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Original Scientific Paper

EFFECT OF ARSENIC ON COW`S UDDER PARENCHIMA

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Summary

During last several decades, arsenic and its compounds have been characterized as major environmental pollutants and thus potential causes of both human and animals poisoning. Water and milk are one of the major sources of arsenic. In this study, the influence of arsenic from drinking water on the concentration of arsenic in cow`s milk was investigated, as well as the influence of arsenic on the udder parenchyma. The study included twenty cows from six farms in Banat. The cows from which blood and milk samples were taken were in good condition and clinically healthy. A statistically significant positive correlation ($r=0.541$ $p=0.000$) between arsenic concentration in drinking water for animals and cow`s milk was observed at all examined farms. Ultrasound examination of udder showed changes in parenchyma in 77 out of 120 cows. Arsenic present in drinking water for animals as well as in cow`s milk may have significant negative impact on cow`s health and may lead to pathological changes in the udder.

Keywords: arsenic, toxic element, udder parenchyma, cow

INTRODUCTION

Although arsenic has been used as a poison in the past, it has become a global problem in the 21st century, primarily due to the pollution of drinking water. It is estimated that about 150 million people are exposed to water contaminated with arsenic (Jang et al., 2016). The major problem lies in the fact that arsenic and its compounds are carcinogenic to humans. Human exposure to this element has been linked to cardiovascular disease, developmental abnormalities, neurological and neurobehavioral disorders, diabetes, hearing loss, portal fibrosis, hematological disorders (anemia, leukopenia and eosinophilia) and numerous cancers such as bladder, lung and column cancers (Tchounwou et al., 2003).

Animals are also exposed to this metalloid. Some of the signs of acute toxicosis are intense abdominal pain, vomiting, diarrhea, weakness, unstable walk, hypodermia, and death (NRC, 2005). In addition to the negative impact of arsenic on the health of cows, the problem is that this metalloid leads to milk contamination. According to study

obtained by the European Food Safety Authority (EFSA, 2014), milk and dairy products are the main source of arsenic intoxication in European children up to 3 years of age.
According to Ordinance on the amount of pesticides, metals and metalloids and other toxic substances, chemotherapeutics, anabolic and other substances that may be found in food (Regulation, 2010; Regulation 1992), the maximum amount of arsenic in milk, butter, hard cheese and other dairy products is 0.1 mg/l, i.e. mg/kg (Official Gazette of the FRY, No. 5/92, 11/92 – amended and 32/2002 and Official Gazette of the RS, No. 25/2010 – other regulations and 28/2011 – other regulations).
The aim of this study was to determine the effect of arsenic from cow`s drinking water on cow`s milk arsenic concentration and the udder parenchyma.

MATERIALS AND METHODS

The study was obtained within six farms located in the Banat region (AP Vojvodina, Republic of Serbia). Twenty cows were randomly selected from each farm. The cows included in this study were two to five years old, in good condition and clinically healthy.
Blood was taken from the tail vein of each cow in an amount of 5 mL. Blood was taken in sterile vacutainer tubes with the addition of EDTA using the principles of antisepsis and asepsis. Milk samples in the amount of 10 mL were taken in sterile plastic bottles, during the first morning milking. Before milking, the teats of the cow`s udder were washed with warm water and wiped with a dry clean cloth. Also, 10 mL of cow`s drinking water was taken from each farm. The water was poured into plastic sterile bottles. All water, blood and milk samples were labeled individually, stored in hand-held refrigerator and transported to the laboratory for analyses.
Arsenic concentration was measured in blood, milk and water samples by atomic absorption spectrometry (AAS). Two mL of each individual blood, milk and water sample was transferred to plastic containers and their weight was measured. The samples from the plastic containers were then transferred to labeled cuvettes with the addition of “pure” water (water from which heavy metals and metalloids were removed) and 5 mL ccHNO_3. Each cuvette was closed and left closed at room temperature for 15 minutes. Samples and blank were placed in ICP-OES apparatus for destruction and burning of samples (according to the scheme of 5 samples), which was then turned on (program 3). When the apparatus was finished destroying and burning the samples, the cuvettes were left at room temperature to cool down. The sample from the cuvette was then transferred to glass flasks, which were filled to a volume of 25mL with “pure” water. The sample from the flasks was then filtered through a funnel with filter paper into the labeled cuvettes. The cuvette were then transferred to an atomic absorption spectrometar. In accordance with Lambert Behr’s law, the radiation intensity of the lamp with a hollow cathode weakens when passing through the atomic vapor of the sample, due to absorption by the atom of the sample.
Ultrasound examination of the udder was used to determine changes in the parenchyma. Prior to the examination, the cow`s udder was cleaned and washed of impurities, and then an ultrasound gel was applied to the ultrasound probe and the udder skin to ensure proper

contact between the probe and the skin. An Esaote pie medical-falco and 6-8 MHz linear probe were used for the ultrasound examination.

All obtained results were processed by standard statistical methods using Microsoft Office Excel 2016. In statistical data processing, standard methods of descriptive and analytical statistics were applied. From the descriptive methods, mean value, maximum and minimum value, as well as standard deviation were used. Pearson`s correlation was applied to assess the statistical significance of the obtained results. A statistically significant value was considered at the level of $p < 0.05$.

RESULTS

The values of arsenic concentrations obtained after testing cow`s drinking water, blood and milk samples at six farms in Banat, are shown in Table 1.

Table 1 Arsenic concentrations (mg/l) in water, blood and milk from six farms

	Sample number	Minimum	Maximum	Mean	Standard deviation
Water	120	0.0017	0.1733	0.0736	0.0594
Blood	120	0.0027	0.1060	0.0566	0.0283
Milk	120	0.0010	0.2800	0.0665	0.0530

The minimum concentration of arsenic in drinking water for cows from all six farms was 0.0017 mg/L, the maximum was 0.1733 mg/L, while the average value of the arsenic concentration in water was 0.0736 ± 0.0594 mg /L.

The minimum concentration of arsenic in the blood of cows from all six farms was 0.0027 mg/L, the maximum was 0.106 mg/L, while the average value of the arsenic concentration in the blood of cows was 0.0566 ± 0.0283 mg /L.

The value of arsenic in cow`s milk ranged from 0.001 to 0.28 mg/L, and the average value in cow`s milk was 0.0665 ± 0.053 mg /L.

Using the Pearson correlation test for arsenic in water and cow`s milk, the statistically significant positive correlation was observed between arsenic concentration in water and milk ($p = 0.541$; $p = 0.000$) (Table 2).

Table 2 Correlation between values in water, blood and milk of cows determined in all six observed farms

		Water	Blood	Milk
Water	Pearson correlation	1	0.526	0.541
	p		0.000	0.000
	Number of samples	120	120	120
Blood	Pearson correlation	0.526	1	0.183
	p	0.000		0.046
	Number of samples	120	120	120
Milk	Pearson correlation	0.541	0.183	1
	p	0.000	0.046	
	Number of samples	120	120	120

A positively strong correlation between arsenic concentration in drinking water and cow's milk indicates that increased concentration of arsenic in water is followed with its increased concentration in milk.
Ultrasound examination of the udder of cows was performed in all 120 cows. Ultrasound examination of 43 cows showed a homogenous structure, uniform echogenicity with anechoic fields, which correspond to blood vessels and the canalicular system of the udder. This finding corresponds to the normal cow udder parenchyma (Figure 1).

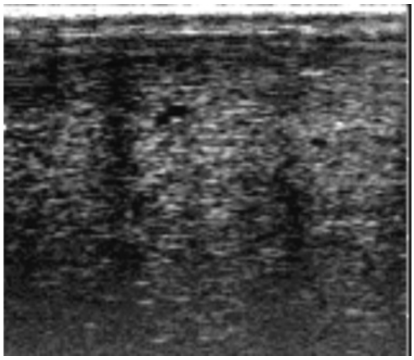

Figure 1 Ultrasound observation of normal udder parenchyma of cows

In 77 of 120 cows, the parenchyma was altered (Figure 2). Ultrasound finding of a pathologically altered udder parenchyma is characterized by visualization of the parenchyma as an inhomogeneous and hyperechoic structure.

Figure 2 Ultrasound observation of altered udder parenchyma of cows

Of the 77 cows that had altered udder parenchyma, 23 (29.87%) did not have high concentrations of arsenic in milk, while 54 (70.13%) cows had high concentrations of arsenic in milk.

DISCUSSION

The maximum contaminant level (MCL) of arsenic in drinking water is 0.01 mg/L (Sarkar et al., 2016). In this study, the minimum concentration of arsenic in cow`s drinking water was 0.0017 mg/L, the maximum 0.1733 mg/L, while the mean value was 0.0736 mg/L. From this, it can be seen that the maximum and mean values of arsenic concentration in drinking water for cows were higher than MCL, which is in accordance with the results of Papić et al. (2012), who, as part of their study in Banat on 244 samples of drinking water, registered a maximum concentration of arsenic of 0.217 mg/L. The maximum contaminant level of arsenic in drinking water for cows is 0.2 μg /L, while according to NRC (2005) this limit is 0.05 mg/L (Bera et al., 2010).

In Serbia, the maximum amount of arsenic in milk is 0.1 mg/L (Official Gazette of the FRY, No. 5/92, 11/92 - amended and 32/2002 and Official Gazette of the RS, No. 25/2010 - other regulations and 28/2011 - other regulations), while according to the Codex Alimentarius standard, the limit of 0.14 mg/L is allowed in the world (Arianejad et al., 2015). The average concentration of arsenic registered in all farms (0.0665 ± 0.053 mg/L) is in accordance with Davidov et al. (2019), who investigated the concentrations of metalloids in milk on three farms in Vojvodina, and the value of arsenic in milk was 0.058 mg/L. On the other hand, Mitrović et al. (2019) observed that the concentration of arsenic in milk taken from Palilula and Surčin was below the detection level, in contrast to the concentration in animal feed in which mean value was 1.08 mg/kg. The mean value of arsenic in milk in this study is in agreement with the results of Kodrik et al. (2011) who measured 0.0521 mg/L of arsenic in the milk of cows near the highway, while the concentration of arsenic in the milk of cows from the uncontaminated area was 0.0233 mg/L. Significantly higher values of arsenic concentration in milk (0.2428-0.684 mg/L) than measured in our study was observed by Licata et al. (2004), as well as Castro-González et al. (2018) and Castro-González et al. (2019) since they published that the average value of arsenic was 0.15 mg/L. On the other hand, slightly lower values (0.0152-0.0259 mg/L) were measured by Arianead et al. (2015), Roy et al. (2009) (0.0179 mg/L in the industrial zone and 0.02414 mg/L in the non-industrial zone), Kabir et al. (2017) (0.012 mg/L) and Castro-González et al. (2017) (0.035 mg/L).

Ultrasound finding of a cow's udder with pathological alterations in the parenchyma depends on the degree of structural changes that occur in the tissue. Inflammatory processes in the udder lead to a change in the echogenicity of the organ. Within our study, ultrasound examination of the udder of cows revealed inhomogeneous and hyperechoic structure of the parenchyma, which corresponds to the findings of other authors (Galfi et al., 2017; Galfi et al., 2015), where the udder parenchyma was observed as inhomogeneous structure, with reduced or increased echogenicity.

CONCLUSION

Based on the obtained study results, it can be concluded that arsenic content in drinking water for cows has a statistically positively correlation with the concentration of arsenic in blood and milk, which means that increased arsenic concentration in water will lead to increased arsenic concentration in blood and milk. This direct effect of arsenic from the drinking water for cows can have a negative impact on the parenchyma of the udder of cows, because the ultrasound examination confirmed the pathologically altered parenchyma.

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