في حليب الأبقار والجاموس الخام المباع في محافظة بغداد

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الخلاصة

تم تحديد هذه الدراسة لتقييم الملوثات البكتيرية وتركيز المعادن الثقيلة في ٨٠ عينة من الحليب الخام (أبقار: ٤٠، جاموس: ٤٠) جمعت من الأسواق المحلية في بغداد، العراق. تم تصنيف نتائج الزرع البكتيري إلى عشر فئات: الايشريشيا القولونية كانت ١٠٠٪(في كل من الأبقار والجاموس)، البكتريا المعوية ٢٣,٧٥٪ (أبقار ٢٥٪، ٢٢,٥٪ جاموس)، الزوائف الزنجارية ١٣,٧٥٪ (أبقار١٥٪،١٢,٥٪ جاموس)، الكلبسيلا ١٥٪ (أبقار ١٧,٥٪، ١٢,٥٪ جاموس)، المكورات العنقودية الذهبية ١٢,٥٪ (أبقار ١٥ ٪، ١٠٪ جاموس)، المكورات العنقودية البشرية ٥٪ (لكل من الأبقار والجاموس)، المتقلبة ١٠٪ (أبقار ١٢,٥٪، ٧,٥٪ جاموس)، الايشريشيا القولونية ١٥٪ (أبقار ٢٥ ٪, ٥ ٪ جاموس)، يرسينيا القولون ٣,٧٥٪ (أبقار ٥٪، ٢,٥٪ جاموس)، السالمونيلا ١٣,٧٥٪ (٢٥٪ أبقار ٢,٥٪ جاموس). كانت متوسطات تراكيز المعادن الثقيلة في عينات الحليب المفحوصة على النحو التالي ٠,٦٢±٠,٢٥، ٠,٢٥±٠,٢٢، ٠,٣١±٠,٢٠ و ٢١±٢ مجم/كجم في عينات حليب الابقار و ٠,٦٠±٠,٣، ٠,٣٣±٠,١٥، ٠,٢٧±٠,١١ و ١٨±٢,٥ مجم/كجم في عينات حليب الجاموس لكل من الرصاص والكادميوم والنحاس والنيكل على التوالي. تعتبر هذه المعدلات العالية من البكتيريا المسببة للأمراض والمعادن الثقيلة في العينات التي تم تحليلها مؤشرا على سوء النظافة والتعقيم أثناء عمليات الحلب وبعدها، كما تشير إلى التلوث البيئي الشديد بالمعادن الثقيلة التي ستؤثر على اللحوم والحليب الذي تنتجه هذه الحيوانات.