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Original Research Article

ASSESSMENT OF ENVIRONMENTAL POLLUTION WITH METALS IN SOME INDUSTRIAL REGIONS OF KOSOVO USING CHICKEN (*Gallus gallus domesticus*) BREAST FEATHERS

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Abstract: The aim of this study was to assess the presence of metals in three regions of Kosovo using chicken (*Gallus gallus domesticus*) breast feathers collected from the industrial regions of Mitrovica and Obiliq and the non-industrial region of Dragash. This study was carried out from September to November 2016, and feathers were collected from 90 individual domestic chickens housed as free range. The concentrations of metals in the chicken feathers were determined with atomic absorption spectrometry (AAS). The range of average measured concentrations of metals (μg g^{-1}) in examined regions were: Zn 109-131, Mn 6.17-31.30, Cu 22.1-27.2, Cr 5.09-19.0, Ni 12.3-15.8, Pb <0.0945-15.5, Cd 11.1-12.3 and As <0.099-7.44. The highest average levels of metals were determined in regions (μg g^{-1}): Dragash: Zn 131, Cu 27.2, Mn 31.3, Cr 19.0, Ni 15.8; Mitrovica As 7.44, Cd 12.3, Pb 15.5. High statistically significant differences ($p<0.001$) were found between the three regions for Pb, As, Mn, and Cr content. There were no significant differences ($p>0.05$) between the Mitrovica and Obiliq regions in terms of Zn content, Mitrovica and Dragash in terms of Cu or between the Obiliq and Dragash regions in terms of Cd content. These results should concern the environmental agencies in Kosovo and encourage them to take concrete steps by periodically checking these pollutants in these two industrial regions. Based on our results, we recommend that programmes for monitoring environmental pollution from metals could use chicken feathers as an important and valuable test material.

Key words: trace metals; AAS; chicken feathers; environmental pollution; Kosovo

Introduction

In recent years, the increase in industrial activities has had an impact on environmental pollution, which has shifted the equilibrium of the global ecosystem (1, 2). Among the pollutants that contribute to this problem are non-essential metals, such as Pb, Hg, Cd, and As (3).

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Non-essential metals are likely to enter a living organism through the food chain (4). These metals can cause various health problems in the organism by weakening the immune system, causing oxidative stress (5), effecting the blood biochemical parameters, reducing reproduction capabilities, reducing body weight, increasing heart diseases, and leading to the manifestation of different mutations and neoplasms (6, 7, 8, 9, 10).

Bioindication and biomonitoring are forms of monitoring of environmental pollution that help to identify sources of essential and non-

essential metals as well as their effects on the living world (11, 12). Using these methods, several authors have investigated various flora and fauna such as plants (13), insects (14), and fish (15, 16) that could serve as bioindicators (17). Birds have been recognized since the 1960s as potential bioindicators of environmental pollution (18). With the aim of measuring environmental pollution from metals and their effects on living organisms, researchers have used different materials taken from birds, such as blood, lungs, liver, musculature, and the gizzard (19, 20, 21, 22, 23). Fairly reliable results for the measurement of environmental contamination with metals have been obtained using avian feathers (24, 25, 26, 27). Specifically, metals present in the feathers reflect their concentration in the circulating blood during the 2-3 weeks of feather formation, which in turn represents both exposure and mobilization from internal tissues (28, 29). Thus, feathers serve as an archive of metal exposure during feather formation (30).

Avian feathers have been confirmed to be a suitable material for measuring effective contamination of metals in the environment (31). The concentration of metals in feathers has been shown to be much higher than that in other organs because feathers contain the protein keratin, which is a protein that metals tend to bind. Also, feathers are considered one of the best ways to eliminate metals from a chicken's body (32, 31, 33).

Concerning environmental pollution with metals, different researchers have analysed different samples in different regions of Kosovo, such as soils (34), earthworms (35), sparrows (36) and the results obtained from these surveys show multiple levels of non-essential metals compared to unpolluted areas. Based on this research, the most polluted region in terms of metals was the Mitrovica region and its surroundings, which is the most polluted region in all of Europe. This result was also confirmed by international agencies (37). Contributing significantly to this pollution in the region of Mitrovica was the Pb and Zn smelter, from which Pb was produced at approximately 730 tons per year at the end of 1990s (38). The Pb and Zn smelter was closed in August 2000 by the NATO military forces (39). However, numerous studies have been carried out in this region after the closure of the foundry, with the results showing alarming levels of metals (Pb, As, Cd, Zn, etc.) and their effects on animals and humans (36, 39). In the Obiliq region, there are two thermal power stations, as well as coal mines, and investigations revealed that in the grey matter that flows from these power plants, the metals were present in very high levels (40). Dragash is a region that does not contain any industrial factory; therefore, we used this region as a control.

Based on the above findings, we conducted a survey to assess the presence of metals in chicken feathers obtained from the following three different regions of Kosovo: Mitrovica, Obiliq and Dragash.

Materials and methods

Study area

The samples were collected from three study regions (areas), including Mitrovica, Obiliq and Dragash, as shown in Figure 1.

The Mitrovica region is located in the northern part of Kosovo, and it has the largest metallurgic and mining complex (Trepca) in Europe for the extraction of Pb, Cd and Zn. Many industrial plants existed in the complex, such as a Pb smelter, fertilizer production plant, refinery, battery factory, Zn electrolysis facility and a sulfuric acid plant. Significant amounts of metal pollutants have been released into the surrounding area, including populated residential areas, and these pollutants have been associated with human health risks (41).

Obiliqi is an industrial region that is located in the centre part of Kosovo. This region contains two thermal power stations, "Kosova A" and "Kosova B", as well as coal mines for coal extraction. Coal contains the metals Pb, Hg, Ni, Cd, As, and others (42), and the combustion from coal-based power plants is one of the most important processes contributing to pollution of the environment and groundwater because of the air ash distribution that contains many metals (43).

Dragash is a non-industrial region and is located in the southern part of Kosovo near Sharr Mountain. Geological analysis has shown that Sharr Mountain is very rich in metals, such as Fe, Cu, Mn, Cr, and Zn (44). These metals can enter living organisms through the food chain (4).

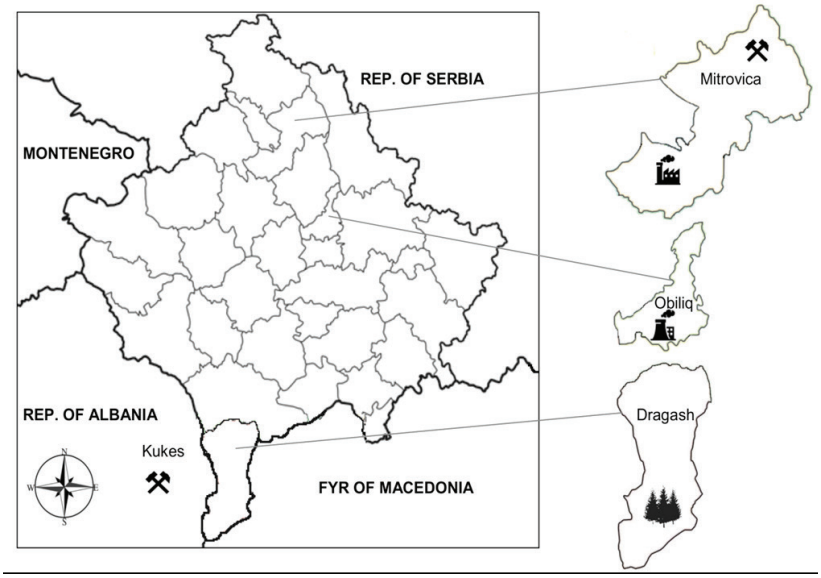

Figure 1: Map of Republic of Kosovo - location of Mitrovica, Obiliq and Dragash

Sampling plan and analytical procedures

The study was performed according to ethical guidelines of the Department of Veterinary Medicine, University of Pristina. In this research backyard chickens *Gallus gallus domesticus* were used, kept in these three regions. Breast feathers were collected by cutting them off with scissors at the skin level for the assessment of metal contamination from chicken which were grown in industrial regions of Mitrovica and Obiliq and the non-industrial region of Dragash. Three farms were sampled in each region and 10 chickens were selected from each farm. The number of chickens was 30 individuals from each region, a total of 90 domestic chickens. All of them were between 1-2 years old, mix breed, females and hatched on selected farms. All collected feathers were dark grey to black. The chickens were kept as free range (out roaming the day and housed at night), and they were fed mostly with maize grains from the same region along with feed they browsed when roaming freely on farm holding. The samples were sent to the laboratory for metal analysis at the Faculty of Agriculture and Veterinary, University of Pristine, Kosovo. Feathers were weighed, stored in plastic bags, labelled for later identification, and kept at –20 °C until they were dissected and analysed.

All feathers were washed three times with acetone followed by deionized water, and then the feathers were dried in an oven at 70 °C for 24 hours. Additionally, all laboratory dishes were cleaned with concentrated sulfuric acid, then washed with distilled water and dried in an oven. The feather samples (0.5 g) were digested with 70 % nitric acid (Sigma Aldrich, Merck, Darmstadt, Germany) and 30 % hydrogen peroxide (Sigma Aldrich, Merck, Darmstadt, Germany) at room temperature overnight. The next day, the samples were completely digested in a digestion unit DK heating digester (VELP Scientifica, Usmate (MB), Italy) at 150 °C for 4 hours until the solutions became clear (45, 46). After cooling, the solution volume was brought up to 50 mL using deionized water. Following digestion, the solutions were filtered through 0.45 µm acid-resistant filter paper Whatman (Sigma Aldrich, Merck, Darmstadt, Germany). The concentrations of metals in chicken breast feathers were measured with an atomic absorption spectrometer (AAS) (MSeries, Thermo, Cambridge, UK) using the flame method. Very low concentrations of Cd and Pb in extracts were determined using a graphite furnace atomic absorption spectrometry (GFAAS), whereas As was determined through hydride generation atomic absorption spectrometry (HGAAS). For quality assurance, internal reference materials (i.e., cow liver and spleen) were used for measuring metals. The recovery rates for the internal reference materials were between 78.5 – 107.3 %, with a low relative standard deviation (RSD) of 13 %. For all analysed metals, the limits of detection (LOD) (based on 3σ of the blank) and the limits of quantification (LOQ) (based on 9σ of the blank) given in Table 1 were calculated based on the DIN-32645 calculation procedure (47).

Table 1: Limits of detection and quantification (µg g-1) for analysed metals for chicken feathers samples at given wavelengths within AAS analysis

Element	Wavelength nm	Limit of detection [3σ]	Limit of quantification [9σ]
		µg g^{-1}	µg g^{-1}
As	193.7	0.033	0.099
Cd	228.8	0.021	0.063
Pb	217	0.0315	0.0945
Ni	232	0.105	0.315
Cr	357.9	0.186	0.558
Mn	279.5	0.063	0.189
Cu	324.8	0.108	0.324
Zn	213.9	0.036	0.108

Table 2: Average concentration value (M) ± standard error of mean (SEM) and concentration range of metals (µg g^{-1}) found in the chicken feathers and the differences in metal concentrations between the three regions of Mitrovica, Obiliq and Dragash

Elements	Mitrovica n 30	Obiliq n 30	Dragash n 30	P Value		
	1	2	3	1:2	1:3	2:3
As	7.44±0.33 (6.00-9.99)	1.50±0.01 (1.04-1.88)	<0.099#	0.000***	0.000***	0.000***
Cd	12.30±0.47 (10.10- 15.69)	11.70±0.12 (9.97-11.82)	11.10±0.12 (10.15-11.80)	0.024*	0.043*	0.904
Pb	15.50±0.45 (13.28-17.72)	2.50±0.13 (1.23-2.79)	<0.0945#	0.000***	0.000***	0.000***
Ni	14.30±0.55 (11.80-17.75)	12.30±0.38 (10.42-14.58)	15.80±0.58 (12.73-19.26)	0.003**	0.042*	0.000***
Cr	11.10±0.21 (9.43-12.20)	5.09±0.19 (4.10-6.21)	19.00±1.12 (14.02-27.14)	0.000***	0.000***	0.000***
Mn	15.20±0.53 (12.74-19.40)	6.17±0.20 (4.97-7.52)	31.30±1.47 (21.39-39.54)	0.000***	0.000***	0.000***
Cu	25.00±0.68 (20.73-28.75)	22.10±0.39 (19.86-23.90)	27.20±1.20 (19.60-32.20)	0.001**	0.072	0.001**
Zn	111.00±2.36 (97.70-120.75)	109.10±2.70 (99.58-128.90)	131.00±6.54 (102.00-172.38)	0.613	0.018*	0.001**

Note: *Significance level: p<0.05*, p<0.01**, p<.001***, # LOQ-limit of quantification*

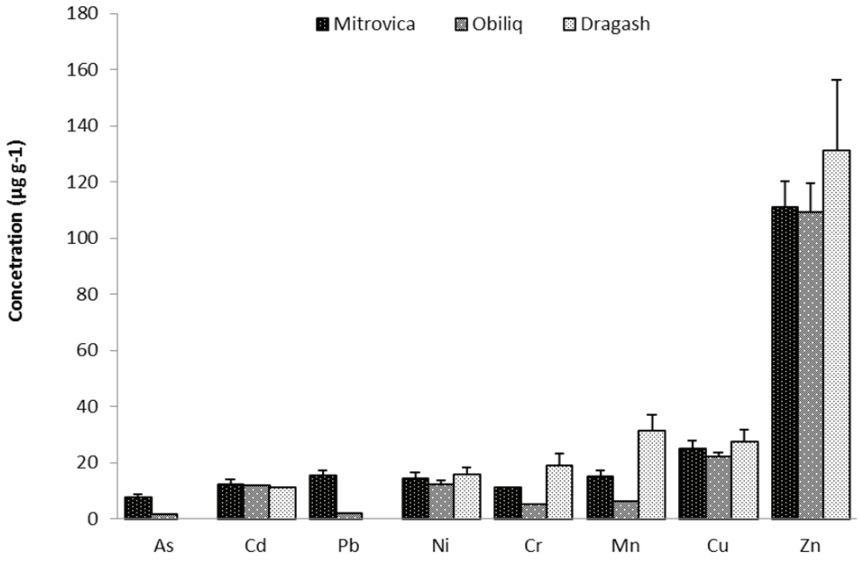

Figure 2: The average and standard deviation of As, Cd, Pb, Ni, Cr, Mn, Cu and Zn in breast chicken feather in the three regions of Kosovo (The sample size is 30 animals for each region presented in the figure)

Statistical analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, USA) software, version 21. The t-test and analysis of variance (ANOVA) were used to assess the differences in metal concentrations between the three regions. The level of significance for the differences was set at $p<0.05$, $p<0.01$ and $p<0.001$.

Results and discussion

The descriptive statistics for the metal (Pb, As, Cd, Cr, Mn, Ni, Cu and Zn) concentrations in chicken feathers from the three regions (Mitrovica, Obiliq and Dragash) determined in this study are shown in Table 2. In a more comprehensive measurement of the quantity of elements in the chicken feathers, we observed the following alignment for almost all the regions: Zn > Cu > Mn > Ni > Cr > Cd > Pb > As. For better visualization of the differences, the metal concentrations in chicken feathers are presented in Figure 2.

In general, the results from our study showed that there was a difference in the metal concentration in chicken feathers between the three studied regions. Among the metals included in the study, higher significant differences in the concentration of metals between the three regions were observed for Pb, As, Cr, and Mn ($p<0.001$).

Concentration profiles and risk to the avian fauna

Lead

Significantly higher average Pb concentration was observed in the Mitrovica region (15.5 µg g^{-1}) compared with the Obiliq region (2.5 µg g^{-1}), whereas the concentration of Pb in the Dragash region was at a minimum level, below the LOQ value. This high variation in the Pb concentration shows the real differences in environmental pollution between the three regions. The occurrence of this variation between the regions can be explained due to the presence of the large metallurgic and mining complex (Trepca) in the Mitrovica region, which is also considered to be the main source of environmental pollution. High concentrations of Pb in chicken feathers have also been reported by other authors from different countries around the world, including South Korea (48) at 3.32 – 20.8 µg g^{-1}, China (46) at 3.95 – 13.8 µg g^{-1}, and Nigeria (49) at 0.64 – 1.62 µg g^{-1}. The study conducted by Salwa et al. (50) found that the concentration of Pb in poultry feathers was 0.869 µg g^{-1}. Although feathers are connected to the blood circulation, Pb accumulates in the keratin protein of the feathers (51). A high level of Pb was also found in human blood samples that were collected for research in the Mitrovica region (52). Indeed, the concentration of Pb in the blood of humans living in the Mitrovica region was shown to be at a relatively alarming level (3.8 µg dL^{-1}) (53).

Arsenic

The highest average As concentration was detected in the Mitrovica region (7.44 μg g^{-1}), followed by the Obiliq region (1.5 μg g^{-1}), and no measurable level was observed for the Dragash region. Industrial development, such as mines, is considered to be the major source of air pollution from As (54). Given that a mining complex is situated in the Mitrovica region, the presence of a higher concentration of As was expected for this region compared to the Obiliq and Dragash regions. Other authors have reported similar levels, including values from Belgium (55) at 0.88 – 23.35 μg g^{-1}, Pakistan (56) at 19 – 21.4 μg g^{-1}, and Malaysia (50) up to 0.472 μg g^{-1}. According to Stafilov et al. (57), the concentration of As in soil from the Mitrovica region was 55 μg g^{-1}.

Chromium

The highest average concentration of Cr was found in the Dragash region (19.0 μg g^{-1}), followed by Mitrovica (11.1 μg g^{-1}) and the Obiliq region (5.09 μg g^{-1}). The differences in the Cr concentration between the regions were found to be statistically significant ($p<0.001$). The significantly higher concentration of Cr in the chicken feathers from the region of Dragash could be related to the fact that this region is located near the city of Kukes in Albania, which is considered to be the region with the largest Cr mine in Europe (58, 59). Similar results for the Cr concentration in feathers have been reported by other studies in different countries around the world, including Malaysia (50) at 2.66 – 4.85 μg g^{-1} and Pakistan (56) at 19 – 21.1 μg g^{-1}.

Manganese

The results of this study showed that the Dragash region also had the highest average Mn concentration in chicken feathers (31.3 μg g^{-1}) compared to Mitrovica (15.2 μg g^{-1}) and Obiliq (6.17 μg g^{-1}). The Dragash region is very rich in minerals containing Mn (60). Similar concentrations have been found in the feathers of birds in other studies, including 7.21 – 65.98 μg g^{-1} (50) and 15.3 – 26.9 μg g^{-1} (31). The Mn concentration in the feathers could be related to the concentrations of ingested contaminants as well as to the contents of contaminants in the air (61).

Nickel

The average concentration of Ni in chicken feathers in the Mitrovica region was found to be 14.3 μg g^{-1}. The highest average concentration of Ni observed in the Dragash region was 15.8 μg g^{-1}, while the lowest concentration was found in the Obiliq region at 12.3 μg g^{-1}. High statistically significant differences were observed when the Obiliq and Dragash regions were compared ($p<0.001$). These differences were less dramatic when Mitrovica to Obiliq ($p<0.01$) and Mitrovica to the Dragash region ($p<0.05$) were compared. From the results of Honda et al. (62), it is known that Ni is related to the pigment of chicken feathers, which makes sense because most of the chickens used for our analysis had a black feather colour. The average level of the Ni concentration in feathers shows a wide range depending on the country in which the study was done. For example, a study conducted by Salwa et al. (50) found the average concentration of Ni to be 1.9 – 2.7 μg g^{-1}. In contrast, a higher Ni concentration was found in the study conducted by Malik and Zeb (31) in Pakistan, at 7.8 – 9.0 μg g^{-1}, whereas an extremely high concentration of Ni was observed in the study of Abdullah et al. (56) in Pakistan, at 41.6 – 84.8 μg g^{-1}.

Copper

Regarding the Cu concentration in the chicken feathers, average concentrations of 25.0 μg g^{-1} for Mitrovica, 22.1 μg g^{-1} for Obiliq and 27.2 μg g^{-1} for the Dragash region were determined. Statistically significant differences between regions Mitrovica and Obiliq as well between Obiliq and Dragash regions were found ($p<0.01$), while no difference was found between the Mitrovica and the Dragash regions ($p>0.05$). The Dragash region is located in the Sharr Mountain area, which contains the metals Cr and Cu (44). Our study results were comparable to study results obtained by other authors, including levels of 10.4 μg g^{-1} (48), 17.0 μg g^{-1} (63), and 6.60 μg g^{-1} (50). In the study conducted by Dauwe et al. (51) in Belgium, higher Cu concentration in poultry feathers of 69 – 88 μg g^{-1} were reported. In contrast, Malik and Zeb (31) in Pakistan found a lower amount of Cu in poultry feathers at 3.7 – 4 μg g^{-1}.

Cadmium

Cadmium is a non-essential metal for living organisms (64). In our study, the average concentrations of Cd in chicken feathers were as follows: 12.3 μg g^{-1} in Mitrovica, 11.7 μg g^{-1} in Obiliq and 11.1 μg g^{-1} in the Dragash region. Significant differences in Cd levels were determined between the regions of Mitrovica and Obiliq and of Mitrovica and Dragash ($p<0.05$). However, there were no significant difference between Obiliq and Dragash regions ($p>0.05$). The results regarding the presence of Cd in feathers are similar to results from researchers in other countries, such as Belgium (51) and Pakistan (56). Our results from the Mitrovica region were also comparable to the results of Zogaj and Düring (65), who confirmed the presence of Cd in various samples from soil, plants, etc.

Zinc

Zinc is an essential metal (66), and the average concentration of Zn in chicken feathers in the Mitrovica region was 111.0 μg g^{-1}. A similar concentration was also observed in the Obiliq region at 109.0 μg g^{-1}, while a substantially higher concentration was found in the Dragash region at 131.0 μg g^{-1}. The presence of Zn in the chicken feathers in the Dragash region was higher compared to the other regions, in which more non-essential metals (Pb, As, etc.) were present.

The average concentration of Zn was shown to be significantly different between the Obiliq and Dragash regions ($p<0.01$). Statistically significant differences were also found between the Mitrovica and Dragash regions ($p<0.05$), whereas the Zn concentration in chicken feathers did not differ between the Mitrovica and Obiliq regions ($p>0.05$). Comparable results were obtained in the study conducted by Salwa et al. (50) who studied the Zn concentration in chicken feathers in 2012 in Malesia (104.0 μg g^{-1}). The Zn concentration in the feathers of other birds in a study by Jungsoo and Tae-Hoe (48) in Korea, varied between 67.9 – 103 μg g^{-1}, while the study results presented by Malik and Zeb (31) from Pakistan, showed a concentration of Zn in the range of 133.8 – 155.2 μg g^{-1}. A considerably greater concentration of Zn in bird feathers was found in the results presented by Abdullah et al. (56) in Pakistan at 226 – 529 μg g^{-1}.

These results support the concept that non-essential metals can have high affinity for sulfhydryl groups and can bind to reduced cysteines in peptides and proteins (67, 68, 69, 70). The current findings also support the data obtained by other researchers related to environmental pollution with metals and their effects on living organisms in the regions of Mitrovica and Obiliq (40, 65, 71).

Conclusions

Our investigation detected high levels of metals in chicken feathers in three studied regions of Kosovo (Mitrovica, Obiliq and Dragash). The outcomes of this research clearly show that the region most polluted by metals was the Mitrovica region, with Pb as the primary metal followed by Cd and As. In the Mitrovica region, Zn was identified as the primary non-essential metal. After the Mitrovica region, the most polluted region was Obiliq, where the primary metal was Cd followed by Pb and As. In Obiliq, we also found higher concentrations of Zn than other non-essential metals. Dragash was the region with the lowest levels of non-essential metals, and among the essential metals, Zn was found at the highest concentrations followed by Mn, Cu, Cr and Ni. The most polluted region was Mitrovica, followed by Obiliq, and the least polluted region was Dragash.

Using avian feathers to determine the degree of environmental pollution is a reliable approach for biomonitoring, as shown by numerous researchers from different countries. Based on our results, monitoring of metals in the feathers of chickens can serve as a valuable method for the evaluation of the environmental pollution. Conducting similar surveys in the future could contribute towards the management of environmental pollution, which would promote the health of all living organisms as well as overall public health.

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OCENA ONESNAŽENOSTI OKOLJA S KOVINAMI NA OSNOVI PREISKAVE PRSNIH PERES PIŠČANCEV (*Gallus gallus domesticus*) V NEKATERIH INDUSTRIJSKIH PODROČJIH KOSOVA

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Povzetek: Namen raziskave je bil oceniti prisotnost kovin v treh področjih Kosova s pomočjo piščančjega (*Gallus gallus domesticus*) perja, vzorčenega v industrijskih področjih Mitrovica in Obiliq ter neindustrijski regiji Dragaš. Raziskava je bila izvedena od septembra do novembra 2016, perje pa je bilo odvzeto 90 piščancem v prosti dvoriščni reji. Koncentracije kovin v piščančjih peresih so bile določene z atomsko absorpcijsko spektrometrijo (AAS). Razpon povprečnih izmerjenih koncentracij kovin (µg/g perja) v preiskovanih območjih je bil: Zn 109-131, Mn 6,17-31,30, Cu 22,1-27,2, Cr 5,09-19,0, Ni 12,3-15,8, Pb <0,0945-15,5, Cd 11,1-12,3 in As <0,09-7,44. Najvišje povprečne koncentracije kovin (µg/g) so bile ugotovljene v področjih Dragaš: Zn 131, Cu 27,2, Mn 31,3, Cr 19,0, Ni 15,8 in Mitrovica: As 7,44, Cd 12,3, Pb 15,5. Statistično pomembne razlike ($p < 0,001$) med tremi področji so bile ugotovljene za vsebnost Pb, As, Mn in Cr. Statistično pomembnih razlik nismo ugotovili v vsebnosti Zn med Mitrovico in Obiliqom, v vsebnosti Cu med Mitrovico in Dragašem ter v vsebnosti Cd med Mitrovico in Dragašem. Rezultati raziskave bi morali biti zaskrbljujoči za okolijske agencije na Kosovu in bi jih morali spodbuditi, da sprejmejo konkretne ukrepe, predvsem občasno preverjanje omenjenih onesnaževal v teh industrijskih področjih. Na osnovi naših rezultatov priporočamo spremljanje onesnaženosti okolja s kovinami z uporabo piščančjega perja kot primernega in uporabnega preskusnega materiala.

Ključne besede: kovine v sledovih; AAS; piščančje perje; onesnaženost okolja; Kosovo

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Original Research Article

HISTOLOGICAL AND STEREOLOGICAL STUDY ON THE KIDNEYS IN SPARROWS LIVING IN WET AND ARID ZONES

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Abstract: The structure of the kidneys of sparrows living in wet and arid zones was compared to each other. A stereological study was conducted to quantify the components of the bird's kidneys. A total of 10 female sparrows living in wet and arid zones were collected. The length of the kidneys was measured before blocking. The transverse serial sections were taken with a thickness of 5 µm. The sections were stained with haematoxylin and eosin (H & E) as well as periodic acid Schiff- alcian blue (PAS- AL).
The kidneys of the sparrows consisted mostly of a cortex with only a small portion as medulla. The medulla was arranged in the form of cones with different lengths, which were randomly distributed within the kidney. The medullary nephron tubules were arranged sequentially. Thick and thin limbs of loop of Henle were separated by the collecting ducts. The histological structure and absolute volumes of the kidneys and components of the nephron were quantified. The absolute volumes of the structures within the right and left kidneys were not significantly different. The volume of the whole kidney ($p<0.001$) in the wet zone was larger than that in the arid zone. Furthermore, the volume proportion of the proximal tubule as well as cortex to whole kidney size was higher than that in wet zone birds. Whereas, the volume proportion of the medulla, distal tubule, cortical collecting tubule, medullary collecting duct and thin limb of loop of Henle to whole kidney size was higher than that in arid zone birds ($p\leq0.001$). This feature indicates that the arid zone bird species had a more high ability to conserve water by producing concentrated urine.

Key words: sparrow; stereology; histology; kidney; environment

Introduction

Many detailed studies have been published concerning the histology of the mammal's kidney, but comparatively, few studies have been conducted into the bird's kidney. The nephron is the functional unit of the kidney and its structure considerably varies between the different vertebrates. Birds and mammals produce hyperosmotic urine due to presence of a loop of Henle. In birds, most nephrons do not contain a loop of Henle, and are referred to as the reptile type of nephrons. In mammals, all the nephrons contain a loop of Henle, but some of them are long and the others are short (1).

Bird inhabiting arid and wet environments have a different diet. To accommodate dietary variations available water and ions, the kidney structure may be expected to vary in birds inhabiting two completely different climatic regions (2). In the avian kidney, the cortex and medulla are arranged in a series of randomly distributed and oriented cones. The avian kidney has both looped and loopless nephrons (3). The ability to conserve ions and/or water may be correlated with the

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Figure 1: Sparrow and the environments of sampling

structure of the nephron. The present study aimed to quantitatively investigate differences in the anatomical structure of the kidneys from sparrows living in either arid or wet zone areas. In addition, the physiological importance of each component of nephron will be discussed. The data can be used in various fields of science, such as histology, biology, physiology and ecology.

Material and methods

Ten adult sparrows (Passer domesticus) were collected from arid and wet zones (5 of each). Shahdad area (Kerman province, Iran) as an arid zone and Qaem Shahr city (Mazandaran province, Iran) as a wet zone were chosen (Fig. 1). The captured birds were accidentally females. All the experiments were conducted by the guidelines provided by Animal Care Committee, Ferdowsi University of Mashhad, and in compliance with the regulations formulated by the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran (Grant No. 3.28795, 3.28801).

Light microscopy

The birds were euthanized with an intraperitoneal injection of barbiturate. Both right and left kidneys were removed from the synsacrum bone. The kidneys were flushed with normal saline and were fixed in 10% neutral buffered formalin for 71 hours. Tissue samples were then dehydrated and cleared by a series of graded alcohols, xylene and eventually embedded in paraffin (Merck; Darmstadt, Germany). Paraffin sections at 5 µm thickness were cut from the cranial division of kidney toward the caudal division. The sections were stained with haematoxylin and eosin (H & E) and periodic acid Schiff- alcian blue (PAS-AL) for histological observation. The latter stain was necessary to distinguish the cortical collecting tubules from the distal tubules, since the lumen of the cortical collecting tubules stains blue due to presence of mucus (4). The detailed histology of the kidney was studied. Microphotographs were taken by a CX21 light microscope (Olympus; Japan) equipped with an Olympus (U-TVO 63XC) camera.

The stereology was used to quantify the components of birds' right and left kidney. For this purpose, the overall length of each kidney from the cranial pole to the caudal pole was measured before blocking. The tissue was cut in an unbiased manner at 10 equally spaced intervals along its entire length (5). Afterward, it was sectioned at a thickness of 5 µm using an RM2145 microtome (Leica; Germany).

According to the systematic random sampling (SRS) method, at least 11 sections were taken from each of the kidneys. By following the staining sections with PAS-Al, several fields from each

section were selected (according SRS) and imaged by a photomicroscope. More than 50 images were recorded for each of the kidneys. The images were placed under a stereological grid with 300 intersection points without any directing. The point counting using the Cavalieri principle was employed to estimate the volume of the kidney, cortex, medulla, glomerulus, proximal tubule, distal tubule, and the loop of Henle and medullary ducts (6). The volume of each part of the nephrons was estimated using the formula:

$$V = \frac{\sum p \times a(p) \times t}{M^2}$$

Where V is the volume of the component measured by the Cavalieri principle, Σp is the total counted points, $a(P)$ is the reference area, t is the interval between the sections and M^2 is the magnification to the power of 2.

Statistics

The mean and standard deviation (SD) of the absolute volume of the structures within the kidneys and proportion of each structure to the whole size of the kidneys were reported. Pair sample t-test was used to compare the volume of the structures within the right and left kidneys. Moreover, the volume of the structures within the kidneys and proportion of them to the whole size of the kidney was compared in wet and arid zone birds using the independent sample t-test. All of the statistical procedures were performed using the IBM statistics software (IBM Corporation, Armonk, NY, Version 21).

Results

Histologically, the sparrows' kidneys consisted of two zones, the cortex and the medulla.

The sparrows' kidney consisted mostly of a cortex with only a small portion as medulla (Fig. 2A, B).

Using a light microscope, there were no differences between the mammalian and reptilian types of nephrons in wet and arid birds. A majority of the nephrons did not have a loop of Henle, and were always only in the cortex area. Most nephron tubules were randomly distributed within the cortex, except for the glomeruli, which were placed most commonly in the peripheral cortex, and a majority of distal tubules were clustered around the intralobular vein (Fig. 3A, B).

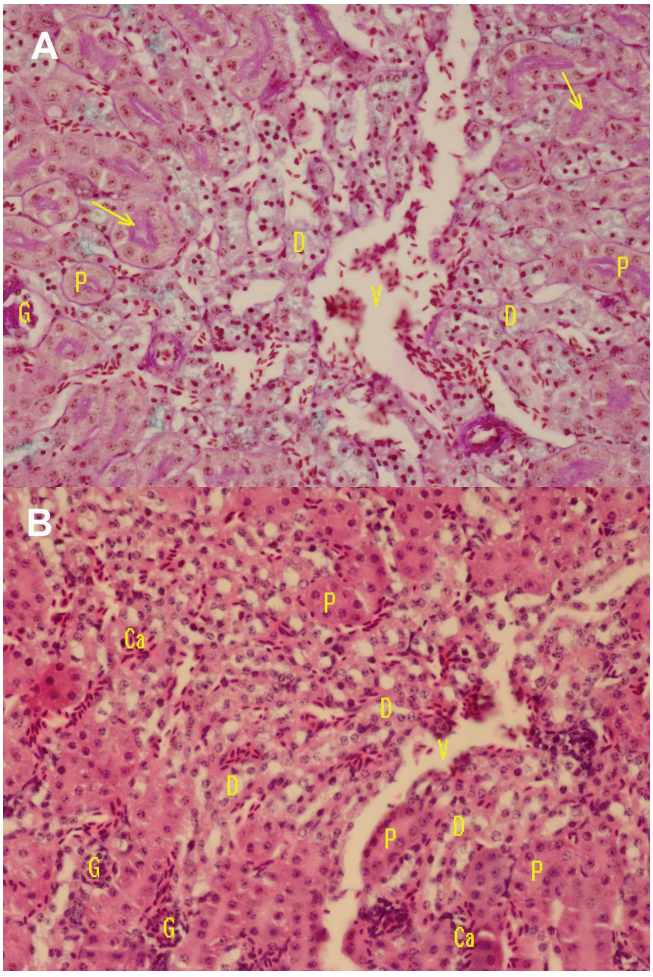

Figure 2: Photomicrograph of the kidney in transverse section of sparrows living in arid (A) and wet (B) zones. Cortex (C); medullary cone (MC); intralobular vein (V), (PAS-AL × 200, 160)

The renal corpuscle consisted of an outer Bowman's capsule separated from glomerulus by Bowman's space. The glomeruli consisted of a packed central core of mesangial cells surrounded by capillary loops (Fig. 3B). Both mammalian and reptilian types of glomerules were studied. The proximal and distal tubules were covered by the cuboidal epithelium. The macula densa was not developed in the sparrows. The luminal surface area of the proximal tubule was enhanced by a thick layer of microvilli forming a brush border (Fig. 3, 4). The cortical collecting tubules were covered by the cuboidal epithelium. The cytoplasm of cell contained a basal nucleus and mucopolysaccharides. The mucopolysaccharide was stained with PAS-AL (Fig. 4).

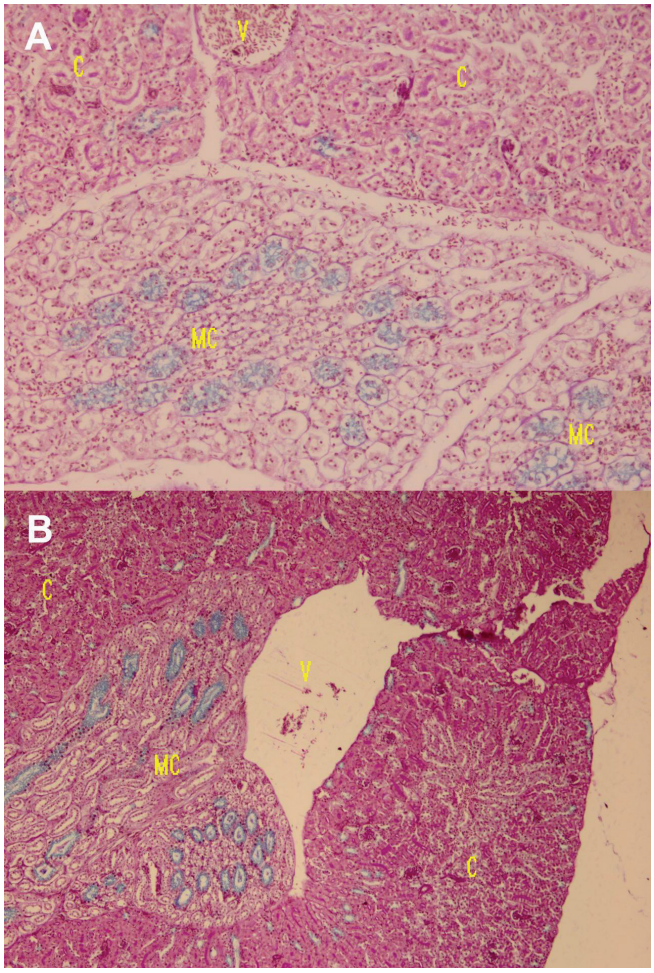

Figure 3: Photomicrograph of the kidney of sparrows living in arid (A) and wet (B) zones, showing the concentric arrangement of distal tubule (D) around the intralobular vein (V). Proximal tubule (P); glomerolus (G); brush border (arrows), (PAS-Al × 400, 640)

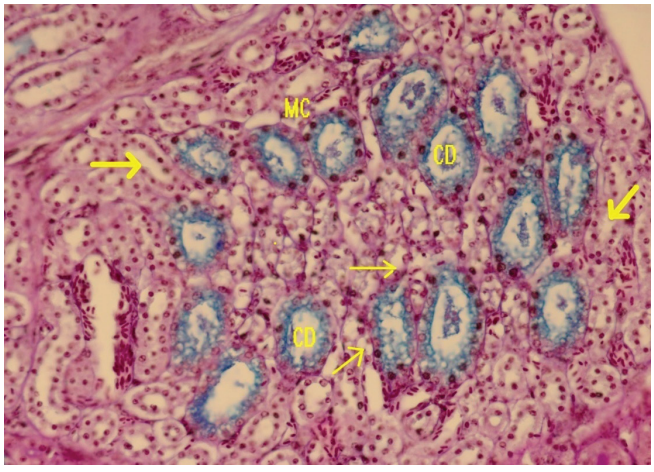

Figure 4: Photomicrograph of the kidney of sparrows living in arid zone. Proximal tubule (P); cortical collecting tubule (CC); glomerolus (G); intralobular vein (V); brush border (arrows), (PAS-Al × 400)

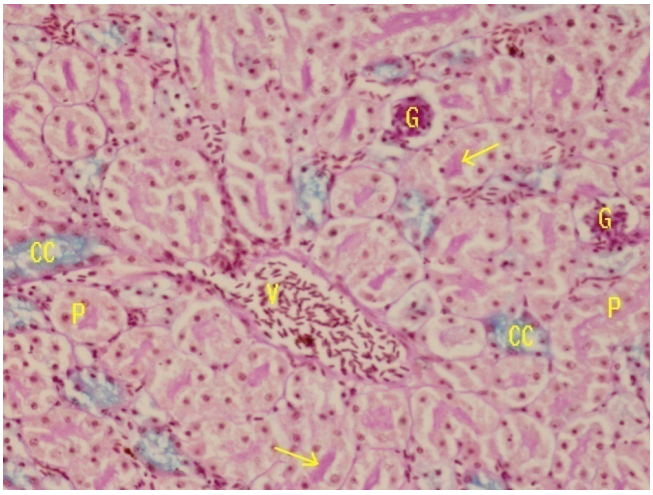

Figure 5: Photomicrograph of the medullary cone (MC) in the kidney of sparrows living in wet zone the wet zone sparrow's kidney, showing the arrangement of tubules within the cone. Thick limb of loop of Henle (thick arrows); medullary collecting ducts (CD), thin limb of loop of Henle (thin arrows) (H & E × 640)

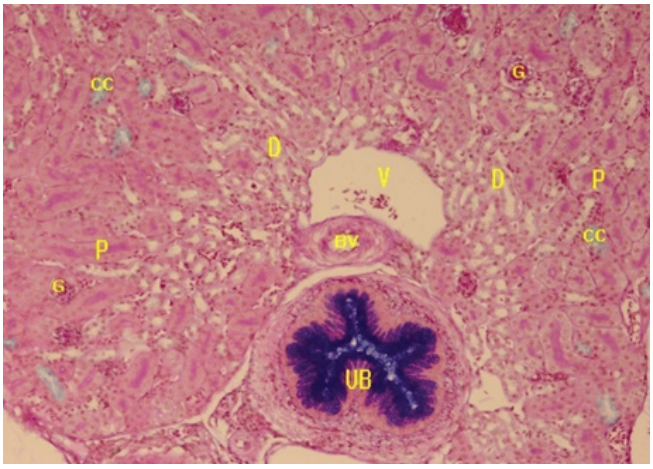

Figure 6: Showing the proximal tubule (P); distal tubule (D); cortical collecting tubule (CC); glomerolus (G); blood vessel (BV); intralobular vein (V) and an ureteric branch (UB) in the kidney of sparrows living in wet zone wet zone sparrow's kidney, (PAS-Al × 320)

Medullary nephron tubules were arranged sequentially. Thick and thin limbs of loop of Henle were separated by the collecting ducts. Thick limbs were limited to the periphery of the medullary cone and surrounded a ring of collecting ducts, which in turn surrounded some thin limbs. The thin and thick limbs of loop of Henle were covered by the simple cuboidal epithelium. The cortical collecting duct continued into the medulla as the medullary collecting duct. The medullary collecting duct consisted

Table 1: Description of the absolute volumes (mm^3) of the left (n=5), right (n=5) and total (n=10) structures of the kidney in wet and arid zone birds

Component		Arid zone				Wet zone			
		n	mean±SD	min	max	n	mean±SD	min	max
Glom.	Left	5	0.64±0.11	0.46	0.78	5	0.71 ±0.30	0.30	0.96
	Right	5	0.64±0.25	0.36	0.98	5	0.74 ±0.15	0.57	0.96
	Total	10	0.64[a]±0.18	0.36	0.98	10	0.72[a]±0.23	0.30	0.96
Prox.tub.	Left	5	4.45[a] ±2.42	2.42	8.14	5	21.80[b]±4.33	15.49	25.42
	Right	5	4.71[a]±1.61	3.32	7.06	5	19.30[b]±3.01	14.51	22.52
	Total	10	4.58[a]±1.94	2.24	8.14	10	20.55[b]±3.75	14.51	25.42
Dist. tub.	Left	5	2.83±0.89	1.70	3.77	5	6.73±2.40	3.90	9.96
	Right	5	3.35±135	1.57	5.08	5	5.68±1.79	3.31	8.30
	Total	10	3.09[a]±1.11	1.57	5.08	10	6.21 [b]±2.07	3.31	9.96
Cor. Coll. Tub.	Left	5	0.85±0.21	0.54	1.12	5	0.69±0.28	0.32	0.96
	Right	5	0.89±0.20	0.65	1.22	5	0.56±0.23	0.20	0.81
	Total	10	0.87 [a]±0.20	0.54	1.22	10	0.63 [b]±0.25	0.20	0.96
Med. Coll. duct	Left	5	0.77±0.30	0.42	1.22	5	0.65±0.24	0.29	0.95
	Right	5	0.84±0.41	0.37	1.39	5	0.67±0.25	0.45	0.99
	Total	10	0.80 [a]±0.34	0.37	1.39	10	0.66 [a]±0.23	0.29	0.99
Thick limb of loop of Henle	Left	5	1.96±0.59	1.23	2.85	5	3.09±0.88	1.96	4.41
	Right	5	2.03±0.83	0.95	3.17	5	2.83±0.70	1.64	3.38
	Total	10	2.00 [a]±0.68	0.95	3.17	10	2.96 [b]±0.76	1.64	4.41
Thin limb of loop of Henle	Left	5	0.62±0.22	0.43	1.01	5	0.53±0.27	0.24	0.85
	Right	5	0.69±0.37	0.32	1.27	5	0.60±0.25	0.35	1.04
	Total	10	0.65 [a]±0.29	0.32	1.27	10	0.57 [a]±0.25	0.24	1.04
Cortex	Left	5	15.16±3.59	10.31	19.35	5	53.46±13.84	36.07	65.99
	Right	5	16.88±5.23	9.58	21.47	5	46.97±5.22	42.35	54.91
	Total	10	16.02 [a]±4.32	9.58	21.47	10	50.22 [b]±10.44	36.07	65.99
Medulla	Left	5	3.86±1.29	2.54	5.99	5	6.84±2.06	4.10	9.65
	Right	5	3.95±1.83	1.78	6.50	5	6.48±1.76	4.58	9.12
	Total	10	3.91 [a]±1.49	1.78	6.50	10	6.66 [b] ±1.82	4.10	9.65
Kidney	Left	5	19.02±4.64	12.85	25.34	5	60.90±15.20	40.55	76.15
	Right	5	20.83±6.83	12.22	27.97	5	53.93±6.24	47.39	62.15
	Total	10	19.93 [a]±5.59	12.22	27.97	10	57.41 [b]±11.55	40.55	76.15

Mean values within a rows followed by different superscript letters are significantly different ($P < 0.05$)

Glomerule (Glom.); Proximal tubule (Prox. tub.); Distal tubule (Dist. tub.); Cortical Collecting Tubule (Cor. Coll. Tub.)

Table 2: Description of the proportion of each component volume to the whole size of the kidney for left (n=5), right (n=5) and total (n=10) structures of the kidney in birds living in wet and arid zones

Component		Arid zone				Wet zone			
		n	mean±SD	min	max	n	mean±SD	min	max
Glom./kidney	Left	5	0.030.004	0.03	0.04	5	0.011±.003	0.006	0.013
	Right	5	0.030.003	0.03	0.03	5	0.013±0.002	0.011	0.016
	Total	10	0.03^{a}±0.004	0.03	0.04	10	0.011^{b}±0.003	0.006	0.013
Prox. Tub./ kidney	Left	5	0.22±0.07	0.14	0.32	5	0.362±0.024	0. 324	0.385
	Right	5	0.23±0.04	0.17	0.28	5	0.357±0.038	0.306	0.412
	Total	10	0.22±0.05	0.14	0.32	10	0.359±0.030	0.306	0.412
Dist. Tub./kidney	Left	5	0.14±0.02	0.13	0.19	5	0.109±0.022	0.078	0.138
	Right	5	0.15±0.02	0.13	0.20	5	0.104±0.025	0.066	0.133
	Total	10	0.15±0.002	0.13	0.20	10	0.106±0.022	0.066	0.138
Cor. Coll. Tub./ kidney	Left	5	0.04±0.007	0.04	0.06	5	0.011±0.004	0.007	0.019
	Right	5	0.04±0.009	0.04	0.06	5	0.010±0.004	0.003	0.014
	Total	10	0.04±0.007	0.04	0.06	10	0.011±0.004	0.003	0.019
Med. Coll. duct/ kidney	Left	5	0.03±0.006	0.03	0.05	5	0.010±0.003	0.007	0.016
	Right	5	0.03±0.009	0.02	0.05	5	0.012±0.004	0.007	0.018
	Total	10	0.03±0.007	0.02	0.05	10	0.011±0.004	0.007	0.018
Thick limb of loop of Henle / kidney	Left	5	0.10±0.01	0.09	0.13	5	0.051±0.009	0.042	0.065
	Right	5	0.09±0.02	0.06	0.13	5	0.052±0.010	0.034	0.061
	Total	10	0.10±0.01	0.06	0.13	10	0.051±0.009	0.034	0.065
Thin limb of loop of Henle /kidney	Left	5	0.03±0.06	0.02	0.04	5	0.008±0.004	0.003	0.013
	Right	5	0.03±0.008	0.02	0.05	5	0.011±0.004	0.005	0.017
	Total	10	0.03±0.007	0.02	0.05	10	0.010±0.004	0.003	0.017
Cortex/kidney	Left	5	0.7±90.03	0.76	0.84	5	0.876±0.026	0.838	0.909
	Right	5	0.81±0.04	0.77	0.88	5	0.871±0.024	0.838	0.893
	Total	10	0.80±0.03	0.76	0.88	10	0.874±0.024	0.838	0.909
Medulla/kidney	Left	5	0.20±0.03	0.16	0.24	5	0.113±0.026	0.080	0.149
	Right	5	0.18±0.04	0.12	0.23	5	0.119±0.025	0.096	0.155
	Total	10	0.19±0.03	0.12	0.24	10	0.116±0.024	0.080	0.155

Mean values within a rows followed by different superscript letters are significantly different ($P < 0.05$)

of a proximal segment and a distal papillary duct covered by the columnar epithelium. The cytoplasm of each cell contained a basal nucleus and mucopolysaccharides. The latter fuses dendritically as they traverse a medullary unit, and eventually forms ureteric branches, draining directly into the ureter. Therefore, each medullary cone terminates in a single branch of the ureter (ureteric branch). Furthermore, the cells of the ureteric branch contained mucopolysaccharide (Figs. 5, 6).

Tables 1 and 2 present the Mean± standard deviation of the absolute volume of the structures within the left and right kidneys, and proportion of the each structure volume to the whole size of the kidney for wet and arid zone birds. The absolute volume of the structures within the kidneys, and the proportion of each component volume to the whole size of the kidney were not significantly different between the right and left kidneys of birds living in wet and arid zones ($p>0.05$). The volume of the whole kidney ($p<0.001$) in wet zone birds was larger than that in arid zone birds (Table 1). Furthermore, the volume proportion of proximal tubule and cortex to the whole size of the kidney in wet zone birds was higher than that in arid zone birds. However, the volume proportion of the medulla, distal tubule, cortical collecting tubule, medullary collecting duct and thin limb of loop of Henle to the whole size of the kidney in arid zone birds was higher than that in wet zone birds ($p\leq0.001$; Table 2).

Discussion

Histologically, the sparrows' kidneys consisted of two zones, the cortex and the medulla. The cortex made up a vast area of the kidney with only a small portion being medulla. There was no difference between the histological structure of the sparrow's kidney in wet and arid zones. This feature is similar to that in rock dove, collared dove and owl (7).

It seems that the avian medullary cones are structurally similar (analogous) to the outer medulla of mammals' kidneys (8). The luminal surface area of the proximal tubule was enhanced by a thick layer of microvilli forming a brush border. Increase of the volume and surface area of the brush border may lead to a greater quantity of glomerular filtrate of the proximal tubule (2). It is accepted that the wide intercellular spaces coupled with the extensive cell membrane infolding in proximal tubule is the characteristic of cells having considerable ion and water reabsorption capacity (9). For example, in house geckos (Hemidactylus sp.), under hydrated conditions, 80% of the filtered water and ions are reabsorbed by elaborated cell membrane infolding, whereas in horned lizard Phrynosoma cornutum and the Galapagos iguanid lizard Tropidurus sp. with no cell membrane infolding, reabsorbtion is approximately 50% of the filtered water and ions (10). In mammals, approximately 70% of all ions are reabsorbed in the proximal tubule (11). The absorption of the proximal tubule in avian, depending on active reabsorption, is approximately 70% of the filtered volume of water (1). In the avian nephron, absorption of sodium chloride from the distal tubule in some cases, may proceed without water reabsorption and increase the cranial concentration gradient along the length of the nephron tubule, thereby allowing the water to be distally reabsorbed along the nephron at the medullary collecting ducts. The distal tubule in the bird's nephron reabsorbs water passively and reabsorbs sodium chloride actively from the nephron tubule as the mammalian's nephron (12). This additional reabsorption helps to conserve the fluid that might otherwise be excreted. Hence, the act of reabsorption by the distal tubule saves both water and solutes (13). The macula densa (MD) in sparrows was not as well developed as in mammals, in which they did not have taller cells, but resembled closely to the macula densa cells found in honeyeaters birds (3), G. domesticus (14), C. japonica (15) and the common starling Sturnus vulgaris (16). The avian cells possess some characteristics of mammalian's MD cells and they are distinguishable from the cells in adjacent portions of the distal tubule. The Golgi system in the avian cells is from apex to the nucleus. The cells in avian MD sites can be considered structurally transitional between the mammal's typical MD cells in mammals and the ordinary cells of the distal tubule (15).

The cortical collecting tubule was covered by the cuboidal epithelium. Each cell contained a basal nucleus and the characteristics of cells having high ion and water reabsorption capacity (9). As with the distal tubules, the cortical collecting tubules also play a role in producing concentrated urine by reabsorbing the water from the tubular lumen. In addition, they are also known to secrete

the mucin, which may help to clear the kidney from the uric acid (13). Since the uric acid is a means of excreting the solutes with minimal water loss, it might be expected that arid zone birds produce more uric acid than wet zone birds as a water conservation strategy.

The countercurrent multiplier mechanism operates between the descending and ascending limbs of Henle via recycling a single solute (NaC) with no water accompaniment, forming an osmotic gradient along the medullary cone (17). In sparrows, the nephron tubules were regularly arranged for the entire length of the medulla, with the thick and thin limbs of loop of Henle separated by the collecting ducts. This situation is different from the arrangement pattern reported in muscicapid passerines, where the tubules were orderly arranged only in the superficial areas of the medulla, and randomly deeper within the medulla (18, 19, 20, 21). The functional significance of the ordered medullary tubule arrangement in passerines is unknown. The separation of the thick and thin limbs of loop of Henle by the collecting ducts appears to complicate the current theory of production of concentrated urine in birds (21). The wide intercellular spaces and few mitochondria in the epithelium of the thin limb of loop of Henle are typical of leaky epitheliums transporting the solutes passively (3). This study found that the volume of the thin limb of loop of Henle was greater in arid zone birds. This indicated that the loops of Henle in arid zone sparrows were longer than those in wet zone sparrows. It is concluded that the loops of Henle are the important areas of water reabsorption. On the contrary, the epithelial cells of the thick limb of loop of Henle in honeyeaters contained more mitochondria, and individual cells were separated by narrow intercellular spaces (3). The avian and mammalian‘s thick limbs are similar in diameter, but avian thin limbs are typically wider (20). In mammals, generally, longer loops of Henle are associated with a high urine concentrating ability (22, 23). The role of the medullary collecting duct is reabsorption of water and possibly some sodium from the tubule (24). It seems that there are no differences in the ultrastructural anatomy of the sparrows’ nephrons. The proximal tubules contain both narrow and wide intercellular spaces filled with interdigitations of the basolateral membrane. The thin limbs of loop of Henle contain the very wide intercellular spaces, which are absent in the thick limbs of loop of Henle. The distal tubule cells contain short, apical microvilli and infolding of the basolateral membrane. In cross-section, the medullary cones of all birds exhibit an outer ring of thick limbs of loop of Henle surrounding an inner ring of collecting ducts, which in turn surrounds a central core of thin limbs of loop of Henle. The Savannah Sparrow has a significant higher volume of medulla compared to wet zone species. Within the medulla, the Savannah Sparrow has a significant higher volume and surface area of capillaries, and a significant higher surface area of thick limbs of loop of Henle and collecting ducts than wet zone species. The data suggest that the salt marsh Savannah Sparrow has the renal morphology necessary to produce more high concentrated urine than wet zone species. A bird living in a salt marsh environment might be expected to concentrate its urine more than wet zone birds owing to limited availability of fresh water and a large amount of potential salt in its diet (24).

The absolute volume of the structures of the kidneys in sparrows is not significantly different between the right and left kidneys. The volume of the whole kidney ($p<0.001$) in wet zone birds was larger than that in arid zone birds. Furthermore, the volume proportion of the proximal tubule and the cortex to the whole kidney size was higher in wet zone birds than in arid zone ones. However, the volume proportion of the medulla, distal tubule, cortical collecting tubule, medullary collecting duct and thin limb of loop of Henle to the whole size of the kidney was higher in birds living in the arid zone environment ($p\leq0.001$). This feature indicates that the arid zone bird species have a higher ability to conserve the water by producing concentrated urine.

We found that the structure of wet zone sparrows’ kidney contained a higher proportion of cortex, whereas in arid zone sparrows, the higher proportion was for the medulla. By different environmental and dietary restrictions, the renal concentrating ability of birds may vary (25). Possible differences in the urinary concentrating ability of species from different zones may be due to differences in the proportion of cortex and medulla. Previous studies demonstrated that arid zone birds had a high volume of renal medulla than wet zone birds (26, 2, 27, 28). Similar results have been found for honeyeater birds (3), the mesic inhabiting white-rumped munia (Lonchura

striata), the collared turtle dove (Streptopelia decaocta), and several species of aquatic birds (28). Birds inhabiting an arid environment and having a largely insectivorous diet have a greater volume of medulla than those inhabiting mesic environments and having a nectarivorous diet (27). The volume of the kidney increased in proportion to the body weight, and these two parameters were strongly correlated with each other. A strong correlation between the kidney weight and body weight has been reported in avian species. The volume proportions of the cortex and medulla vary according to the bird's order, body size and environment. High renal concentrating ability improves the efficiency of renal conservation of water, and thus the species with larger proportions of renal medulla should have effective conservation of water. The quantitative structural characteristics of the avian kidney appear to be influenced by several factors, since the kidney volume per body weight in grams, and the volume proportions of the cortex and medulla can be correlated with the order, body size and habitat. Furthermore, a relatively large medulla seems to be the adaptive advantage of species, which have to mitigate the water scarcity or excessive evaporative water loss by maximum renal water conservation (28).

The absolute volume of the structures within the house sparrow's kidneys is not significantly different in different seasons. Thus, kidney morphology may not be affected by seasonal changes (13).

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HISTOLOŠKA IN STEREOLOŠKA ŠTUDIJA LEDVIC VRABCEV, ŽIVEČIH V VLAŽNIH IN SUHIH OBMOČJIH

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Povzetek: V opisani raziskavi smo primerjali strukturo ledvic vrabcev, ki živijo v vlažnih in sušnih območjih. Izvedena je bila stereološka raziskava, s katero smo količinsko opredelili različne tkivne strukture v ptičjih ledvicah. Zbrali smo vzorce 10 samic vrabcev, ki živijo v vlažnih in sušnih območjih. Pred nadaljnjo histološko obdelavo vzorcev smo izmerili dolžino ledvic. Pripravljene so bile prečne serijske rezine ledvic debeline 5 µm. Rezine so bile obarvane s hematoksilinom in eozinom (barvanje H&E) ter s PAS histokemijsko reakcijo z dodatkom alcianskega modrila (PAS-AL). Ledvice vrabcev so večinoma sestavljala področja skorje in manjši deli sredice. Sredico so sestavljali stožci različnih dolžin, ki so bili naključno razporejeni znotraj ledvice. Središčne cevke nefrona so potekale ena ob drugi. Debelejši in tanjši deli zanke nefrona so bili ločeni z zbirnimi cevkami. Histološka zgradba ledvic, absolutna prostornina ledvic in sestavni deli nefrona so bili kvantitativno ovrednoteni. Absolutna prostornina struktur se med levo in desno ledvico ni značilno razlikovala. Prostornina celotne ledvice vrabcev v vlažnem območju je bila večja od prostornine ledvic vrabcev iz sušnega področja ($p < 0,001$). Poleg tega je bil prostorninski delež proksimalne cevke in področje skorje v primerjavi s celotno velikostjo ledvic večji pri pticah iz sušnega področja kot pri pticah iz vlažnega področja. Prostorninski delež sredice, distalne cevke, zbirne cevke v skorji, zbirnega kanala v sredici in tankega dela zanke nefrona v celotni velikosti ledvic pa je bil višji pri vrabcih iz vlažnih področij kot pri vrabcih iz sušnih področij ($p \leq 0,001$). Opisane značilnosti ledvic kažejo na to, da bi lahko imele ptice iz sušnih območij večjo sposobnost ohranjanja vode s proizvodnjo koncentriranega urina.

Ključne besede: vrabec; stereologija; histologija; ledvica; okolje

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Original Research Article

MILK QUANTITY, COMPOSITION AND HYGIENE TRAITS OF ROUTINELY MACHINE MILKED LIPIZZAN MARES

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Abstract: The aim of the study was to research milk yield, composition and hygienic quality of routinely produced milk from Lipizzan mares. It was the first known case of adapting mares of this breed to the routine machine milking procedure. Three Lipizzan mares included in the routine machine milking produced commercial quantities of mare's milk.
Milk from Lipizzan horse breed was for the first time obtained and analysed in such quantity. The mares were milked consecutively three times per day during five days in a week, and during weekends, they were joined with their foals. The average values were as follows: daily milk yield (MY) was 8.24 kg, fat content (FC) was 4.027 g/kg, protein content (PC) was 15.064 g/kg, lactose content (LC) was 63.218 g/kg, somatic cell count (SCC) was 6.556 x 10^3/ml, total bacteria count (TBC) was 114.689 x 10^3/ml, and average freezing point (FP) was -0.505 °C. Consecutive milking significantly influenced PC, LC, SCC, and TBC. FC tended to increase with days in milk, whereas PC, SCC, and TBC decreased during the lactation stage. Significant differences between mares were found in PC, LC, SSC and TBC. Interaction between consecutive milking and mare was significant only for FC. The study confirmed that warmblooded horse breed can be used for commercial milk production along with coldblooded breeds. Routine machine milk production with use of Lipizzan mares could add an economic value to the indigenous breed and serve as an additional reason for its preservation.

Key words: milk composition; milk yield; days in milk; consecutive milking; hygienic quality

Introduction

The Lipizzan horse, established in 16th century in Lipica, Slovenia, is Slovenian indigenous breed and one of the oldest cultured horse breeds in the world. It is mainly used as a riding horse for leisure, sport dressage, classical dressage, and for carriage driving. According to Lipizzan International Federation, the majority of the Lipizzan population is bred by the private breeders in 19 countries and on nine state studs in the Central and Eastern Europe. Successful long-term preservation of indigenous horse breeds could only be performed by their economic utilization. According to Potočnik (1) the preservation with an economic increase of equine breeding could be achieved by milk production and its processing. There has been an increasing interest on the use of mare's milk in human nutrition, cosmetic and pharmaceutical industry in the recent years (2). Because of its nutritional characteristics, mare's milk is used as a low-allergenic substitute for bovine milk (3), as a substitute for human and bovine milk for premature new-borns (4) and as a healing agent for several diseases and disorders (5). It has been reported that use of mare's milk acts beneficially against skin diseases, supports

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general physical health, immune system and stomach function, keeps vitality of muscles, joints and bones, and aids with metabolism problems, liver diseases, cardiovascular diseases and cancer (2). Fat component of mare's milk is considered as a valuable ingredient in the cosmetics due to its high polyunsaturated fatty acids content, while proteins are used as an active component for skin hydration and prevention of skin ageing (6).

Production, composition and hygienic quality of milk may change depending on the lactation stage, age, parity and milk management (7, 8). Many studies investigated and reviewed some of these factors and their influence on the production of mare's milk (e.g. 8, 9, 10, 11). However, there is still a lack of knowledge regarding this topic. Hygienic status of mare's milk is most commonly described by somatic cell count and information on total bacteria count is difficult to find (8). Published data originates mostly from milk samples taken from mares that were not included in a routine milk production. Such samples in majority represent only first jets of milk and cannot equate with the quality of milk samples from completely milked udder.

This study was undertaken to examine the routinely produced milk from Lipizzan mares and it was the first known case of adapting mares of this breed to the routine machine milking procedure. Milk yield, composition and hygienic quality of milk were researched.

Material and methods

Animals and management

The experiment was carried out at the Equine Education and Research Centre Krumperk (EERC) of Department of Animal Science at Biotechnical Faculty, University of Ljubljana, Slovenia. From May 2011 on, EERC was the first registered establishment for raw mare's milk production and ethics approval was not required for the study. Milk samples were collected from three Lipizzan mares (Famosa XI, Thais VII and Bistrica IV) aged seven years. Two of them were in the second parity, and one was in the third parity. The body weight of the mares in the experiment was estimated using chest girth measurement (12). The chest girth measurement of Famosa XI and Bistrica IV was 187 cm and their estimated body weight (BW) was 516 kg, whereas the chest girth measurement of the Thais VII was 199 cm and her estimated BW was 607 kg. All mares were fed with the same feed; their diet consisted of 12 kg of first cut hay mid quality, 800 g beet pulp, 1500 g oats, 2200 g feed mixture (2871 kcal/kg, 14% crude protein), 300 g wheat bran, and 50 g mineral-vitamin mixture. Mares were adapted to the routine machine milking before the start of experiment. They were milked consecutively three times per day during five days in a week, and during weekends they were joined with their foals. Mares foaled from March to April and the routine milking started at the end of their second month of lactation. Milk samples for the analyses were taken from mid-August to the end of September.

Milk sampling

Milking was carried out by a Milkline milking machine for goats (model Economy 1E) adapted to the mares, with a mean vacuum level of 42 kPa, a pulsation rate of 120 cycles/min, and a pulsation ratio 1:1. Before the start of milking routine (3 h before first milking) foals were separated from the mares with the fences, which allowed nasal and visual contact with mares. Mares were milked three times per day, always at the same time. Separation was carried out at 07:30 a.m., first milking started at 10:30 a.m., second at 01:30 p.m., and third milking at 04:30 p.m. After the third milking, foals were re-joined with the mares. Milk yield (MY) and composition were noted for each mare after each milking on each test day (TD) during five weeks of the trial. The average daily MY was expressed as an estimated daily milk synthesis in the mare's udder. The milk from completely milked udder was sampled for the first time on the TD for each mare at the 113th, 161st and 157th day of lactation (1st week of trial). Samples were cooled and stored at 4°C till the next morning when the analysis was performed. Sampling was carried out in the 7-day intervals (once per week) until the end of the experiment. Altogether 45 representative samples (15 samples per mare) were analysed for contents of fat (FC), protein (PC), lactose (LC) as well as somatic cell count (SCC), total bacteria count (TBC) and freezing point (FP).

Analysis of milk

Samples were analysed at the accredited laboratory of the Institute of Dairy Science at Biotechnical Faculty (University of Ljubljana, Slovenia). FC, PC and LC were determined using a mid-infrared spectrometer Milkoscan FT 6000 (Foss Electric, Hillerød, Denmark) according to the reference standard ISO-IDF 9622 (13). Freezing point was determined using a mid-infrared spectrometer Milkoscan FT 6000 equipped with Integrated Milk Testing (IMT) software. TBC was determined with Bactoscan FC 65+ (Foss Electric, Hillerød, Denmark) according to the reference standard ISO-IDF 21187 (14). SSC was determined with a fluorooptо-electronic counter Fossomatic 500 (Foss Electric, Hillerød, Denmark) in accordance with the reference standard ISO-IDF 13366-2 (15).

Statistical analysis

Data were analysed by statistical package SAS/STAT (16). Mixed procedure was used for the analysis of variance. Statistical model was as follows:

$$y_{ijk} = \mu + \beta\left(x_{ijk} - \bar{x}\right) + A_i + M_j + AM_{ij} + e_{ijk}$$

and described overall mean (μ), mare (A_i), consecutive milking (M_j) and their interaction (AM_{ij}) as fixed effects, days in milk (x_{ijk}) as linear regression and random residual (e_{ijk}). Differences (determined at α=0.05) between least square means (LSM) were estimated using Bonferroni multiple comparison test. Average stage of lactation in the experiment was 151 days ($\bar{x}$). The analysis of variance for SCC and TBC was performed using this values as logarithms ($\log_2$ for SCC and $\log_{10}$ for TBC), while they were discussed as antilogarithms. The traits included in the model are presented as least square mean values with standard errors (LSM±SE) and are shown at the following figures only when they were significantly different.

Results

The results for milk yield, chemical composition and hygienic quality of Lipizzan mare's milk are shown in Table 1. The average estimated daily milk yield was 8.24 kg, with a minimum of 5.44 kg and a maximum of 10.56 kg. The milk production was 1.52 kg/100 kg of BW.

Among milk composition traits, FC was the most variable trait with a coefficient of variation (CV) 73.39%. The average FC was 4.0 g/kg, the average PC was 15.1 g/kg, whereas the average LC was 63.2 g/kg.

Hygienic status of mare's milk was evaluated throughout SCC and TBC. The mean values for SCC were generally low (6.55 x 10^3/ml), with the minimum of 2.000 x 10^3/ml and maximum of 17.0 x 10^3/ml. Contrary to that, it was found that the average TBC in milk of Lipizzan mares was high (114.69 x 10^3/ml). Coefficient of variation for the TBC in mare's milk was higher (127.01%) than for the SCC (48.74%). The mean value for FP was -0.505 °C, ranging from -0.528 °C to -0.443 °C.

The present study indicated that consecutive milking (Figure 1) significantly influenced PC, LC, TBC, and FP. PC significantly differed ($P \leq 0.001$) between consecutive milking: 1.52% in the milk from the first milking, 1.56% in the milk from the

Table 1: Milk yield, chemical composition and hygienic quality of Lipizzan mare's milk

Trait	Mean	SD	CV (%)	Minimum	Maximum
Milk yield (kg)	8.24	1.87	22.69	5.44	10.56
Milk yield (kg/100 kg BW)	1.519	0.368	24.22	0.929	2.039
Fat content (g/kg)	4.027	2.955	73.39	0.300	12.800
Protein content (g/kg)	15.064	1.062	7.05	12.500	17.100
Lactose content (g/kg)	63.218	2.137	3.38	57.600	66.100
SCC (10^3 /ml)	6.556	3.195	48.74	2.000	17.00
TBC (10^3 /ml)	114.689	145.667	127.01	7.0000	650.000
Freezing point (°C)	-0.505	0.020	3.97	-0.528	-0.443

SD: standard deviation; CV: coefficient of variation; SCC: somatic cell count; TBC: total bacteria count

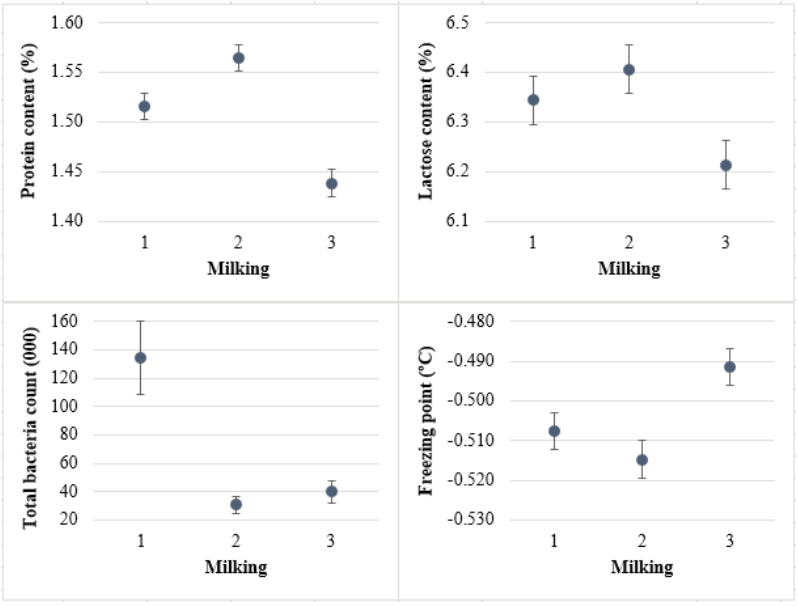

Figure 1: Influence of consecutive milking on protein content (%), lactose content (%), TBC (10^3/ml), and freezing point (°C) in mare's milk

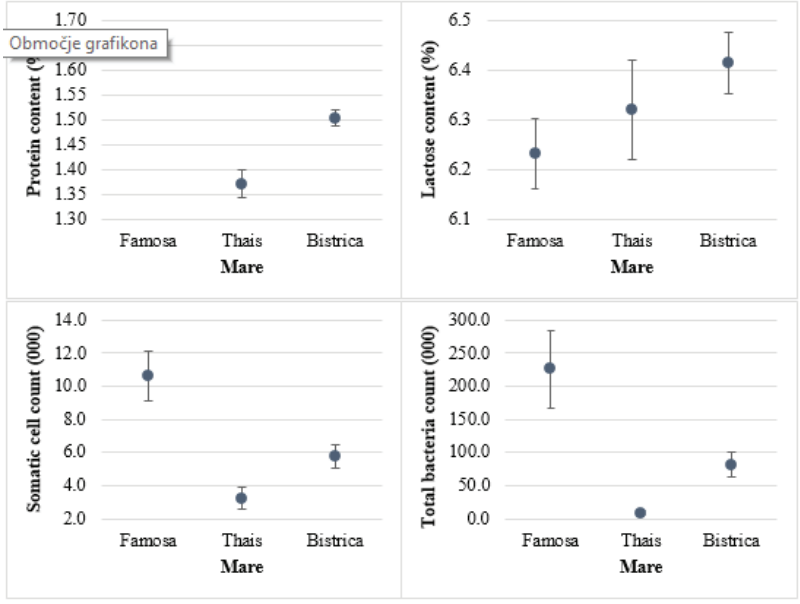

Figure 2: Influence of mare (Famosa, Thais, and Bistrica) on protein content (%), lactose content (%), SCC (10^3/ml), and TBC (10^3/ml) in mare's milk

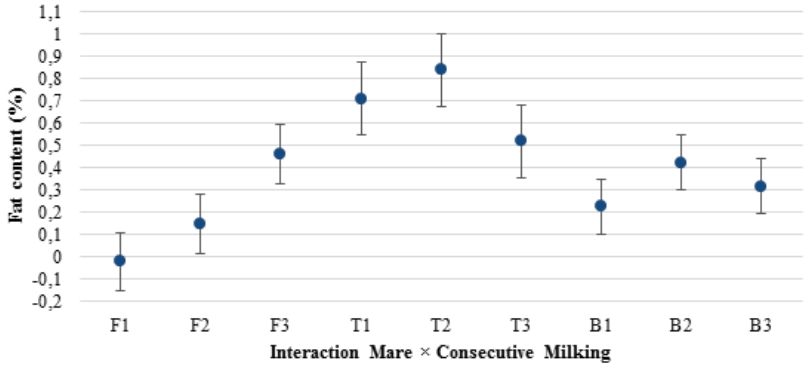

Figure 3: Interaction between mare (F=Famosa; T=Thais; B =Bistrica) and consecutive milking (first = 1, second = 2, and third = 3) on fat content (least square mean ± standard error) in mare's milk

second milking, and 1.44% in the milk from in the third milking. LC was significantly higher (P = 0.026) in the samples from the second milking (6.41%) compared to the ones from the third (6.21%) milking. The highest TBC was found in the samples from first milking (134.37 x 10^3/ml) and it significantly differed ($P \leq 0.001$) from the content of samples from the second (30.63 x 10^3/ml) and the third (39.96 x 10^3/ml) milking. The lowest FP of mare's milk was found in the second milking (-0.515 °C).

The regression coefficient indicated that FC tended to increase during lactation stage, whereas PC, SCC, and TBC decreased. Results of this study, as well confirmed that PC of mare's milk would slowly decrease during lactation. By expectation PC would decrease for 0.01% in average with every additional day in milk. For every additional day in milk, SCC and TBC would decrease for 0.09 and 8.58 x 10^3/ml in average, meaning that hygienic quality of mare's milk during the lactation would slowly increase.

The results showed individual differences between mares' milk in PC, LC, SSC and TBC (Figure 2). PC significantly differed ($P \leq 0.001$) between all mares. Thais VII had the lowest PC (1.37%), whereas Bistrica IV (1.50%) and Famosa XI (1.65%) had higher PC in milk. LC was significantly higher (P = 0.040) in milk from Bistrica IV (6.41%) than in milk from Famosa XI (6.23%). The highest SCC was found in milk from Famosa XI (10.60 x 10^3/ml), and it significantly differed (P = 0.001) from the SCC found in milk from Thais VII (3.24 x 10^3/ml) and Bistrica IV (5.75 x 10^3/ml). TBC also significantly differed ($P \leq$ 0.001) between all mares included in the study. It was found that Thais VII had the lowest TBC (9.00 x 10^3/ml), whereas Bistrica IV (81.04 x 10^3/ml) and Famosa XI (225.31 x 10^3/ml) had the highest TBC in milk. Among all analysed traits significant interaction between consecutive milking and mare was found only in FC (P = 0.038) (Figure 3).

Discussion

Compared to our results, Caroprese et al. (9) reported slightly lower daily milk yield (7.69 kg) from the machine milked light Murgese mares (average live weight of 550 kg). However, it should be considered that Murgese mares were previously not subjected to any milking procedures. According to Caroprese et al. (9) participation of untrained horses and milkmen resulted in a lower milk yield. Consequently, the adaptation to milking routine plays a crucial role in an optimal milk extraction. It is premised that the production of mare's milk is proportional to the mare's BW (2-3.5 kg milk/100 kg of BW) for sustainment of the rapid growth of the foal (17, 18). Similar data on the milk production, confirming the range from 2 kg to 3.5 kg of milk /100 kg of BW, were reported for heavy mares by many authors (4, 19, 20, 21). The milk production in the present study, on the contrary, was slightly lower (1.52 kg/100 kg of BW). Since the Lipizzan horse is a light, early matured breed with more narrow growth curve (BW can increase significantly even after 2 years of age), these lower values were somehow reasonable.

The average FC was lower than reported by Santos and Silvestre (21) for Lusitano mares (5.9 g/kg) and Centoducati et al. (4) for Italian Draft mares (11.07 g/kg). Until now, there were no evidenced data on the milk traits of Lipizzan mares, so the lower FC could be characterised to the breed. In addition, Caroprese et al. (9) investigated milk production in hand and machine-milked Murgese mares and reported significant difference of the FC. They found higher FC (1.63%) in samples from machine milked mares compared to the hand milked ones (1.06%) due to more thorough emptying of udder and milk extraction from alveoli and galactophorous ducts. Regardless the fact that mares in this study were completely adapted to the routine machine milking, another explanation of a low FC and its high CV could be due to the insufficient oxytocin release during the machine milking. The average PC was lower than reported by Oftedal et al. (22) for Thoroughbred and Standardbred mares (20.0 g/kg) or Santos and Silvestre (21) for Lusitano mares (18.4 g/kg), and similar to the one reported by Centoducati et al. (4) for Italian Heavy Draft mares (15.5 g/kg). Due to the higher content of lactose, mare's milk is considered to be much sweeter than the other types of milk used for human consumption. In the present study, the average LC was slightly higher than LC reported by Smolders et al. (23) for light mares (62.0 g/kg) and Santos and Silvestre (21) for Lusitano mares (60.8 g/kg).

Hygienic status of mare's milk was evaluated throughout SCC and TBC. Compared to the other species (24, 25, 26) it could be confirmed that

milk of Lipizzan mares had very low SCC. The mean values for SCC were lower than reported by Centoducati et al. (4) in milk of Italian Heavy Draft mares (14.5 x 10^3/ml), Čagalj et al. (27) in milk of Croatian Cold Blooded mares (22.5 x 10^3/ml), and higher than the ones evidenced by Markiewicz-Kęszycka et al. (8) in Polish Cold Blooded mares (3.5 x 10^3/ml). It is considered that mares have a generally good health status of the mammary gland and good microbial quality of milk due to the low volume of mare's udder, high resistance to pathogens, and high concentrations of lactoferrin, lysozyme and immunoglobulin in milk (8). Evaluation of the hygienic status of milk reported by Markiewicz-Kęszycka et al. (8) in Polish Cold Blooded horse (maximum of 72.0 x10^3/ml) and Čagalj et al. (27) in Croatian Cold Blooded horse (maximum of 58.0 x 10^3/ml) confirmed that mares have a very low content of TBC. Contrary to that, it was found that the average TBC in milk of Lipizzan mares was high. Coefficient of variation for the TBC in mare's milk was higher than for the SCC. It is possible that this result is due to the inadequate hygiene measures taken during milking.

FP of milk is directly related to the concentration of water-soluble substance, from which the main effect on FP arises from lactose and minerals. The mean value for FP was similar to the previously reported mean value for FP (-0.49 °C) in milk of Polish Cold Blooded horse with minimum and maximum values ranging from -0.55 °C to -0.47 °C (8).

MY in mares depends on and increases with the time elapsed between the last suckling of foal and milking (28). It has been pointed out that daily oscillation in the levels of physiological variables has been under influence of many factors (e.g. locomotor activity, body temperature, heart rate, blood pressure, hormonal and urinary secretion) so milk composition would also be expected to exhibit daily variation (29, 30). The present study indicated that PC, LC, TBC, and FP were significantly influenced by consecutive milking. Although the major mare's milk constituents were not investigated during the whole day-night cycle as in the study of Piccione et al. (31) who researched daily rhythmicity of concentration of major constituents of asinine milk, peaks of PC were observed at similar time during the day, that is around 01:30 p.m..–However, contrary to our results, Piccione et al. (31) reported peak in LC during the night.

The highest TBC was found in the samples from the first milking and it significantly differed from the content of samples from the second and the third milking. Contrary to that, Piccione et al. (31) observed no daily rhythmicity of TBC in asinine milk. The lowest FP of mare's milk was found in the second milking. Since the mare's milk lower FP is directly connected to its higher LC (27) it was somehow expected that the higher LC in the second milking would affect the FP.

Although it is known that FC in milk is highly variable, the general trend based on literature data is an inverse function of lactation stage (32). Observed changes in the FC of Lipizzan mare's milk are contradicted to those in literature (17, 33, 34). Namely, it could be expected that with each additional day in lactation, FC in mare's milk would increase by an average of 0.01%. This might be attributed to the machine milking which, compared to hand milking results in milk with higher FC (9). Several authors confirmed that PC of mare's milk is influenced by the stage of lactation as it decreases over the lactating period (17, 21, 33, 34).

The results showed that every additional day in milk SCC and TBC would decrease meaning that hygienic quality of mare's milk during the lactation would slowly increase. Danków et al. (35) reported similar trend in Wielkopolska mare's milk for SCC and TBC measured from the first day of lactation (194 x 10^3/cm^3 for SCC and 46 x 10^3/cm^3 for TBC) up to the 150th day of lactation (41 x 10^3/cm^3 for SCC and 37 x 10^3/cm^3 for TBC).

Among the factors associated with changes in the mare's milk composition, the effect of individual animal has been scarcely investigated. Mare significantly affected MY, FC, and PC in Slovenian draft horse, whereas consecutive milking affected only PC and FC (36). Our study also confirmed individual differences between Lipizzan mares in PC, LC, SSC and TBC. Doreau et al. (37) and Csapó et al. (38) reported low and insignificant inter-animal variation in mare's milk components. However, Doreau et al. (37) indicated that the individual differences among milk composition traits could be related to the udder characteristics or to requirements of the offspring (as in lactating cows), although no evidence was found to support this theory.

Conclusions

This study provided information on milk yield, composition and hygienic quality of milk produced by Lipizzan mares that were included in the routine machine milking process carried on an establishment registered for raw mare's milk production. This was the first time that commercial quantities of milk from Lipizzan horse breed were obtained and analysed. The study confirmed that warmblooded horse breed can also be used for commercial milk production along with coldblooded breeds, and thus enabled future comparison. Fat content showed to be the most variable milk component while overall low somatic cell count indicated high hygienic quality of mare's milk. Analysis revealed that consecutive milking, stage of lactation, individual animal, and interaction between mare and consecutive milking affect the milk traits. Results indicated that routinely machine milked Lipizzan mares are suitable for commercial milk production. (Routine) milking would represent another way of economically justified use and additional reason for preservation of this indigenous horse breed. However, the need for in-depth knowledge on sustainable milk production system and conduction of a milking experiment with larger number of Lipizzan mares to provide an agreement and/or new insights with the findings of this study still exist.

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KOLIČINA, SESTAVA IN HIGIENSKA KAKOVOST MLEKA PRIDOBLJENEGA Z RUTINSKO STROJNO MOLŽO LIPICANSKIH KOBIL

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Povzetek: Pridobili in raziskali smo komercialno količino namolženega mleka kobil lipicanske pasme. Rutinska mehanska molža treh kobil je potekala na raziskovalni ustavnovi, registrirani za prirejo kobiljega mleka. Rezultati so pokazali, da je bila povprečna dnevna količina mleka (KM) 8,24 kg, mleko pa je v povprečju vsebovalo 4,027 g maščobe/kg, 15,064 g beljakovin/kg in 63,218 g laktoze/kg. Povprečno število somatskih celic v mleku je bilo 6,556 x 10^3/ml, povprečno skupno število mikroorganizmov 114,689 x 10^3/ml, povprečna zmrziščna točka (ZT) mleka pa -0,505 °C. Rezultati analize variance so pokazali, da se količina beljakovin in laktoze ter število somatskih celic in skupno število mikroorganizmov statistično značilno razlikujejo glede na zaporedno molžo ter med posameznimi kobilami ter da se količina beljakovin, število somatskih celic in skupno število mikroorganizmov v mleku zmanjšuje z napredovanjem laktacije, medtem ko se vsebnost maščob povečuje. Interakcija med zaporedno molžo in kobilo je statistično značilno vplivala le na vsebnost maščob. Gre za prvo raziskavo mleka kobil lipicanske pasme, kar omogoča nadaljno primerjavo med pasmami. Uporaba kobil te slovenske avtohtone pasme konj za komercialno rutinsko prirejo mleka bi predstavljala njeno dodatno gospodarsko vrednost in s tem razlog za ohranitev.

Ključne besede: sestava mleka; količina mleka; stadij laktacije; zaporedna molža; higienska kakovost mleka

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Original Research Article

DETERMINATION OF THE IN VITRO EFFECT OF LEMONGRASS (*Cymbopogon flexuosus*) OIL AGAINST FISH PATHOGENIC BACTERIA ISOLATED FROM CULTURED OLIVE FLOUNDER (*Paralichthys olivaceus*)

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Abstract: The antibacterial activity of essential oil from lemongrass (*Cymbopogon flexuosus*) (LGO) was tested against strains of *Edwardsiella tarda* (n=4), *Photobacterium damselae* (n=1), *Lactococcus garvieae* (n=1), *Streptococcus iniae* (n=4) and *S. parauberis* (n=4) isolated from cultured olive flounders in Korea. Disk diffusion assay, MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) tests showed the strains of *Streptococcus* spp. were the most sensitive. LGO was bactericidal (MBC/MIC=1-4) for all strains of *L. garvieae*, *S. iniae* and the FP5060 strain of *E. tarda*, and the FP5228, S527 and S1466 strains of *S. parauberis* suggesting sensitivity to LGO. Gram-negative bacteria were less sensitive than Gram-positive bacteria. Since antibacterial activity of LGO was effective against fish pathogenic bacteria tested in this study, the use of LGO could be helpful in treating such bacterial infections in fish.

Key words: lemongrass oil; olive flounders; fish pathogenic bacteria; antimicrobial activity

Introduction

During the last decades, there has been a continuous growth in aquaculture industries in Korea. Infectious diseases cause high mortalities when appearing in intensive fish farming. The rapidly expanding aquaculture industry has suffered from heavy economic losses due to bacterial pathogens, particularly infectious bacterial diseases including streptococcosis, lactococcosis and edwardsiellosis are major problems for olive flounder aquaculture in Korea (1, 2). Antibiotics are widely used to prevent bacterial infections in fish. However, continuous use of antibiotics leads to drug resistance and thereby to a reduced efficacy of the drugs (3). Antibiotics accumulate in the environment and fish and pose a potential risk to humans and to the environment (4). Thus, it is essential to develop antibacterial treatments that are made from natural substances.

For thousands of years, traditional medicines based on plants and plant extracts have been extensively utilized in humans and animals as remedies to control bacterial, viral, and fungal diseases (5). Natural products especially from

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plants have been investigated for their therapeutic and prophylactic effects on several fish diseases (6). Essential oils (EOs) are one kind of plant products which have been used for their aromatic, flavor, bactericidal, preservative and medicinal properties (7). Due to their safety and their recognized antimicrobial activity, there has been a considerable interest in plant essential oils to be used as possible alternatives to control bacterial infection in fish (8, 9).

Lemongrass (*Cymbopogan citrates* and *C. flexuosus*) is a plant belongs to the family Germineae, cultured in almost tropical and subtropical countries as a source of EO. Moreover, it is known to possess pharmacological properties, including antimicrobial and disinfectant functions (10, 11). The main active constituent of essential oil from lemongrass (LGO) is citral (65–95%) (12). The effect of LGO has been studied against various microbes of environmental, clinical and food origin (13, 14)

However, until now no study has been conducted to investigate the antimicrobial property of LGO against fish pathogenic bacteria isolated from olive flounder. Therefore, this study was carried out to examine the potential of LGO as an alternative to commercial antibiotics in aquaculture use.

Materials and methods

As test strains, five Gram-negative and nine Gram-positive bacterial strains isolated from Korean cultured olive flounder (*Paralichthys olivaceus*) were used. The Gram-negative strains were *Edwardsiella tarda* (FP5060, ED47, Yoshida and ED45), *Photobacterium damselae* (FP4101) and the Gram-positive strains were *Lactococcus garvieae* (FP5245), *Streptococcus iniae* (FP5228, S186, S530 and S131) and *S. parauberis* (FP5228, S124, S527 and S1466) obtained from Geyongsang National University (Jinju, Korea) and National Institute of Fisheries Science (Busan, Korea). The 100% pure lemongrass (*C. flexuosus*) oil (Aromarant Co. Ltd., Rottingen, Germany) was purified from the leaves of lemongrass grown in China.

The disc diffusion assay was conducted to detect the antimicrobial activity. Different concentrations of LGO (1:1, 1:2, 1:5 and 1:10; 1 part of the LGO in respective parts of the methanolic solution) was dried on sterile disks (ADVANTEC®, Japan), and each disc was placed on a Mueller Hinton agar (MBcell, Seoul, Korea) plate smeared with the test organism. *Escherichia coli* ATCC 25922 strain was used as the reference strain. Plates were incubated for 24 h at 27 °C to determine the antimicrobial effect. The determination of MIC was done using broth micro dilution method with some modification using different concentrations of oil (5% DMSO was used to dissolve LGO). The MIC was measured after 24 h incubation and each test was repeated three times. In order to determine the MBC, the culture medium from wells which have LGO concentration higher than MIC was smeared on separate Tryptic Soy Agar (TSA) (MBcell, Seoul, Korea) plates and incubated for 24 h at 27 °C (15). The concentration, at which no growth was observed on TSA plate, was determined as the MBC.

Antibiotic susceptibility was determined by disc diffusion method using OXOID™ antibiotic disks (Oxoid Co. Ltd., Seoul, Korea) mention in Table 2. Resistance profiles (resistant, intermediate or susceptible) were assigned using criteria described by Clinical and Laboratory Standards Institute (16). Each test was repeated three times.

Results

The Inhibition Zone diameters (IZDs) (mm) of Gram-negative bacteria ranged from 8 to 32 mm and the IZDs of Gram-positive bacteria ranged from 7 to 53 mm at 1:1 of LGO (Table 1). *MIC* of LGO for bacterial strains *ranged* from 0.016 to 0.5% (V/V) (Table 3). Mean MBC/MIC was1 to 8 (Table 1).

The multiple antibiotic resistant index (MRI %) of the isolates ranged between 0–57.1. *E. tarda* (ED45 and ED47) showed the highest MRI % (57.1), followed by both *L. garvieae* (FP5245) and *S. iniae* (FP3287) (35.7) (Table 2).

Discussion

The results from the disk diffusion, MIC and MBC tests support the general characterization of Gram-positive and Gram-negative bacteria. Gram-negative bacteria are less susceptible to the inhibitory effects of essential oils compared Gram-positive bacteria, because they possess an outer membrane surrounding the cell wall which

Table 1: Susceptibility pattern of lemon grass oil (LGO) against fish pathogenic bacteria

Bacterial strain	Inhibition zone (mm) with different LGO dilutions added on disc				MIC % (V/V)	MBC % (V/V)	MBC/MIC
	1:1	1:2	1:5	1:10			
Photobacterium damselae (FP4101)	12	8	0	0	0.25	2	8
Edwardsiella tarda (FP5060)	8	0	0	0	0.5	4	8
E. tarda (ED47)	27	20	16	18	0.032	0.125	4
E. tarda (Yoshida)	25	19	16	17	0.032	0.125	4
E. tarda (ED45)	32	23	18	15	0.063	0.25	4
Lactococcus garvieae (FP5245)	20	18	10	7	0.25	0.5	2
Streptococcus iniae (FP5228)	22	19	11	8	0.125	0.25	2
S. iniae (S186)	40	35	30	20	0.063	0.25	4
S. iniae (S530)	38	32	30	24	0.125	0.5	4
S. iniae (S131)	50	48	30	24	0.032	0.125	4
Streptococcus parauberis (FP5228)	53	50	28	18	0.016	0.032	2
S. parauberis (S124)	7	0	0	0	0.125	0.5	8
S. parauberis (S527)	38	30	25	18	0.032	0.032	1
S. parauberis (S1466)	45	35	26	19	0.016	0.063	4

Table 2: Susceptibility pattern of lemon grass oil (LGO) against fish pathogenic bacteria

Bacteria	Antibiotics[a]		MRI %
	Sensitive	Resistant	
Photobacterium damselae (FP4101)	AMX, AMP,CTX.CRO,TC,CHL, OFX, NAL, CN,IMI,SXT	VA	7.14
Edwardsiella tarda (FP5060)	AMX,CTX.CRO,TC,CHL, OFX,NAL, IMI,SXT	AMP, CN, VA	21.42
E. tarda (ED47)	AMX, CTX, CRO,IMI	AMP, TC, CHL, VA, NAL SXT, OFX,CN	57.14
E. tarda (Yoshida)	AMX, AMP,CTX.CRO,TC,CHL, OFX, NAL,CN,IMI, SXT	VA,	7.14
E. tarda (ED45)	AMX, CTX, CRO, IMI	AMP,TC,CHL, VA, NAL, SXT, OFX, CN	57.14
Lactococcus garvieae (FP5245)	AMX, TC,DA, E, VA	AMP, CTX, CRO, CHL OFX	35.71
Streptococcus iniae (FP3287)	AMX, TC, CHL, E, VA	AMP, CTX, CRO, DA, OFX	35.71
S. iniae (S186)	AMX, AMP ,CTX, CRO, TC, CHL E, DA, VA,OFX	0	0
S. iniae (S530)	AMX, AMP ,CTX, CRO, TC, CHL E, DA, VA, OFX	0	0
S. iniae (S131)	AMX, AMP ,CTX, CRO, TC, CHL E, DA, VA,OFX	0	0
Streptococcus parauberis (FP5228)	AMX, CTX, CRO, TC E, DA VA OFX	AMP, CHL	14.28
S. parauberis (S124)	AMX, CTX,CRO,VA, CHL, OFX	AMP, TC, E, DA	28.57
S. parauberis (S527)	AMX, CTX, CRO, TC, CHL E, DA, VA, OFX	AMP	7.14
S. parauberis (S1466)	AMX,CTX, CRO, TC, CHL DA, VA, OFX	AMP, E	14.28

[a]Antibiotics- AMP=ampicillin (10 µg), CTX=cefotaxime (30 µg), CRO=ceftriaxone (30 µg), TC=tetracycline (15 µg), CHL=chloramphenicol (30 µg), E=erythromycin (15 µg), DA=clindamycin (10 µg), VA=vancomycin (30 µg), OFX=ofloxacin (5 µg), NAL=nalidixic acid (30 µg), CN=gentamicin (10 µg), IMI=imipenem (10 µg) and SXT= trimethoprim-sulfamethoxazole (25 µg), AMX=amoxicillin (30 µg).

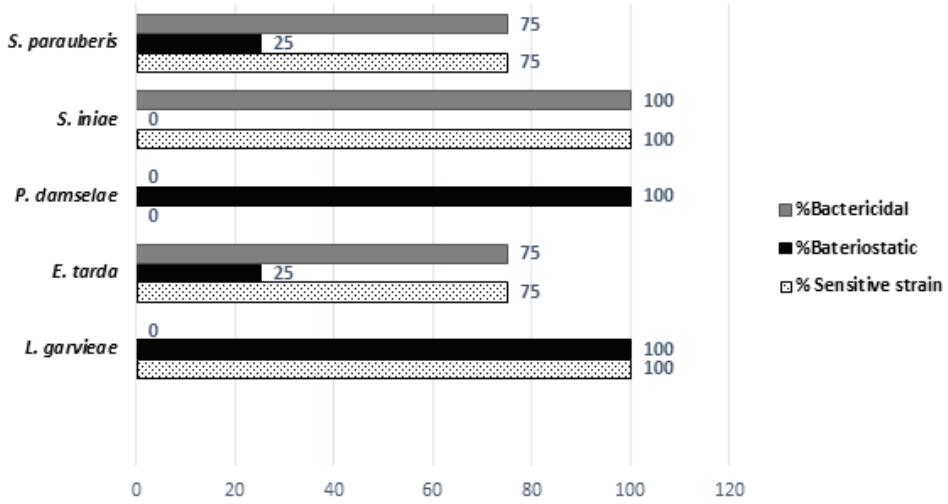

Figure 1: Comparison of the effect of LGO against microbial strains. The graph depicts the bacteriostatic and bactericidal activity of LGO against fish pathogenic bacteria while showing the sensitive strains

restricts the diffusion of hydrophobic compounds through its lipopolysaccharide covering (17). Previous studies have reported similar results of LGO where *Micrococcus* spp. and *Streptococcus* spp. as Gram-positive and *E. tarda*, *E. coli* and *Proteus* spp. as Gram-negative strains were tested (13, 18). In this study, Gram-positive (*S. iniae, S. parauberis, L. garvieae*) were also more sensitive than Gram-negative (*E. tarda, P. damsalae*).

All the strains were sensitive at every concentration of LGO except *P. damsalae* (FP4101), *E. tarda* (FP5060) and *S. parauberis* (S124) in disk diffusion test. The maximum effect of LGO was found at 1:1 concentration and the minimum effect was observed at 1:10 concentration of LGO. In the case of *P. damsalae*, it was sensitive for only 1:1 and 1:2 concentrations. Since the MIC value of *P. damsalae* was comparatively higher than other sensitive strains, it can be considered as less sensitive against LGO. *E. tarda* and *S. parauberis* strains with growth inhibition only at 1:1 concentration of LGO were also less sensitive. Meanwhile, *S. parauberis* (FP5228) was inhibited by the LGO with the largest zones of inhibition (Table 1).

Plant EOs can be classified into two categories, considering the MBC/MIC ratio. The EOs which exhibit MBC/MIC ratio ≤4 are denoted as bactericidal for tested bacteria, while the EOs which demonstrate MBC/MIC ratio >4 are represented as bacteriostatic (19). In the present study, LGO was bactericidal for all the sensitive strains while bacteriostatic for resistant strains. There was no any previous report about the MBC results of LGO against same bacteria tested. However, LGO demonstrated bactericidal activity at every concentration against eleven fish pathogenic bacteria in this study. There were both bactericidal and bacteriostatic strains in same bacterial species. A previous study reported cinnamon, bay, clove EOs were both bacteriostatic and bactericidal against different strains of *L. monocytogenes* (20). The test organisms were inhibited by LGO at comparatively low concentrations in MIC as compared to disk diffusion method (14). The results obtained by each of these methods differ due to many factors between assays including differences in microbial growth, exposure of microorganisms to the oil, the solubility of oil or oil components, etc (14, 21).

The bacteriostatic and bactericidal activity of LGO against fish pathogenic bacteria compared with the most sensitive strains showed 1/1 (100%) of *L. garvieae*, 3/4 (75%) of *S. parauberis*, 4/4 (100%) of *S. iniae* and 3/4 (75%) of *E. tarda* were very sensitive to LGO. On the other hand, 1/1 (100%) of *P. damsalae*, 1/4 (25%) of *E. tarda* and 1/4 (25%) of *S. parauberis* (S124) were resistant (Figure 1). All microbes are not equally susceptible to LGO, although *Streptococcocus* spp. and *E. tarda* were comparatively more susceptible to LGO (22).

All the test strains showed the difference in their sensitivity against different antibiotics (Table 2). The comparative effects of LGO and the standard antibiotic discs on the various test organisms are demonstrable indications of the oil as an antibacterial agent. A similar kind of observation was reported with some selected pathogenic bacteria and had suggested that

the test organisms particularly Gram negative were found to be more susceptible to LGO than standard antibiotics (14).

Nowadays, treatment of fish for various infections poses a serious problem in Korea and many regions of the world (2). The present study established that LGO has potential antibacterial property, and is a good candidate for further research to develop a new antibacterial drug against fish pathogenic bacteria. Moreover, in order to gain more perceptivity into the application of LGO for fish bacterial diseases, the stability and safety of LGO in the aquatic environment should also be further investigated.

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IN VITRO UČINEK OLJA LIMONSKE TRAVE (*Cymbopogon flexuosus*) PROTI RIBJI PATOGENI BAKTERIJI, IZOLIRANI IZ GOJENEGA MORSKEGA LISTA (*Paralichthys olivaceus*)

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Povzetek: Protibakterijsko delovanje eteričnega olja limonske trave (*Cymbopogon flexuosus*; LGO) je bilo preizkušeno glede njihovega delovanja proti sevom bakterij *Edwardsiella tarda* (n = 4), *Photobacterium damselae* (n = 1), *Lactococcus garvieae* (n = 1), *Streptococcus iniae* (n = 4) in *S. parauberis* (n = 4) pridobljenih iz gojenih morskih listov v Koreji. Difuzijska metoda z diskom ter testi MIC (najmanjša zaviralna koncentracija) in MBC (najmanjša baktericidna koncentracija) so pokazali, da so bili sevi *Streptococcus* spp. najbolj občutljivi. LGO je imel baktericidno delovanje (MBC / MIC = 1-4) na vse seve *L. Garvieae* in *S. Iniae* ter na seva FP5060 in FP5228 E. Tarda in seva S527 in S1466 *S. parauberis*. Gram-negativne bakterije so bile manj občutljive od gram-pozitivnih bakterij. Ker je bilo antibakterijsko delovanje LGO učinkovito proti ribjim patogenim bakterijam, preizkušenim v tej študiji, bi lahko uporaba LGO pomagala pri zdravljenju tovrstnih bakterijskih okužb pri ribah.

Ključne besede: olje limonske trave; morski list; patogene bakterije rib; protimikrobno delovanje

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Case Report

CARDIAC MYXOMA IN A DOG

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Abstract: Cardiac tumours occur very rarely in domesticated animals and humans. The most common cardiac tumours in dogs are hemangiosarcoma and chemodectoma. Myxoma, on the other hand, occurs extremely rarely. In this report, we present a case of an 11-year-old, spayed, mixed-breed dog with a myxoma arising from the tricuspid valve. The dog presented with syncope, tachypnea, tachycardia, exercise intolerance and progressive ascites. Two-dimensional echocardiography showed a polypoid intracavitary mass in the right heart. At necropsy, a polypoid, red mass was observed arising from the tricuspid valve. Histologically, the mass was composed of spindle-shaped and stellate cells with small hyperchromatic nuclei surrounded with myxoid matrix. After performing immunohistochemistry, the neoplastic cells were found to be strongly positive for vimentin. Based on gross, microscopic, and immunohistochemical staining features, the neoplasm was diagnosed as a cardiac myxoma.

Key words: myxoma; heart; dog; two-dimensional echocardiography; histopathology; immunohistochemistry

Introduction

Cardiac tumours occur very rarely in dogs and humans, with a reported prevalence of 0.19% in both species (1, 2). The most common cardiac tumours in dogs are hemangiosarcoma and tumours of the aortic arch body (chemodectoma and paraganglioma) (3, 4, 5). In addition, lymphoma (6) and ectopic thyroid carcinoma (7) are also frequently reported. Other cardiac tumours like myxoma (8, 9, 10, 11, 12, 13, 14), mast cell tumour (4), leiomyoma (15) and leiomyosarcoma (16), fibroma (17), fibrosarcoma (18), rhabdomyoma (19), rhabdomyosarcoma (20), melanoma (4), thyroid adenoma (7), myxosarcoma (21), osteosarcoma (22), peripheral nerve sheath tumour (23) and malignant mesenchymoma (24) have been rarely reported.

The World Health Organization (WHO) defines a cardiac myxoma as a neoplasm composed of stellate to plump cytologically bland mesenchymal cells set in a myxoid stroma (25). Although this benign tumour is the most frequent primary cardiac tumour in humans and accounts for 50% of all cardiac tumours (26). It is, however, rarely reported in dogs.

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The purpose of this report is to comprehensively describe a case of a dog with cardiac myxoma affecting the tricuspid valve. History, clinical presentation, haematology and biochemistry, ultrasound, as the results of necropsy, histopathology and immunohistochemistry are presented.

Case presentation

An 11-year old, mixed-breed, spayed female dog weighing 8 kg was presented for evaluation due to a single episode of syncope that happened after exercise 14 days prior to admission. After that episode, the dog became lethargic and exercise intolerant.

Physical, routine haematological and biochemical examinations and two-dimensional echocardiography were performed. On physical examination, tachypnea (44 breaths/min) and tachycardia (164 beats/min) were detected. A systolic diastolic murmur, grade IV/VI, was heard over the tricuspid valve. The dog presented with a moderately distended abdomen. Routine haematological examination showed increased haematocrit (61%, reference range 37-55%), polycythemia (10.10 x 10^{12} /l, reference range 5.50-8.50 x 10^{12} /l) and increased haemoglobin (19.4 g/dl, reference range 12.0-18.0 g/dl). Furthermore, slightly elevated serum alanine aminotransferase activity (ALT) (108 U/l, reference range 10-100 U/L) was detected.

A homogenous isoechogenic intracavitary mass in the right ventricle and atrium was observed with two-dimensional echocardiography (Figure 1a). Abdominal ultrasonography demonstrated liver congestion and free abdominal fluid. Fluid was obtained with aspiration and was characterized as modified transudate.

Based on the results of the physical examination, blood work and ultrasonography findings, intracavitary neoplasm in the right portion of the heart with right heart decompensation was suspected.

The owner declined any further diagnostics and therapy, and the dog was euthanized 14 days later due to progression of ascites and respiratory distress.

Necropsy was done at the Institute of Pathology, Wild Animals, Fish and Bees at Veterinary Faculty University of Ljubljana. At the necropsy, a dark red, polypoid, soft elastic mass measuring 3 x 2.5 x 1.5 cm was found arising from the parietal cusp of the tricuspid valve (Figure 1b). The right atrium and ventricle were severely dilated. The liver, kidneys, spleen and lungs were severely congested, and the lungs was severely oedematous. The abdominal cavity contained one litre of modified transudate.

Samples of the mass were fixed in 10% buffered formalin and routinely embedded in paraffin for histopathological examination. Tissue sections, each 4-µm thick, were first deparaffinised and then stained with haematoxylin and eosin (HE), toluidine blue and periodic acid-Schiff (PAS). Stained sections were then examined under a light microscope. In addition, immunohistochemistry was conducted on the samples to confirm the mesenchymal origin of the neoplastic cells. Immunohistochemical staining was performed on the 4-µm sections of formalin-fixed, paraffin-embedded tissue samples. A mouse monoclonal antibody raised against human vimentin (clone VD9; Dako, Glostrup, Denmark), diluted 1:100, was used for immunolabelling. Antigen retrieval was performed by microwave treatment at medium power (550 W) for 20 minutes in 0.1 M citrate buffer (pH 6.0). The sections were incubated with primary antibodies for one hour at room temperature in a humid chamber. Endogenous peroxidase activity was quenched with Peroxidase-Blocking Solution, Dako REAL™ (DAKO) for 30 minutes at room temperature. Afterwards, the visualization kit DAKO REAL™ EnVision™ Detection System Peroxidase/DAB+, Rabbit/Mouse (DAKO) was applied according to the manufacturer's instructions. Sections were counterstained with Mayer's haematoxylin and mounted. Sections of normal canine skin were used as positive controls. Sections treated without primary antibodies served as negative controls.

Upon microscopic examination, the cardiac mass was composed of mildly anisocytotic spindle-shaped and stellate neoplastic cells with a small amount of cytoplasm and small, hyperchromatic nuclei that displayed only mild anisokaryosis. No mitoses were observed (Figure 2a). The neoplastic cells were embedded in an abundant extracellular matrix that was weakly PAS-positive and mildly metachromatic with toluidine blue (Figure 2b). Furthermore, small multifocal haemorrhages, numerous macrophages containing hemosiderin, and a single small group of neutrophilic granulocytes and plasma cells were

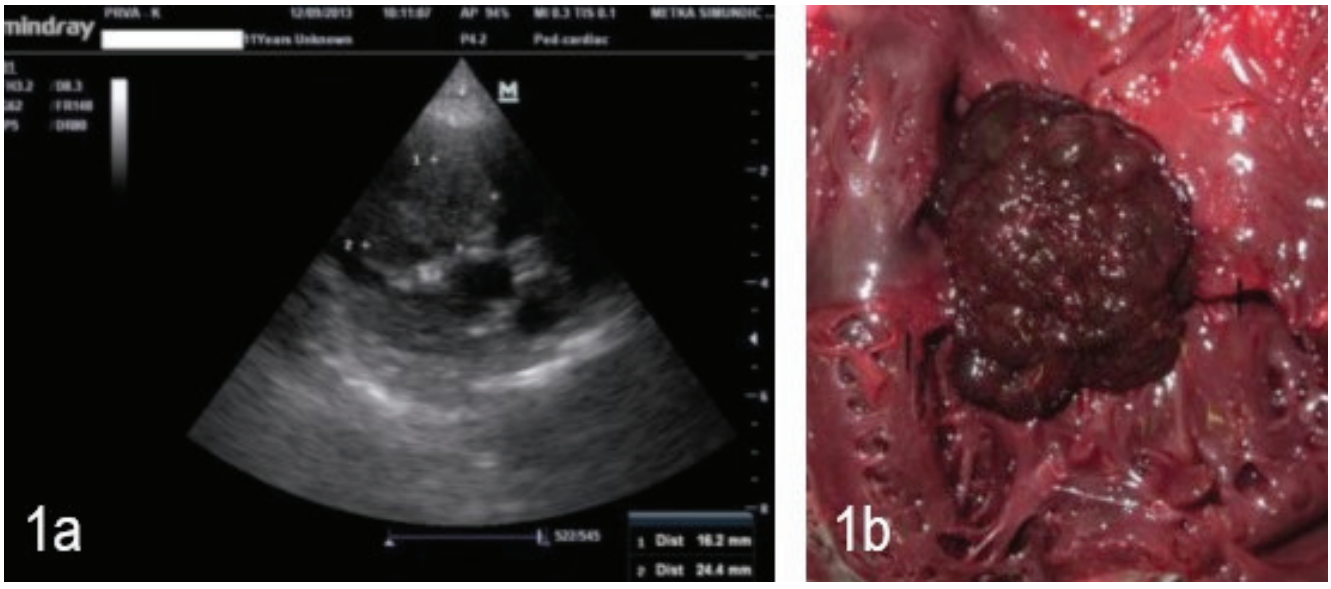

Figure 1: Pictures of cardiac myxoma. (1a) Homogenous isoechogenic intracavitary mass in the right ventricle and atrium was observed with two-dimensional echocardiography. (1b) Polypoid mass measuring 3 x 2.5 x 1.5 cm was found arising from the parietal cusp of the tricuspid valve. The cusps of the tricuspid valve are marked with arrows

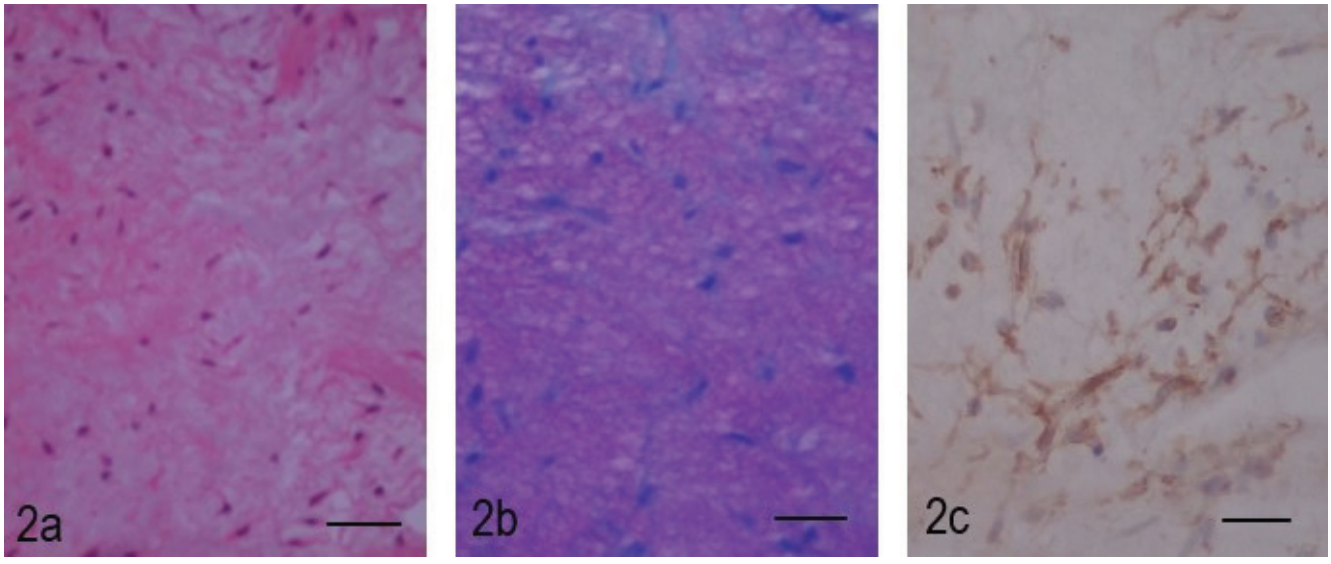

Figure 2: Pictures of cardiac myxoma. (2a) The neoplastic mass was composed of mildly anisocytotic spindle-shaped and stellate neoplastic cells with a small amount of cytoplasm and small, hyperchromatic nuclei that showed only mild anisokaryosis. HE scale bare length 100 µm. (2b) The neoplastic cells are embedded in an abundant extracellular matrix that is metachromatically stained with Toluidine blue. Toluidine blue staining, scale bare length 100 µm. (2c) Immunostaining for vimentin shows strong positively expressed vimentin. Mouse monoclonal anti-vimentin antibody, horseradish peroxidase-labelled polymer (EnVision + Kit), counterstained with Mayer's haematoxylin, scale bare length 100 µm

found in the tumour. Immunohistochemically, the neoplastic cells were strongly positive for *vimentin* (Figure 2c).

Based on gross, microscopic, and immunohistochemical staining features, the neoplasm was diagnosed as cardiac myxoma.

Discussion

Cardiac myxoma is a very rare tumour in dogs, and to the authors knowledge, only seven cases have been reported so far (8, 9, 10, 11, 12, 13, 14). As reported in the literature, myxoma is a

tumour that occurs in relatively older dogs (8 – 13 years). It is significant to note that our patient, which was an older female dog with a tumour in the right heart, is in accordance with the reported data. In humans, the occurrence of myxoma has a clear gender predisposition since approximately 70% of affected cases are females (27). Although reports in the current peer-reviewed veterinary literature show that myxoma is a tumour with no gender predisposition, we found that in all reports, myxoma was found in the right heart of female dogs, whereas in male dogs, the left heart was affected.

Clinical presentation in dogs with cardiac tumours depends on the tumour's anatomic localization, size, effects on the haemodynamic properties, as well as the mobility of the mass. Most common cardiac tumours cause pericardial effusion leading to ascites, exercise intolerance and syncope, but they also can cause arrhythmias, pulmonary congestion and sudden death (14). Reported clinical signs of myxoma in the right part of the heart include congestion of systemic circulation, ascites and arrhythmias. In some reports, tumour emboli in the pulmonary arteries and, consequently, respiratory distress were found (8, 9, 11). In the presented case, syncope was the only presenting problem. During the physical examination, ascites, presumably due to the right heart failure, and loud systolic/diastolic heart murmur were also found. However, the intensity and dynamics of the heart murmur could point toward a high level disruption of the laminary flow, presumably due to a huge intracardiac mass in this case. The presence of a heart murmur in canine cardiac myxoma is not a consistent finding because it is only reported in four of all reported cases. The common finding in these four cases is a high intensity (grade IV-V /VI) murmur of either a systolic or pansystolic duration (8, 10, 11, 13).

Syncope and cardiac murmur are very unspecific clinical signs and are common findings in canine patients with various cardiac and non-cardiac diseases. Therefore, these symptoms can be easily misinterpreted.

In human medicine, transthoracic or transoesophageal ultrasonography (28), CT and/or MRI scans can be used for the characterization of cardiac masses (29, 30) since differential diagnoses include thrombus and valvular diseases such as degeneration or vegetation. Due to the widespread accessibility of echocardiography, the diagnosis of cardiac tumours is becoming more frequent in veterinary medicine (31). In the presented case, transthoracic two-dimensional echocardiography was essential for the confirmation of the diagnosis; it revealed a mass arising from the tricuspid valve and filling the right atrium and ventricle, which is a common location for myxoma in canines. Namely, in two of seven reported cases of canine cardiac myxoma, the tumour originated in the left heart. Specifically, myxoma was found arising from the interventricular septum and obstructing the aorta in one case (14) and affecting posterior papillary muscle and the chordae tendineae of the mitral valve and left ventricle in the other case (13). In one of the other five cases, the tumour originated from the pulmonary valve and impaired the right ventricular outflow (10); the remaining four tumours originated from the tricuspid valve (8, 9, 11, 12). On the other hand, in humans, 60-86% of cardiac myxoma originate from the left atrium, 15-28% from the right atrium, and 8% from the right ventricle; furthermore, 1.6-8.5% of cardiac myxoma are biatrial and 1.6% are multifocal (35).

Canine cardiac myxoma haematological and biochemical results were only presented in four reports. In two of these reports (10, 13), the blood work was unremarkable. Specifically, moderate anaemia with mild neutrophilia was found in one case (9), while significantly elevated activity of ALT with borderline elevation of alkaline phosphatase was reported in the other case (11). Contrary to these reports, polycythaemia was the hallmark of blood work alterations in our case. It can be explained by impaired blood inflow to the right heart and, consequently, decreased blood flow through the lungs. This results in the decreased oxygenation of blood, which leads to a compensatory increased production of erythrocytes. Therefore, this alteration is probably a consequence of chronic hypoxia, since there are no data regarding the possible production of erythropoietin in cardiac myxoma leading to polycythemia, which is otherwise a well-known paraneoplastic syndrome in hepatic, renal and adrenal tumours (32). Similar to results reported by Machida et al (2003), elevated serum ALT activity was found, albeit the elevation in our case was only marginal. This elevation was probably caused by hepatic congestion induced from right-side heart failure (33).

Currently, surgery has been the treatment option for cardiac myxoma in humans (34) and

veterinary medicine in which only two such cases were reported in dogs. One of these cases resulted in a very successful outcome and two-year survival time (35). In the other case, however, the patient died 36 hours after surgical removal of the tumour (11).

Conclusion

Intracardiac tumours are very uncommon occurrences in dogs. However, they can be a rare aetiologic factor for syncope, arrhythmia and ascites, and therefore cardiac myoma should be included in the list of differential diagnosis in a case of listed clinical signs. Echocardiographic examination is essential for the clinical diagnosis of intracardial tumours, but the final diagnosis can only be made with histopathology.

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MIKSOM SRCA PRI PSU

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Povzetek: Tumorji srca so pri živalih in ljudeh redki. Pri psih se v srcu najbolj pogosto pojavljata hemangiosarkom in kemodektom, pojavnost miksomov pa je izjemno nizka. V prispevku predstavljamo primer miksoma triksupidalne zaklopke pri psički mešanki, stari 11 let. Psička je prišla na pregled zaradi enkratne sinkope in ker je bila bolj mirna in utrujena. Ugotovili smo tahikardijo, tahipnejo in progresivni ascites. Z dvodimenzionalno ultrazvočno preiskavo smo v desnem delu srca ugotovili polipoidno intrakavitarno maso. Pri raztelesbi smo ugotovili, da je polipoidna, temno rdeča novotvorba izraščala iz parietalnega lista triksupidalne zaklopke. Mikroskopsko je bila novotvorba zgrajena iz vretenastih in zvezdastih celic, z majhnimi, hiperkromatičnimi jedri, ki jih je obdajal miksoidni matriks. Z imunohistokemičnim barvanjem smo potrdili, da so novotvorbne celice izražale vimentin. Novotvorbo smo na osnovi makroskopskih in mikroskopskih značilnosti diagnosticirali kot miksom srca.

Ključne besede: miksom; srce; pes; dvodimenzionalna ultrazvočna preiskava; histopatologija; imunohistokemija

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