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Seasonal variations in the blood concentration of selected heavy metals in sheep and their effects on the biochemical and hematological parameters

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HIGHLIGHTS

- The environmental effect on the health of animals (serum chemistry) was observed.
- Season affected the concentration of heavy metals (lead and copper) significantly.
- Hematological parameters were negatively influenced by heavy metals content.

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ABSTRACT

The main objective of this study was to assess the concentration of various heavy metals (Cd, Pb, Zn, Cu, Hg) in the blood of sheep, followed by biochemical and hematological analysis in order to reveal possible associations. Blood was collected in two different seasons: winter (fed by fodder) and spring (grazing animals). The higher concentrations of Pb ($p < 0.01$), Cu ($p < 0.05$) and Hg, but lower of Cd and Zn were found in spring. Evaluation of the biochemical and hematological parameters during different seasons showed a possible environmental effect on the health of animals. A statistically significant increase of Ca ($p < 0.001$), Mg ($p < 0.05$), urea ($p < 0.001$), TP ($p < 0.05$), glucose ($p < 0.01$), AST ($p < 0.001$), ALT ($p < 0.001$), ALP ($p < 0.01$), cholesterol ($p < 0.001$), bilirubin ($p < 0.05$), triglycerides ($p < 0.001$) and a decrease of P ($p < 0.05$), HGB ($p < 0.05$), MCHC ($p < 0.05$) and RDWc ($p < 0.05$) in spring was detected. The results of this study showed statistically significant correlations between Pb and ALP ($r = 0.53$) level in winter and between Pb and Ca ($r = -0.73$) in the spring. The hematological analysis revealed a significant correlation between Zn and RBC ($r = 0.61$), MCV ($r = -0.74$), MCH ($r = -0.71$) and between Pb and MCH ($r = -0.55$), PCT ($r = -0.66$), PDWC ($r = -0.55$) in the winter. A high positive significant correlations were found between Cd and RDWC ($r = 0.77$) and Cu and RDWC ($r = 0.75$). The significance of this work is the use the data in the preventive diagnosis of metabolic and production diseases. The collected data may serve as a control indicator to detect toxic hazards related to the heavy metal occurrence on animal health status.

1. Introduction

Out of all environmental components, heavy metals burden is closely correlated with natural (volcanic activity, erosion, fires,

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biological weathering of soil) (Cui et al., 2005; Navarro et al., 2008; Gadd, 2010), as well as with anthropogenic inputs (industry, mining and metal processing, agriculture) (Pacyna et al., 2009; UNEP, 2013). These actions have been consistently shown to exhibit a negative impact on the food chain quality and safety. Increased contamination affects the health status of animals as well as humans (Loutfy et al., 2006; Massanyi et al., 2014). Among the most discussed and monitored factors, heavy metals significantly contribute to environmental contamination, among which cadmium (Cd), copper (Cu), mercury (Hg), lead (Pb) and zinc (Zn) are the most notable (Angelovicova and Fazekašová, 2014; Arvay et al., 2014, 2015; Massanyi et al., 2014). Meanwhile Cd, Pb and Hg are three nonessential metals that have been investigated in great detail over the years (Buchwalter, 2008). Chronic ingestion of heavy metals may lead to severe alterations of animal and human health, especially if their dietary content exceeds the permissible levels. Heavy metal toxicity may result in neurological disorders, cancer, liver and kidney damage, as well as numerous other health complications (Zheng et al., 2007; Lai et al., 2010; Bortey-Sam et al., 2015), which have significantly increased the mortality rate over the past decades (Formicki et al., 2008).

Monitoring of the environmental contamination in relation to historic mining and metal production is an important activity which serves to quantify the risks arising from significant associations between the occurrence of contaminants in the biotic and abiotic system (Arvay et al., 2014). Assessment of the heavy metal content in the blood of livestock represents a commonly used method to determine the extent of food chain contamination. Increased concentrations of heavy metals in the blood have a significant impact on numerous biochemical and physiological changes in the organism (Massanyi et al., 2014), which are translated into organ damage, particularly alterations to the liver and kidneys (Gasparik et al., 2004; Kramarová et al., 2005), ovaries and testes (Massanyi et al., 2007; Tvrda et al., 2011; Toman et al., 2012; Krockova et al., 2013) and thus on the overall health status of animals and humans (Bortey-Sam et al., 2015).

Different locations of the Spiš region (Cadastral area of the village Poráč N: 48°53′24″; E: 20°43′48″) are characterized by extreme concentration of Hg, Cd, Pb, Zn and Cu in all environmental compartments (Angelovicova and Fazekašová, 2014; Angelovicova et al., 2014; Arvay et al., 2014). This area carries a legacy of mining and processing of ore with high contents of such elements, especially Hg. As such, it is one of the most Hg contaminated areas in Central Europe, as evidenced by numerous studies (Kalac et al., 1996; Svoboda et al., 2000; Slavik et al., 2015).

A characteristic feature of this area is an extensive sheep breeding whose milk is used for food purposes. Therefore the main objective of this study was to assess the concentration of selected heavy metals (Cd, Pb, Zn, Cu and Hg) in the blood of sheep, followed by an extensive biochemical and hematological analysis. The data may help in the preventive diagnosis of metabolic and production diseases of livestock as well as general health status. Such disorders usually proceed in a latent form with less developed clinical symptoms, which are economically extremely serious, especially in highly productive animals (Vrzgula 1991). Furthermore, our study aimed to establish a direct correlation between the heavy metal content, biochemical and hematological parameters in order to reveal possible associations between the winter (fed by fodder) and spring (free grazing) season as well as environmental contamination in a specific area.

2. Material and methods

2.1. Animal management

Adult sheep (n = 15) of two breeds (Merino and East Friesian) housed at the sheep farm Poráč (central Spiš – Slovakia; N: 48°53′24″; E: 20°43′48″) were used in our study (Fig. 1). All animals were healthy and in good health condition.

2.2. Blood sampling

Blood samples were taken in two seasons. The first collection was conducted in December (winter season – animals fed by fodder) and the second one was performed in May (spring season – grazing animals). Blood samples were taken by a qualified veterinarian from *vena jugularis* (Danko et al., 2011) and placed into two tubes. Samples for biochemical and heavy metal assessment were placed into tubes without additive, and tubes containing EDTA as an anticoagulant, were used for the hematological analysis.

In order to quantify Cd, Pb, Zn, Cu and Hg the blood samples (blood clot) were kept at −20 °C until analysis. For the biochemical analyses the blood samples were centrifuged for 10 min at 3000 × g and blood serum was collected. Whole blood samples were used for hematological analyses (Massanyi et al., 2014).

2.3. Biochemical and hematological analyses

Sodium (Na), potassium (K) and chloride (Cl) ions were determined with an EasyLite analyzer (Medica, Bedford, MA, USA) provided with an ion-selective electrode (Kolesarova et al., 2008). Calcium (Ca), phosphorus (P), magnesium (Mg), urea, total proteins (TP), glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), bilirubin (Bili), cholesterol (Chol) and triglycerides (TG) were measured using commercial kits DiaSys (Diagnostic Systems GmbH, Holzheim, Germany) on the Randox RX Monza analyzer (Crumlin, United Kingdom).

Selected hematological parameters (WBC – total white blood cell count, LYM – lymphocytes count, MID – cell population of middle dimensions including monocytes and eosinophils, GRA – granulocytes count, LYM % - lymphocyte percentage, MID % – cell population of middle dimensions including monocytes and eosinophils percentage, GRA % – granulocytes percentage, RBC – red blood cell count, HGB – hemoglobin, HCT – hematocrit, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, RDWc – red cell distribution width (%), PCT % – platelet percentage, MPV – mean platelet volume, PDWc – platelet distribution width) were determined with the hematology analyzer Abacus Junior VET (Diatron®, Wien, Austria) as per the manufacturer indications (Massanyi et al., 2014).

2.4. Detection of heavy metals

2.4.1. Pre-analytical procedure

High-purity chemicals were used for all operations. For detection of heavy metals, the blood clot samples were kept at −18 °C until analysis. The thawed samples (~0.5 g) were mineralized in a closed system of microwave digestion using Mars X-Press 5 (CEM Corp., Matthews, NC, USA) in a mixture of 5 mL HNO_3 (SupraPUR®, Merck, Darmstadt, Germany) and 5 mL deionized water (0.054 μS/cm) from Simplicity 185 (Millipore SAS, Molsheim, France). Digestion conditions for the applied closed microwave digestion system comprised of the temperature, which ran up to 160 °C during 15 min and subsequently was kept at a constant temperature for