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Za věcnou a jazykovou správnost příspěvků odpovídají jejich autoři.

OBSAH

SEKCE 1: HYGIENA A TECHNOLOGIE POTRAVIN

Whole genome sequencing – a tool for characterization of potentially persistent strains of *Listeria monocytogenes* obtained from two dairy producing plants
Tomáštíková Z., Zobaníková M., Gelbíčová T., Karpíšková R. 5

Testing of efficiency of UV light for *Listeria monocytogenes* decontamination
Brychta T., Vorlová L., Karpíšková R. 8

Diversity of livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA): The Czech Republic situation
Henok A.T., Karpíšková R., Koláčková I. 11

Detection, seroprevalence and antimicrobial resistance of *Yersinia enterocolitica* in fruits and vegetables purchased at supermarkets in the Czech Republic
Verbíková V., Bořilová G., Nesvadbová M., Morávková M. 15

Impact of two modified atmosphere packaging on antioxidant capacity of breast muscle from organic chickens
Abdullah F.A.A., Buchtová H., Hostovský M. 18

The impact of processing technologies and isolation procedures on the quality of animal DNA in food and feed products
Pospíšilová E., Piskatá Z., Bořilová G., Nesvadbová M., Morávková M. 21

QuEChERS or SPE: Which method of sample preparation is better for the determination of fluoroquinolones in honey?
Dluhošová S., Kaniová L., Borkovcová I., Vorlová L. 24

Stanovení oxidu siřičitého v sušených meruňkách
Pospíšil J., Vorlová L. 27

Využití kvantových teček pro značení alergenů hořčice a sóji v modelových vzorcích
Luňáková L., Petrášová M., Pospiech M., Tremlová B., Javůrková Z. 30

Určení druhu škrobu pomocí skenovací elektronové mikroskopie
Běhalová H., Javůrková Z., Rosíková K. 33

SEKCE 2: VÝŽIVA, DIETETIKA HOSPODÁŘSKÝCH ZVÍŘAT A HYGIENA VEGETÁBILIÍ

Selected blood biochemical indicators of the dwarf lop rabbits in relation to a different diet composition
Šimek V., Zapletal D., Kudělková L., Tvrdoň K. 39

Production of nutrients green mass by three varieties of white lupine
Sedláková K., Straková E., Suchý P., Kroupa L. 42

SEKCE 3: VETERINÁRNÍ EKOLOGIE

Odpadní vody rezervoárem *E. coli* rezistentní k beta-laktamovým antibiotikům
Kutilová I., Komárková M., Tomčová E., Dolejská M. 47

SEKCE 5: VEŘEJNÉ VETERINÁŘSTVÍ A OCHRANA ZVÍŘAT

Neinvazivní monitoring stresu u lemurů kata (*Lemur catta*)
Malíková M., Voslářová E., Večerek V., Schwarzenberger F. 53

Vliv přepravní vzdálenosti na úhyny krůt přepravovaných na jatky
Machovcová Z., Voslářová E., Bedáňová I., Malena M., Večerek V. 56

Effects of the travel distance on transport-related mortality in calves
Šímová V., Večerek V., Voslářová E., Bedáňová I. 58

Microchip status and gender of sheltered dogs as factors affecting their length of stay
Žák J., Voslářová E., Večerek V., Bedáňová I. 61

Comparison of successfully adopted dogs and dogs returned to the shelter
Vitulová S., Voslářová E., Večerek V., Bedáňová I. 64

SEKCE 6: VETERINÁRNÍ TOXIKOLOGIE A TOXIKOLOGIE POTRAVIN

The effect of selected tricyclic antidepressants on fish early-life stages
Sehonová P., Plhalová L., Blahová J., Doubková V., Maršálek P., Prokeš M., Tichý F., Skládaná M., Večerková L., Farková D., Svobodová Z. 69

Effect of salt bath on biochemical indices of rainbow trout after treatment of proliferative kidney disease
Enevová V., Blahová J., Modrá H., Palíková M., Otisková T., Svobodová Z. 72

SEKCE 7: CHOROBY VOLNĚ ŽIJÍCÍCH ZVÍŘAT A ZVÍŘAT ZOOLOGICKÝCH ZAHRAD

Antibiotická rezistence aeromonád v kaprovém rybníkářství
Jelínková E., Kohoutová L., Dolejská M., Čížek A., Palíková M. 77

The occurrence of protozoan parasites in different wild and domestic mammals in South Africa
Lukášová R., Halajian A., Bártová E., Kobédová K., Swanepoel L.H., O'Riain J. 80

Analysis of the full G gene sequence of the viral haemorrhagic septicaemia virus isolate from the Czech Republic
Pojezdal Ľ., Pokorová D., Palíková M., Reschová S., Navrátil S., Veselý T. 83

***In vitro* reproductive toxicity of Cd, Hg and Pb to fallow deer (*Dama dama*): a model study of Sertoli cells and ovariocytes**
Abdelsalam E., Kováčová V., Pikula J. 86

Endoparasitic infections in Central European bats
Linhart P., Banďouchová H., Piaček V., Abdelsalam E.E.E., Kováčová V., Pikula J. 89

Posouzení zdravotního stavu dravců a sov v záchranných centrech: biochemická a parazitologická studie
Piaček V., Kováčová V., Heger T., Pikula J. 92

SEKCE 1

Hygiena a technologie potravin

Whole genome sequencing – a tool for characterization of potentially persistent strains of *Listeria monocytogenes* obtained from two dairy producing plants

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Summary
Listeria monocytogenes is the causative agent of human listeriosis transferred mainly via contaminated food. These bacteria have been found to persist for long time period in food processing plant environment with recurrent contamination of food products which may pose significant risk for consumers. Detailed characterization of these strains should be done in order to assess their properties. In our study 87 L. monocytogenes food and food environment isolates were obtained repeatedly during 12 years period from two cheese producing plants. All isolates were characterized by serotyping and macrorestriction analysis followed by pulsed field gel electrophoresis and selected isolates were further investigated using clonogrouping by polymerase chain reaction (PCR) and whole genome sequencing (WGS). Our objective was to compare the properties of strains obtained from two producing plants in order to determine their features and to verify the use of WGS for determination of persistent strains.

Keywords: *serotyping; clonogrouping; macrorestriction analysis*

Introduction

Listeria monocytogenes is the causative agent of human listeriosis transferred mainly via contaminated food. These bacteria have been found to persist for long time periods in food processing plants (Khan *et al.* 2016) with repeated or permanent contamination of food products originated from these facilities. Detailed characterization of these strains should be done in order to assess their properties. In our study we focused on two dairy producing plants in the Czech Republic with positive findings of *L. monocytogenes* from final products, semi-products and food processing environment. The aim of this study was to compare properties of the strains obtained in order to determine their features and to verify the use of WGS for determination of persistent strains.

Material and Methods

In total 87 *L. monocytogenes* isolates originating from producer A and producer B were investigated in this study. All isolates were serotyped by the multiplex PCR (Doumith *et al*. 2004) in combination with the slide agglutination method using commercial antisera (Denka Seiken, Japan) and were further typed by macrorestriction analysis using *AscI* enzyme (New England BioLabs, USA) according to the EU Reference Laboratory protocol (Roussel *et al.,* 2014) followed by the analysis with Bionumerics 5.1 software (AppliedMaths, Belgium). For seven strains from producer A and one strain from producer B PCR clonogrouping (Chenal-Francisque *et. al.,* 2015) and WGS were performed. dsDNA for WGS was extracted using the MagAttract HMW DNA isolation kit (Qiagen, Germany) followed by preparation of the sequencing libraries (Nextera XT chemistry, Illumina Inc., USA), which were sequenced using the MiSeq sequencer (Illumina Inc., USA) in the IABio (Prague, Czech Republic) and AGES (Vienna, Austria). Data obtained were analysed by the RidomSeqsphere+ software version 3.0 (Ridom GmbH, Germany) using the cgMLST (core genome Multi Locus Sequence Typing) based on the analysis of 1701 target genes of *L. monocytogenes* (Ruppitsch *et al.* 2015).

Results and Discussion

Potentially persistent strains of *L. monocytogenes* were isolated from two cheese producing plants during 12 year period both from the food products (ripened cheeses) and from the environment (surfaces of devices, water intended to washing cheeses, etc.). All strains belonged to one serotype and three different pulsotypes (Tab. 1). Two highly similar *L. monocytogenes* pulsotypes (91% similarity) were detected from producer A and one pulsotype from producer B. Comparison of pulsotypes of strains from producer A and producer B show high level of similarity (84.2%) suggesting their possible genetic relatedness. Results of PCR clonogrouping and results of typing by WGS (detection of clonal complex, MLST - multi locus sequence typing and cluster type) for eight randomly selected strains are described in Tab. 2. Strains were not categorized (U) by PCR clonogrouping as this method is designed for the detection of only the most common clonal complexes. Typing by WGS revealed the identical clonal complex and MLST type but different cluster types for strains from producer A and producer B (Tab. 2). Comparison of nucleotide sequences of 1701 genes revealed identical deletion in allele 49 of the *apbE* gene (one base deletion between 1079 to 1087 bp of the allele) coding thiamine biosynthesis protein in all eight sequenced strains form both producers. This deletion is described to be detected in *L. monocytogenes* originating from food processing environment and mutation in *apbE* gene has been associated with the adaptation of strains to specific stress conditions (Hingston *et al.*, 2017).

Table 1: Total number of investigated isolates and results of serotyping and macrorestriction analysis for strains obtained from producer A and producer B.

Producer	A	B
manufactured dairy product	blue veined cheese	smear-ripened cheese
No. of isolates obtained from the environment	73	3
No. of isolates obtained from food products	4	7
Monitoring period	12 years (2004 – 2016)	11 years (2004 – 2015)
Serotype	1/2a	1/2a
Pulsotype	719, 719B	713

Table 2: Results of PCR clonogrouping and whole genome sequencing of strains obtained from producer A and producer B.

Producer	Ev. No. of strain	Year of isolation	Source of isolation (producer)	Serotype	Clonal complex (PCR)	Pulsotype (PFGE)	Clonal complex (WGS)	MLST type (WGS)	Cluster type (WGS)
A	L 3075	2011	E	1/2a	U	719	CC204	ST204	4024
	LV 208	2014	E	1/2a	U	719	CC204	ST204	4024
	LV 294	2014	E	1/2a	U	719	CC204	ST204	4024
	LV484	2015	E	1/2a	U	719	CC204	ST204	4024
	LV 515	2015	E	1/2a	U	719B	CC204	ST204	4024
	LV 544	2015	F	1/2a	U	719	CC204	ST204	4024
	LV 562	2015	E	1/2a	U	719B	CC204	ST204	4024
B	LV 599	2015	F	1/2a	U	713	CC204	ST204	4015

E - plant environment, F - food product (cheese), U - uncategorized

Figure 1: Macrorestriction analysis based dendrogram displaying three *L. monocytogenes* pulsotypes detected at two producers. *S*. Braenderup H9812 was used as a molecular weight marker, A and B – designation of producers, 719, 719B and 713 – designation of pulsotypes.

Conclusion

Whole genome sequencing represents a valuable tool for determination of potentially persistent strains of *L. monocytogenes.* The method enables various characteristics of *L. monocytogenes* using only single approach. Comprehensive analysis of genomes of specific strains and their mutual gene by gene comparison allows searching for probably significant mutations.

Acknowledgement

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Testing of efficiency of UV light for *Listeria monocytogenes* decontamination

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Summary

This study has been aimed at two experiments. First experiment was the observation of the bactericidal effect of UV light (254 nm) on bacterial populations of Listeria monocytogenes (mixture of 3 most common serotypes) with exposition times from 1 – 20 minutes. Test was performed on Petri disheswith ALOA agar with concentration of bacterial contamination of 100 CFU/cm^2. It was shown that even 1 minute exposition to UV light is enough to tested bacteria on smooth surface of Petri dish.

Second experiment was aimed at possible reduction of bactericidal effect of UV light (254 nm) on bacterial populations of the same mixture of 3 most common serotypes of Listeria monocytogenes by shadow created by crumbs, fluff and pin placed directly onto ALOA agar and by paperclip and polyetylen bag put above Petri dish. We discovered that crumbs and pin placed directly onto the agar created shadow big enough to protect the bacteria from inactivation. No reduction of inactivation was observed in case of paperclip, polyetylen bag and fluff.

Keywords: *UV light; UV light effect reduction; Listeria monocytogenes decontamination*

Introduction

There are many ways for destroying bacteria in the environment, from working surfaces, production machines, packaging and other materials. UV irradiation can be one way. UV light is part of electromagnetic radiation spectra between alpha rays and visible light. UV light composes of 3 different types of light with wavelenght between 40 and 400 nm. These are UVA (wavelenght 315 – 400 nm), UVB (wavelenght 280 – 315 nm) and UVC (wavelenght 100 – 280 nm) (WHO, 2010a).

UVC part of spectra is almost not present in natural environment due to complete absorption in atmostphere. It is the part with bactericidal effect on bacteria due to ability to penetrate the cell and cause DNA damage (Anonymous, 2009).

One advantage is that UV light doesnt cause any changes in sensory properties of foodstuff or in its composition (Stermer et al., 1987, Wallner-Pendleton et al., 1994).

For right use of UV light there are some limitations that have to be kept in mind for succesful sterilisation. In our experiment we tested time neded to deactivate 3 strains mixture of *Listeria monocytogenes* and different situations that can reduce the effect of UV light.

Materials and Methods

All used strains of *Listeria monocytogenes* were acquired from meat industry swabbing. As surface for comtamination were used Petri dishes with ALOA agar. We used bacterial suspension with *Listeria monocytogenes* level of approx. 100 cells per cm^2 of Petri dish. Source of UV light was germicidal lamp NBV 2x30PLW (ULTRAVIOL sp.j., Polsko).

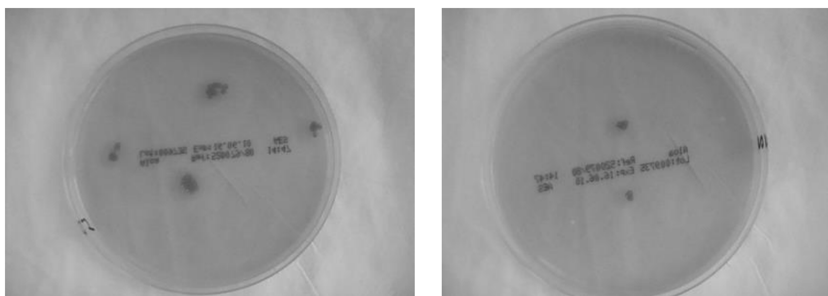
2 different experiments were performed. In the first one we tested how will shadow created by different materials limit the UV light sterisation effect. Shadow was created by crumbs, fluff and pin placed directly onto ALOA agar and by paperclip and polyetylen bag (plastic bag) put above Petri dish. Lamp was placed aprox. 1 meter above dishes with 45° and 90° angle.

In second experiment we tested time needed to sterilise surface of Petri dish with innoculum mentioned above. Time of exposure was 1 – 20 minutes with lamp placed 1 meter above

dishes and angle of 90°. After exposure all shadow-materials were sterile removed and incubated according to ČSN EN ISO 11290-2.

Picture 1: Materials creating shadow place on Petri dish.

Results
From all tested materials no effect on UV irradiation eficacy was detected in case of plastic bag, fluff and paperclip. On the other side pin and crumbs lowered effect of sterilisation.



Picture 2: Growth of *Listeria monocytogenes* under pin and crumbs.

In second part of experiment we found that 1 minute long exposure with lamp placed 1 meter above dishes is enough to kill all bacteria in the suspension place on surface of ALOA agar.

Discussion
We proved that *Listeria monocytogenes* is susceptible for UV light even after short exposure with no difference shown at 45° or 90° angle. No reduction of UV light germicidal effect was observed when shadow was created by plastic bag and paperclip placed above Petri dish and fluff put directly onto agar. Reduction of UV light germicidal effect was observed when shadow was created by pin and crumbs placed onto agar surface (Picture 2).

Our results show some capabilities of UV light: Materials that UV light can penetrate **do not lower** its bactericidal effect. Materials that UV light cannot penetrate **lower** its bactericidal effect. Moreover, materials that UV light cannot penetrate **do not lower** its bactericidal effect if they are thin/small enough and far enough from the target surface so the light can bend and reach the target surface as there is no obstacle as was shown in case of paperclip.

As our results show direct UVC light has bactericidal effect already after 1 minute exposition time. That was proven in similar experiment conducted with different bacteria (*Deinococcus radiodurans, Pseudomonas fluorescens, Serratia marcescens, Staphylococcus epidermidis*) but with same conditions (Menetrez et al, 2010) that showed 4 log reduction of tested strains.

Comparison study was performed aiming at susceptibility of different bacteria to UV light that showed that *Listeria monocytogenes* is most resistant to UV light bactericidal effect from all tested bacteria (Rowen et al., 1999).

UV light is method usable for surface desinfection when there are no shadow effect that can reduce its efficacy. It is suitable for surface treatment of fresh meat (Stermer et al., 1987, Wong et al., 1998), packaged meat (Djenane et al., 2001), fresh fish (Huang et Toledo, 1982) and eggs (Kuo et al., 1997). In these experiments was also shown that on surfaces with a structure that creates shadows bacteria absorb lower doses of UV light than in case of smooth surfaces. Real efficacy will be lower than was shown in our experiment.

Conclusions

UV light is method of desinfection that doesnt change colour, taste and odour of foodstuff. We proved that UVC germicidal lamp is effective for desinfection after short exposition time. But its important to realize that its effect can be lowered or entirely blocked by different materials and dirt that are present directly on the treated area. Proper mechanical cleaning is vital to achieve full desinfecting effect.

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Diversity of Livestock Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA): The Czech Republic Situation

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Summary
LA-MRSA are mostly linked with the clonal complex (CC)398; sequence types (ST) 398, harboring S. aureus protein A gene (spa) types most frequently, t011, t034, t108, t567, t899, t1254, t1451, t2011, 2510. Nevertheless, other clonal lineages such as ST9 (t1430), CC30/ST39 (t007), ST97 (t3992, t5487), CC5 (t002) and CC88 are detected from pigs and other livestock animals across the globe. To evaluate the clonal diversity of LA-MRSA in the Czech Republic, samples from livestock animals, food of animal origin and the environment have been collected. Multiplex PCR, spa typing, and MLST have been used to characterize the strains. In this study 12 different spa types belonging to five sequence types (ST) were detected. Ninety four percent of tested strains are belonging to CC/ST398 for which t011, t034, t2123 and t2346 are the major spa types. In addition, non-ST398 strains such as CC1(t127), ST5(t3598), ST8(t064) and ST361(t315) were detected, which are known as human associated clones. The diversity of LA-MRSA has grown and detecting lineages of human origin in animals and vice-versa become more common. Thus, livestock animal and its products will be a potential for the evolvement of MRSA in human population. Monitoring of pigs as well as other food-producing animal species and their products is therefore recommended.

Keywords: *CC398; MLST; PCR; spa typing*

Introduction

In the last few decades, many bacterial species have developed resistance to antimicrobial agents. It has been now escalated by WHO to one of the top health challenges facing the 21st century. *Staphylococcus aureus* is a bacterium that colonizes a variety of animal species and known to rapidly develop resistance to antimicrobial agents (Rasmussen et al., 2012). Nowadays, colonization of livestock animal by multidrug resistant *Staphylococcus aureus* (LA-MRSA) has drawn attention not only because of its importance in veterinary medicine but also the evidenced zoonotic potential and increment of its infectious processes in human (Köck et al., 2013).

LA-MRSA strains are mostly linked with clonal complex (CC) 398 notable sequence types (ST) 398, ST752 or ST753, harboring the staphylococcal cassette chromosome *mec* (SCC*mec*) and *S. aureus* protein A gene (spa) types t011, t034, t108, t567, t571, t899, t1254, t1451, t2011, t2510 and close relatives (Köck et al., 2013). In most European countries, CC398 is the most frequently identified type from livestock. However, the epidemiology has been found to differ in different geographic areas across the globe (Butaye et al., 2016). Moreover, European baseline report mentioned also other clonal lineages of LA-MRSA such as CC9/ST9 (t1430), CC30/ST39 (t007) and CC97/ST97 (t3992, t5487) from pig production and CC9 (t1430) and CC5 (t002) from veal calf (EFSA, 2009). Several studies from Asia have demonstrated that CC9/ST9 (t1430), seems to be the prominent. In the United States, the diversity of LA-MRSA is higher than that identified in Europe or Asia, with the reports of both CC398 as well as a variety of "human" associated strains (Butaye et al., 2016). Isolates belonging to MRSA ST5 and ST88 SCC*mec* IV, and not ST398, so far have been found in African livestock (Lozano et al., 2016). This study has been conducted to assess the diversity and molecular characteristics of LA-MRSA in livestock animals, food of animal origin and the environment in Czech Republic.

Materials and Methods

Isolates: Isolates used in this study originated from samples collected in the period 2012-2017 from livestock animals, food of animal origin and environment sample and were taken on farm, slaughterhouse and retail levels. Strains were stored in deep freezer at -80 °C.

Isolation and identification: Strains were obtained in accordance with the Commission Decision 2008/55/EC. Suspected *S. aureus* colonies were confirmed using the polymerase chain reaction method detecting species specific fragment SA442 (Martineau et al., 1998), and *mec*A gene (Oliveira and de Lencastre, 2002).

***Spa*-typing and MLST:** PCR method was used for the detection of the sequence type ST398 (van Wamel, 2010). Spa typing was performed according the methodology published on the website SeqNet.org maintained by the European Network of Laboratories. Primers according to Stegger et al. (2012) were used for spa typing and 7 housekeeping genes for MLST were used according to Enright et al. (2000). Sequencing was performed in a sequencing facility of Eurofins MWG Operon (Ebersberg, Germany).

Results and Discussion

In total, 757 strains were analyzed that collected from livestock animals (221), food of animal origin (414) and environmental samples (122). A majority (38%) of strains were from pigs' others 18, 9.6, 7 and 9.5 percent were obtained from cattle, goat, sheep and poultry respectively. From a total of 159 MRSA positive strains 12 different *spa*-types were identified. Detected *spa*-types with their sequence types are summarized in table one. Most of the strains were spa-types associated with ST/CC398 (94%) such as t011, t034, t2123, t2346, t4652, t899, t1255, and t4659. The *spa*-types t011, t034, t1255, t899, and t3495 are widely distributed in most of Europe countries. However, spa-type t2346 principally prevalent in central Europe such as Germany, Czech Republic and Austria. *Spa*-type t4652 and t2123 which are reported in this study are rarely identified and only reported from Germany and Netherlands (EFSA, 2009; Köck et al., 2013). However, according to Ridom *spa* server, *spa*-type t4659 have been reported only from the Czech Republic (http://spa.ridom.de/spatypes.shtml).

Out of 105 *spa*-typed strains 9 (8.5%) isolates were not belonging to the *spa*-types related to CC398. These were attributed to four *spa*-types that clustered in four different STs such as ST361, CC/ST1 (t127), CC/ST8 (t064), and CC/ST5 (t3598). *Spa*-types belonging to lineages ST361, CC/ST1(t127), and CC/ST8(t064) are commonly identified in Europe and are known as human strains (Köck et al., 2013). A study from Sweden reported CC/ST5 (t3598) as MSSA human strain but in our study, we identify it from livestock product and it harbored *mec*A gene which allows a bacterium to be resistant to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics. Detecting human strains from food of animal origin may be a sign of cross-contamination during processing. However, colonization of livestock animal with human strains and jumping of animal strain to human host are increasing time to time. In addition to this, host specific strains are widening their host range (Köck et al., 2013; Lozano et al., 2016). Thus, colonization of pigs and other livestock animals with MRSA no matter which strain belongs to, will be potential for the evolvement of strains (Butaye, Argudín, & Smith, 2016). Therefore, it is paramount important assessing the existing situation, close monitoring of animal husbandry and food of animal origin processing line to safeguard the public and minimize the risk of development of wide host range antibiotic resistant strains.

Table 1: Number of strains with MLST and spa types from different sample origins.

Sample Origin	Strains identified (n=757)	*mec*A positive strains (n=159)	MLST (no. of isolates) (n=156)	*Spa*-types (no. of isolates) (n=105)
Livestock Animal				
Pig	109	59	ST398 (59)	t011(24), t034(21), t2123(7), t2346(2), t4659(1)
Cattle	34	18	ST398 (11) ST361(6) ST1(1)	t011(3), t034(1), t2346(1), t1255(1) t315(1) t127(1)
Goat	38	12	ST398 (12)	t011(2)
Sheep	25	1	ST398(1)	t011(1)
Poultry	10	-	-	-
Other food animals	5	-	-	-
Food of Animal Origin				
Pork, hum, bacon	179	19	ST398(18)	t011(6), t034(8), t2346(1), t4652(2), t899(1)
Cattle meat & milk	102	25	ST398(24) ST5(1)	t011(5), t034(1) t3598(1)
Goat milk	35	9	ST398(6) ST8(1)	t011(2) t064(1)
Sheep milk	29	1	ST398(1)	t011(1)
Poultry meat & liver	62	1	ST398(1)	t011(1)
Other meat	7	-	-	-
Environment				
Milking equipment, feeder, handrail, cart, fence etc.	122	14	ST398(14)	t011(5), t1773(1), t2123(3)

Conclusion

Though alike other European countries, clear majority of strains detected in this study are belongs to CC/ST 398 with the most common *spa* type of t011, clonal diversity of LA-MRSA in livestock animals are growing. Lineages other than ST398 which are commonly known as human strains are also detected in this study. In addition to the impact of LA-MRSA in livestock production, our finding illustrates that livestock animals may have a role for perpetuation and widespread distribution of human strains in the community. So, its paramount important taking breakthrough measure before LA-MRSA become a major health system challenge of the country.

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Detection, seroprevalence and antimicrobial resistance of *Yersinia enterocolitica* in fruits and vegetables purchased at supermarkets in the Czech Republic

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Summary
The aim of this study was the detection of Yersinia enterocolitica in fruits and vegetables purchased in the supermarkets in the Czech Republic during a period of one year 2014. A total of 312 samples including cut green leaves (n=94), frozen vegetable (n=57), untreated vegetable (n=54), fresh and frozen fruit (n=72) and sprouts (n=35) samples were analysed. The samples were examined using cultivation methods according to ČSN EN ISO 10273 and identification was proved by sequencing analysis of RpoD fragments (RNA polymerase sigma factor RpoD). Isolates which were confirmed as Y. enterocolitica were subjected to further characterization (biotyping, serotyping and antimicrobial susceptibility). Biotyping revealed only the biotype 1A in all isolates. Serotyping test determined that two isolates belong to serotype O:08. The antimicrobial susceptibility of the Y. enterocolitica isolates was tested using the agar dilution method. According to agar dilution method all tested isolates were susceptible to ciprofloxacin, gentamicin and enrofloxacin. On the other hand, all isolates were resistant to erythromycin, ampicillin and streptomycin. Although the main source of pathogenic Y. enterocolitica is considered to be pork and pork products, the results of the study show that unprocessed or minimally processed fruits and vegetables can be another source of Y. enterocolitica and pose a health risk to consumers.

Keywords: *vegetable; fruit; Yersinia enterocolitica; zoonosis; antimicrobial resistance*

Introduction

Yersinia enterocolitica (*Y. enterocolitica*) is one of the major food-borne enteropathogen, and is responsible for a wide variety of clinical manifestations ranging from mild gastroenteritis to invasive syndromes like terminal ileitis and mesenteric lymphadenitis (Estrada, 2012). *Y. enterocolitica* is a heterogeneous species, which can be divided into six biotypes (1A, 1B, 2-5) and nearly 50 serotypes (Quinghua et al., 2015). The most common bioserotypes associated with human yersiniosis are O:3/4, O:5,27/2, O:9/2, O:8/1B (Bottone, 1999). Pigs are suggested as the most important animal reservoir of *Y. enterocolitica* and consumption of insufficiently cooked pork and organs is the main source of infection (Bottone, 1999). Nevertheless, contaminated water, dairy products, seafood and fresh vegetables may serve as another possible sources of infection (Lee et al., 2004). The increasing trend of antibiotic resistance in microorganisms is one of the most notable challenge in human´s and animal´s therapy that leads to the fact that antibiotic treatment is less effective and increase mortality. Hence, testing of antibiotic susceptibility in bacterial agents occurring in food product is important. In this work we focused on detection of *Y. enterocolitica* from food of non-animal origin and test the antimicrobial susceptibility of these isolates.

Materials and Methods

A total of 312 samples of fruits and vegetable were collected during a period of one year 2014 from different supermarkets in the Czech Republic. The samples were examined using cultivation methods according to ČSN EN ISO 10273. Isolates which were urea-positive, oxidase-negative and catalase-negative (suspected *Yersinia* spp.) were identified using sequencing analysis of *RpoD* fragments (RNA polymerase sigma factor *RpoD*). Isolates confirmed as *Y. enterocolitica* were biotyped according the biotyping scheme (Wauters et al.,

1988) and serotyped using commercial antisera (O:3 O:5, O:8, O:9, O:25; SIFIN). The susceptibility of the *Y. enterocolitica* isolates to selected 10 antibiotics was assessed by MIC (minimal inhibition concentration) using the agar dilution method according to guidelines (EUCAST 2015; EUCAST 2016; BASC 2014). The following panel of 10 antimicrobial agents included: ampicillin, tetracycline, nalidixic acid, chloramphenicol, erythromycin, ciprofloxacin, gentamicin, enrofloxacin, streptomycin and kanamycin (all Sigma-Aldrich, USA).

Results

Out of 312 samples of fruits and vegetables, *Yersinia* sp. was isolated from 53 (17.0%) samples. Eighteen (34.0%) from 53 isolates were confirmed by sequencing analysis as *Y. enterocolitica.* Biotyping of *Y. enterocolitica* isolates revealed only biotype 1A. Testing of selected serotypes (O:03, O:09, O:05, O:08 and O:25) confirmed only serotype O:8 in two isolates. According to agar dilution method all tested isolates of *Y. enterocolitica* were susceptible to ciprofloxacin, gentamicin and enrofloxacin. The highest resistance rates were observed for erythromycin (100%), ampicillin (100%) and streptomycin (38.9%). Out of 10 tested antibiotics, multi-drug resistance was found in 12 isolates of *Y. enterocolitica.*

Discussion

Although, *Y. enterocolitica* is considered as the one of the most important zoonotic agents, there is a limited information concerning occurrence of this bacteria in fruits and vegetables. In this study, *Y. enterocolitica* was found in 5.8% (18/312) of sample. All isolates were confirmed as biotype 1A that is generally regarded as non-pathogenic (Fábrega and Vila, 2012). However, strains of biotype 1A represent a large fraction of strains from patients with yersiniosis and may be considered as opportunistic pathogens in some cases. This biotype was reported as predominant biotype in food also in other studies from Poland and China (Zadernovska et al., 2017; Quinghua et al., 2015).

In recent years, the authority of EFSA stress the importance of monitoring of antibiotic resistance in bacteria transmitted through the food. Several studies have reported that *Y. enterocolitica* produces β-lactamases A and B, which confer resistance to ampicillin and first-generation cephalosporins (Fábrega and Vila 2012; Laukkanen and Fredriksson et al., 2012; Quinghua et al., 2015). *Y. enterocolitica* is usually susceptible to aminoglycosides, chloramphenicol, tetracycline, the third generation cephalosporins and fluoroquinolones. (Crowe et al., 1996). In this study, antibiotic susceptibility to ciprofloxacin, enrofloxacin and gentamicin was detected in all isolates. High-level antibiotic resistance (100%) was confirmed to ampicillin and erythromycin. Similar data were also reported by Lee et al., 2004 and Simonova et al., 2007.

Conclusions and Recommendations

Although the main source of pathogenic *Y. enterocolitica* are pork and pork products, the results of our study showed that fruits and vegetables could be another source of *Y. enterocolitica* that may pose a health risk to consumers. The most common antibiotic resistance was observed to erythromycin, ampicillin and streptomycin. Hence, good hygienic practice (handling, heat treatment) before consumption of minimally processed vegetable and fruit is highly recommended.

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Impact of two modified atmosphere packaging on antioxidant capacity of breast muscle from organic chickens

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Summary

The aim of study was to detect the antioxidant capacity of organic chicken breast storage under modified atmosphere packaging. 24 samples were packed in MAP-O_2: 80% O_2 +20% CO_2, 24 samples in MAP-N_2: 30% CO_2 + 70% N_2. 48 samples in AIR as control (for each MAP types were 24 samples) and storage at (2 ± 2 °C). Antioxidants capacity were evaluated by using free radical scavenging ability 2,2-diphenyl-1-picrylhydrazyl (DPPH assay) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)(ABTS assay). Samples were analyzed at 2nd, 10th and 14th day of storage period. Higher antioxidants capacities were detected via ABTS comparing to DPPH assay in breast chicken storage under MAPs and AIR. Antioxidant capacity tends to increase from 2nd to 10th day of storage.

Keywords: *organic broiler; DPPH; ABTS; MAP; meat*

Introduction

Recently several studies dealt with impact of antioxidant against oxidation processes in poultry meat by using both dietary (feed-based) and technological (formulation/packaging) strategies (Estevez, 2015). Dietary antioxidant strategy (i.e., provide antioxidants in animal feed) had effective role on lipids stabilization in membranes and decrease lipid oxidation in meat during storage period, but this effective role can differ between type of muscles (Ahn et al., 2006). Grass consumption by organic chickens in free range area, improved the amount of alpha-tocopherol and carotenoids in their meat and thus increased antioxidant capacity (Castellini and Mourvaki, 2007). The aim of our study is to evaluate the impact of two MAPs (MAP-O_2: 80% O_2 +20% CO_2, MAP-N_2: 30% CO_2 + 70% N_2) on the antioxidant capacity in the chicken breast muscle from organic production system by using two detection methods including DPPH and ABTS.

Material and Methods

Total of 96 breast of fresh chicken were obtained from organic production farm one day post-slaughter. Of the total of 48 samples were packed in mixture of gases (24 samples with MAP-O_2: 80% O_2 +20% CO_2, 24 samples with MAP-N_2: 30% CO_2 + 70% N_2. 48 samples as control (for each MAP types were 24 samples) were packaged using polyolefin film stretched over the tray (AIR). All the samples were stored in a cooling chamber with regulated temperature (+2 ± 2 °C) for 14 days. The experiment was conducted on 2nd day and repeated on 10th and 14th days of storage. At each sampling day, six samples from MAPs obverse twelve control samples were taken for antioxidant capacity analysis. Samples were taken from the same local of breast muscle (*m. pectoralis major*). Preparation of meat extract was performed depending on Jung et al. (2010) with some modifications. 5 ml of 5% Trichloroacetic acid added to 1 gram of meat sample and after homogenization (1130×g/1 minute) in ice bath, 3.33 ml of chloroform were added then centrifuged at 3500×g for 5 min. Fresh solution of radical stock solution was prepared daily. ABTS radical (ABTS•+) was produced by reacting ABTS (7 mM) stock solution with 2.45 mM potassium persulfate (final concentration) at a ratio of 1:0.5 and the mixture was stabilized in the dark at room temperature for 12–16 h before use. ABTS•+ solution was diluted with sodium acetate buffer (pH 5.2±0.1) to an absorbance of 0.70 (±0.02) at 734 nm. Solution of DPPH ($0.025 g.l^{-1}$) was prepared by dissolving 0.0025 g of DPPH in 100 ml of methanol. The original methods were

adapted for microtiter plates. To 0.015 ml of sample or Trolox standards (final concentration 0–0.2 mM) in methanol a 0.285 ml of diluted ABTS•+ or DPPH solution was added and the absorbance was read at 734 nm (ABTS•+) or at 515 nm (ABTS•+). TEAC values are expressed as micromol (µmol) Trolox equivalents per gram of the sample (Miller et al., 1993; Re et al., 1999; Heilerova et al., 2003). Statistical data (mean ± s.d.) were conducted by using Microsoft offices Excel 2003. Statistical significance ($P < 0.05$) was estimated by t-test and ANOVA analysis of variance, with post hoc Tukey test for finding differences between independent variances using SPSS 20 statistical software (IBM Corporation, Armonk, USA). Values in the same row with different letters a, b are significantly among 2nd, 10th, 14th day of storage, values in the same column with different letters A,B are significantly different among type of storage, $^{*}P < 0.05$.

Results

Generally, the antioxidant capacities which are detected by ABTS assay in chicken breasts storage under three types of atmosphere was higher [1.05-1.32 TEAC (µmol/g ww)] that detected by DPPH assay [0.59-1.19 TEAC (µmol/g ww)] during storage period (Tables 1 and 2). The antioxidant capacity in all samples had elevation tendency from 2nd day to 10th of storage and particularly (significantly) in the samples under MAP-N2 and AIR which are detected by DPPH assay.

Table 1. Antioxidant capacity of breast chicken storage under MAP-O_2, MAP-N_2 and AIR detected by DPPH method.

Type of storage	2nd day	10th day	14th day	Stat. sign.
AIR	0.84 ± 0.45^{aA}	1.13 ± 0.16^{bB}	1.07 ± 0.23^{bA}	*
MAP-O_2	1.16 ± 0.40^{aB}	1.19 ± 0.18^{aB}	1.06 ± 0.24^{aA}	NS
MAP-N_2	0.59 ± 0.39^{aA}	0.97 ± 0.17^{bA}	1.05 ± 0.30^{bA}	*
Statistical significant	*	*	NS	-

Table 2. Antioxidant capacity of breast chicken storage under MAP-O_2, MAP-N_2 and AIR detected by DPPH method.

Type of storage	2nd day	10th day	14th day	Stat. sign.
AIR	1.20 ± 0.42^{aA}	1.32 ± 0.35^{aA}	1.15 ± 0.38^{aA}	NS
MAP-O_2	1.05 ± 0.30^{aA}	1.28 ± 0.30^{aA}	1.12 ± 0.50^{aA}	NS
MAP-N_2	1.09 ± 0.49^{aA}	1.31 ± 0.27^{aA}	1.24 ± 0.16^{aA}	NS
Statistical significant	NS	NS	NS	-

Discussion

The ABTS assay assesses lipophilic and hydrophilic antioxidants, whereas DPPH estimate only lipophillic compounds (Kim and Lee, 2009). Combination between DPPH and ABTS antioxidants are distinct because DPPH· is a free radical, while ABTS·+ is a cation radical (Lee et al., 2005). DPPH assay is more suited to lipophilic compounds or high lipid content substances due to its performed in an organic solvent system, whereas ABTS assay is suitable with both aqueous and organic solvent systems (Arnao et al., 2001). The higher ability of ABTS assay to detect antioxidant capacity in comparison to DPPH in different foods is attributed to the better reflection of highly pigmented and hydrophilic antioxidants via ABTS assay than DPPH assay (Kim et al., 2002). Antioxidant capacity tends to increase from 2nd to 10th day of storage. It could be due to exposure of "hidden" antioxidants amino acids resulting from protein denaturation. Destruction of cell membrane can be lead to release some antioxidant compounds, thus increased their reactivity against radical probes (Serpen et al., 2012).

Conclusion

The results of study indicated that ABTS assay is more capable to detected antioxidants capacity than DPPH assay due to higher antioxidants yielded. Denaturation of protein which probably occurs due to pH change (production of carbonic acid from CO_2 absolution in muscle as well as lactic acid formation through lactic acid bacteria metabolism) could be led to variation in antioxidant capacity from 2nd day to 10th day of storage period.

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The impact of processing technologies and isolation procedures on the quality of animal DNA in food and feed products

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Summary
The aim of this study was to find suitable methods for the homogenization and isolation of nucleic acids from samples of both raw and thermally or mechanically treated muscle tissue and different types of meat products and feeds. The effect of different processing methods (high temperature, pressure, mechanical processing, addition of other raw materials) on DNA from animal tissues (chicken, pork, beef) in products manufactured in technology workshops was investigated. Subsequently, several extraction techniques for DNA isolation from model meat products were tested. DNA quality and quantity were assessed by absorbance measurements and determination of the ratio of absorbance using a spectrophotometer. Gel electrophoresis was performed in order to determine the degree of DNA fragmentation induced by technological processing. The amplification ability was tested by PCR analysis.

Keywords: *DNA isolation; PCR analysis; food authenticity*

Introduction

Authentication of species origin in food and feed products is important for encouraging the enforcement of the law, and also protecting consumers in terms of health, economic or religious concerns. A total control is not always guaranteed by labelling. Therefore, analytical methods must be used for species identification. The analysis is targeted mostly on the detection of proteins or DNA molecules which are extracted from tissues. Due to protein denaturation caused by the heating or canning (high temperature in combination with high pressure) process (Mackie et al., 1999), DNA is a more suitable molecular marker for species authentication because it is more resistant to thermal treatment. Actually, DNA is also degraded into small fragments during the thermal process but these are still detectable. Moreover, DNA is largely independent of the tissue source or sample damage (Bossier et al, 1999, Lockley and Bardsley, 2000). The critical step is the extraction of high quality DNA in sufficiently large quantities from heterogeneous food matrices. Inhibitor compounds can interfere with PCR by decreasing or even completely inhibiting the activity of DNA polymerase (DiPinto et al., 2017). The aim of this study was to determine the degree to which DNA was influenced by the technological processes used in food industry (mechanical, thermal, chemical or enzymatic treatment) in self-prepared samples from the muscle tissue of pigs, chicken and cattle, and how the subsequent sample preparation and DNA extraction procedure can affect its qualitative and quantitative parameters.

Material and Methods

<u>*Sample preparation*</u>

Authentic fresh meat samples from pork (*Sus scrofa domesticus*), chicken (*Gallus gallus*) and beef (*Bos taurus*) were purchased at local markets or were provided by a local slaughterhouse. Model samples included single species-samples, mixed meat mixtures and products containing a defined percentage of muscle of the species studied (chicken, pork or beef). Additionally, two samples of commercial feed with a defined composition - granules and cans were tested. Finally, a set of 25 reference samples was selected.

DNA extraction
Six commercial kits based on the affinity of the DNA to bind to the silicate membrane column were used: DNeasy Blood a Tissue Kit (Qiagen), DNeasy mericon Food Kit (Qiagen), Food DNA Isolation Kit (Norgen Biotek), UltraPrep Genomic DNA Food Mini Prep kit (AHN-Bio), High Pure PCR Template Preparation Kit (Roche) and NucleoSpin Food (Macherey-Nagel), one commercial kit based on the magnetic separation (Chemagic DNA Tissue 10 kit (PerkinElmer) and one phenol-chloroform extraction were provided. The extraction procedures were performed according to the protocols supplied by the manufacturers.

DNA quantification and purity
The quality of extracted DNA was compared by measuring concentration and purity using a UV spectrophotometer (NanoDrop™ 1000, Thermo Scientific). DNA extracts were quantified by measuring the absorbance at 260 nm (A260). DNA purities were estimated by calculating the A260/A280 ratios. Samples calculated to have A260/A280 ratios of 1.7 - 2.0 were assumed to be pure samples, free from protein and other contaminants. Measurements were performed at room temperature following sufficient mixing of all samples.

DNA fragmentation and PCR analysis
To verify the degree of DNA integrity, which is usually disrupted in processed samples, gel electrophoresis to determine DNA fragmentation was performed. To check the suitability of the extracted DNA for the subsequent PCR analysis, primers aimed at amplifying fragments of defined sizes (cattle 274 bp, swine 398 bp, chicken 227 bp) according to Matsunaga et al. (1999) were tested.

Results and Discussion

DNA concentration and purity were determined spectrophotometrically by measuring DNA absorbance and A260/A280 ratios. The DNA was considered to be satisfactorily pure when A260 to A280 ratios were within the range of 1.7 – 2.0. Contamination of DNA with proteins usually reduces the A260 to A280 ratio to values lower than 1.7. The average DNA concentrations (ng/µl) for particular extraction techniques are described in Figure 1.

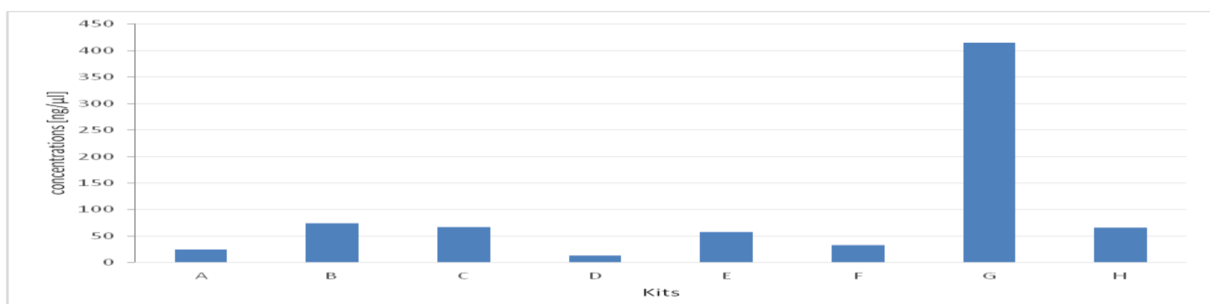

Figure 1. Average values of DNA concentrations for particular kits (A-H).

The ratio A260/A280 of almost all extracts ranged between 1.7-2.0 (Table 1), except kits C, E and F. We obtained the information about the approximate purity of the isolated DNA, but not about the potential fragmentation induced during the isolation procedure.

Regardless of the extraction method, considerable levels of DNA degradation were observed in all samples. The results of PCR amplification of meat product extracts demonstrated that there are significant differences in the performance of each method. In the final phase, total financial requirements and labour intensiveness of the extraction using different kits were assessed (Table 2).

Table 1: Group rate values A.

Extraction kit*	Value "A"		
	<1.7	1.7 – 2.0	>2
A	2	48	0
B	3	46	1
C	39	11	0
D	2	24	24
E	45	5	0
F	49	1	0
G	1	45	4
H	20	22	8

*A – DNeasy Blood & Tissue Kit (Qiagen), B – DNeasy mericon Food Kit (Qiagen), C – Chemagic DNA Tissue 10 Kit (PerkinElmer), D – Food DNA Isolation Kit (Norgen Biotek), E – UltraPrep Genomic DNA Food Mini Prep Kit (AHN-Bio), F – High Pure PCR Template Preparation Kit (Roche), G – Fenol-chloroform extraction, H – NucleoSpin Food (Macherey-Nagel)

Table 2: Financial requirements and labour intensiveness of the extraction using different kits.

Extraction	Cost per	Sample	Manual	Average value of DNA	Absorbance range
A	++	25	+	24	48/50
B	+++	200	+	74	46/50
C	++	10	++	70	11/50
D	+++	200	+	15	24/50
E	++	200	+	56	5/50
F	++	25	+	33	1/50
G	+	100	+++	425	45/50
H	+++	200	+	67	22/50

[a] 0-50 +, 50-100 ++, 100-150 +++

Conclusion

In the present study, the efficiency of eight extraction methods was assessed and compared for the DNA isolation from meat products with different composition and technological treatments. Furthermore, the comparison of the effectiveness of various kits with regard to financial requirements, labour intensiveness, time consumption and the input amount of the raw material was performed. According to acquired data and total evaluation kit B kit (DNeasy mericon Food Kit, Qiagen) is the best for DNA-extraction of the products.

Acknowledgment

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QuEChERS or SPE: Which method of sample preparation is better for the determination of fluoroquinolones in honey?

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Summary
Our study was focused on suitable preparation of honey samples for the determination of fluoroquinolone antibiotics. We used honey samples spiked by standard working solutions containing 5, 10 and 20 μg/ml of marbofloxacin, norfloxacin, ciprofloxacin, enrofloxacin and flumequine and 0.5, 1 and 2 μg/ml of danofloxacin. We tested the quick, easy, cheap, effective, rugged and safe (QuEChERS) method and solid phase extraction (SPE) for optimisation and recovery and we compared them. The analyses were determined by ultra performance liquid chromatography (UPLC) with fluorescence detection.

Keywords: *dispersive extraction; antibiotics in honey; optimisation; recovery; UPLC*

Introduction

Honey is a natural sweet product of animal origin with a positive effect on human health. According to the current legislation, honey must not contain any heterogeneous substances and impurities. Using antibiotics is banned in beekeeping in the countries of the European Union (EU) and maximum residue levels (MRLs) have not been established for honey. The situation is different in states outside the EU where the antibiotics are used in the incidence of American foulbrood (*Paenibacillus larvae*) and European foulbrood (*Melisoccocus plutonius*) (Reybroeck et al., 2012).

Quinolones are synthetic broad-spectrum antimicrobial drugs with bactericidal activity against Gram-positive and Gram-negative bacteria. Enrofloxacin, ciprofloxacin, marbofloxacin and flumequine are the most commonly used in beekeeping. These quinolones belong to the group of fluoroquinolones (FQs) (Galarini et al., 2015; Jin et al., 2017). Their frequent use in veterinary medicine is associated with negative effects on consumers of animal products containing residues of these substances. The other studies describe the increased resistance of microorganisms to antimicrobial substances, allergic reactions and toxicity. For this reason, it is necessary to analyse content of veterinary drug residues in animal products (Lombardo-Agui, et al., 2012; Tayeb-Cherif et al., 2016).

In our study, we focused on optimising the honey sample preparation enriched with FQs for chromatographic analyses by QuEChERS and SPE methods. We compared them and according to results we chose the most appropriate method applicable to the determination for our conditions.

Materials and Methods

Chemicals:

All chemicals were analytical reagent grade. Standards of FQs marbofloxacin (MAR), norfloxacin (NOR), ciprofloxacin (CIP), danofloxacin (DAN), enrofloxacin (ENR), flumequine (FLU), trifluoroacetic acid (TFA) (Sigma-Aldrich, Germany); acetonitrile (ACN), methanol (Merck, Germany); ethylene diaminetetraacetic acid disodium salt dihydrate (Complexone III), citric acid monohydrate, anhydrous sodium sulphate, sodium chloride (Penta, Czech Republic); chromatographic column Acquity UPLC BEH C18 2.1 x 100 mm, 1.7 μm particle size, SPE column Oasis MAX 500 mg, 6 ml (Waters, Ireland); SPE column Strata XA 500 mg, 6 ml (Phenomenex, USA). HPLC grade water was prepared using Aqua

Osmotic device 03 (Tisnov, Czech Republic). The mobile phases were filtered through the membrane filter (0.2 µm) and degassed in an ultrasonic bath for 15 minutes. As a model honey was used floral honey without FQs from the market.

Preparation of standard solutions:
Standard stock solutions of FQs were prepared by dissolving in methanol at concentration levels 1 mg/ml (MAR, NOR, CIP, ENR, FLU) and 0.1 mg/ml (DAN). Mixed standard working solutions (5, 10, 20 µg/ml for MAR, NOR, CIP, ENR, FLU and 0.5, 1, 2 µg/ml for DAN) were diluted into water. Stock solutions were stored in refrigerator at 4 °C for one month. Working solutions were made fresh each day of analysis.

Sample preparation:
QuEChERS: We applied procedure according to Jin et al. (2017) with modification. 2 g of honey were weighed into a propylene tube. Working solutions of FQs, water, citric acid and Complexone III were added. The mixture was mixed until dissolved. ACN, sodium chloride and sodium sulphate were added. The mixture was well stirred and centrifuged for 10 minutes at 5,000 rpm. ACN layer was filtered through the membrane filter 0.22 µm into vials.

SPE: 2 g of honey were dissolved in 6 ml of water, mixed standard working solutions of FQs were added. The mixture was completed to volume 10 ml. The procedures of application notes (Anonymous, 2014) were used to purify the honey matrix. For concentration (enrichment of analytes) by SPE two types columns were used. Analytes were eluted with methanol after equlibration and conditioning of SPE columns and sample loading. After evaporation, analytes were reconstituted in the mobile phase A (0.2% TFA). The samples were filtered through the membrane filter 0.22 µm into the vial.

UPLC conditions:
Chromatographic analyses were performed using the separation module Acquity with fluorescence detector; software Empower 2 was used to quantitative determination (Waters, Ireland). The analytes were separated on Acquity UPLC BEH C18, 2.1 x 100 mm column, 1.7 µm particle size. The mobile phases were (A) 0.2% TFA and (B) methanol : ACN (1 : 5); the flow rate 0.5 ml/min, column temperature 35 °C, injection volume 1 µl. The fluorescence detection ran at wavelengths (ex./em.) 280/450 nm (NOR, CIP, DAN, ENR), 294/514 nm (MAR) and 312/366 nm (FLU). A calibration curve method was used to evaluation.

Results and Discussion
Recoveries of QuEChERS and SPE methods are demonstrated in Table 1.

Table 1: Recoveries [%] of QuEChERS and SPE for three concentration levels of FQs.*

	5 (0.5) µg/ml			**10 (1.0) µg/ml**			**20 (2.0) µg/ml**		
	QuE	**MAX**	**XA**	**QuE**	**MAX**	**XA**	**QuE**	**MAX**	**XA**
MAR	102	54	97	92	52	88	80	51	72
NOR	137	47	104	109	34	97	73	47	74
CIP	126	49	100	105	41	94	75	51	74
DAN	208	92	123	161	54	118	94	59	76
ENR	160	76	115	135	77	105	99	71	77
FLU	202	54	123	129	70	76	107	65	65

* QuE = QuEChERS; MAX = SPE column Oasis MAX; XA = SPE column Strata XA

QuEChERS is often used method for determination of contaminants in various food matrices. It has many advantages in the contrast to the commonly used SPE, such as low consumption of organic solvents, lower price and reduced time for sample preparation. Nevertheless, this method is not adequate for using in honey matrices in low concentration of FQs. Recoveries approached to 200 %. This method showed false positive results of unknown origin. For SPE method by using two different SPE columns recoveries were more acceptable. They were from 34 to 92 % for SPE column Oasis MAX and from 65 to 123 % for SPE column Strata XA. Recoveries depended on concentration of FQs spiked in honey and on FQ itself.

Conclusion

This is a pilot study for the optimisation of suitable methods for the preparation of honey samples containing FQs. To prove and verify these methods we used wide range of concentrations which usually do not occur in honey. QuEChERS was suitable for FQs in higher concentration, while the SPE can be preferable for lower concentrations. It is necessary to carry out experiments in the range of the trace level concentrations of FQs in honey and confirm methods by liquid chromatography tandem mass spectrometry (LC-MS/MS).

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Stanovení oxidu siřičitého v sušených meruňkách
Determination of sulfur dioxide in dried apricots

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Summary
The primary objective of this study was to evaluate sulfur dioxide content in dried apricots available on the Czech market. The secondary objective was to verify any possible effect of rinsing with drinking water on the total content of sulfur dioxide. Three groups of samples (n=14) were collected in March and April 2017 and processed within 24 hours (within 8 hours after opening respectively). The samples were measured in accordance with the spectrophotometric method, using the commercial analytic kit Megazyme K-TSULPH. The method is based on the reaction with Ellman's reagent. The total sulfur dioxide concentration ranged between 1,008 and 3,184 mg.kg^{-1} with average content of 1,798 ± 628.6 mg.kg^{-1}. The maximum legislative limit of 2,000 mg.kg^{-1} was exceeded in five samples (35.7 %). In all but one sample no significant changes of SO_2 content were observed after rinsing ($p>0.05$).

Keywords: *Ellman's reagent; DTNB; food additive; dried fruits*

Úvod
Oxid siřičitý (dále SO_2) je pro své antioxidační a antimikrobiální účinky často využíván jako konzervační látka v potravinářském průmyslu. Do potravin se přidává ve formě siřičitanových a disiřičitých solí či v původní formě. Část přidaného SO_2 může být následně v potravině navázána na sloučeniny obsahující karbonylové skupiny, zatímco část zůstává volně v podobě molekul SO_2 či jako HSO_3^- ion přítomný ve vodném roztoku (Montes et al., 2012). Oxid siřičitý a metabolity jeho sloučenin mohou ve vyšších koncentracích, či u vnímavých jedinců vyvolávat celou řadu nežádoucích zdravotních účinků (Vally et al., 2009). Z tohoto důvodu je také celosvětově jeho obsah v potravinách sledován a upraven řadou legislativních norem. Z hlediska označování potravin, musí být dle Nařízení č. 1169/2011 na obalu výrobku uveden, pokud jeho množství, vyjádřené jako celkový obsah SO_2, přesáhne 10 mg.kg^{-1}. Obsah SO_2 a jeho sloučenin v potravinách je v zemích EU upraven Nařízením č. 1333/2008. V rámci národní legislativy je pak upraven Vyhláškou č. 4/2008 Sb., kterou se stanoví druhy a podmínky použití přídatných látek a extrakčních rozpouštědel při výrobě potravin. Tato vyhláška, harmonizovaná s výše uvedeným nařízením, uvádí nejvyšší povolené množství SO_2 pro sušené meruňky 2000 mg.kg^{-1}. Výrobci doporučují sušené meruňky skladovat v suchu a chladu, konzumovat po oplachu vodou a v krátké době od otevření obalu. Z tohoto důvodu jsme do studie zařadili i sledování vlivu oplachu na celkový obsah SO_2.

Materiál a metodika
5 g rozmělněného vzorku bylo s přesností na tři desetinná místa naváženo na analytických vahách Boeco BAS 31 Plus (Boeckel + Co GmbH, DE). Spolu se 40 ml destilované vody byl vzorek homogenizován po dobu 30 sekund dispergátorem DI 25 basic (IKA-Werke GmbH, DE) a poté analyticky převeden do odměrné baňky o objemu 100 ml. Dále bylo do vzorku za stálého míchání napipetováno 5 ml činidel Carrez 1 (36 g.l^{-1}), Carrez 2 (72 g.l^{-1}) a 10 ml 0,1 M NaOH (4 g.l-1). Po doplnění odměrné baňky destilovanou vodou k rysce a důkladném promíchání se vzorek nechal 15 minut odstát a poté byl zfiltrován přes filtrační papír Whatman č. 1 (Whatman International Ltd, UK). Čirý, lehce nažloutlý filtrát byl analyzován při 405 nm na dvou-paprskovém spektrofotometru Specord 200 Plus (Analytik Jena AG, DE), s využitím komerčního setu pro stanovení oxidu siřičitého K-

TSULPH (Megazyme Inc, IE). Měření bylo prováděno v kyvetách o délce hrany 1 cm, při konstantní teplotě 25 °C a proti prázdné kyvetové komoře (tzv. proti vzduchu).

Vzorky (n=14) sušených meruněk ošetřených SO_2 byly získány z tržní sítě ČR ve třech odběrech v průběhu března a dubna 2017. Vzorky byly zpracovány a analyzovány do 24 hodin od získání, respektive do 8 hodin od otevření obalu. Vzorky byly připravovány a měřeny duplicitně a u každého vzorku byla stanovena koncentrace SO_2 před oplachem a po něm. Oplach byl prováděn proudem pitné vody po dobu 3 minut. Ke každé sadě vzorků byly změřeny dva slepé vzorky a dva standardy SO_2 pro jednobodovou kalibraci. Kvantifikace výsledků byla prováděna primárně pomocí jednobodové kalibrace na standardní roztok SO_2 o koncentraci 300 mg.l^{-1}. U vzorků s nadlimitními hodnotami byla naměřená data ověřena pomocí klasické vícebodové kalibrace v rozsahu koncentrací 0 – 400 mg.l^{-1}. Ke zpracování a kvantifikaci dat byly použity programy Excel (Microsoft Corp., US) a Mega-Calc (Megazyme Inc, IE). Ke statistickému zpracování dat byly použity programy Excel a Unistat (Unistat Ltd, UK).

Výsledky

Průměrná koncentrace SO_2 ve sledované skupině výrobků činila 1798 ± 628,6 mg.kg^{-1}. Minimální stanovená koncentrace byla 1008 mg.kg^{-1}, maximální naměřená koncentrace dosahovala 3184 mg.kg^{-1}. V rámci pokusu byl zjišťován i vliv oplachu meruněk na celkový obsah SO_2. Z výsledků statistické analýzy vyplývá, že námi zvolený druh oplachu nemá statisticky významný vliv ($p>0,05$) na koncentraci SO_2 ve výrobku.

Diskuse

Z hlediska výskytu SO_2 v sušených meruňkách je odborná literatura na data chudá. Většina odborné literatury se věnuje vývoji nových detekčních metod, jejich srovnávání s již zavedenými metodami, či modelaci absorpce a desorpce SO_2 během stárnutí výrobku v závislosti na výchozí koncentraci SO_2 a podmínkách skladování. Tyto studie jsou však prováděny na referenčních materiálech, či laboratorně připravovaných vzorcích a nelze je tedy použít ke srovnání s hodnotami zjištěnými u reálně analyzovaných vzorků potravin. Jedním z mála literárních zdrojů, který se zabývá i sušenými meruňkami z tržní sítě je výzkumná práce, kterou publikovali Nury et al. v roce 1959, tedy ještě před stanovením limitu 2000 mg.kg^{-1} na národní i mezinárodní úrovni. U sířených meruněk z tržní sítě Spojených států amerických byly zjištěny koncentrace v rozpětí 3090 – 3230 mg.kg^{-1}. V současné době probíhá pod hlavičkou Evropského úřadu pro bezpečnost potravin (EFSA) projekt na re-evaluaci potravinářských aditiv, včetně SO_2 a jeho forem. Ve svém vyjádření (EFSA, 2016) uvádí Komise pro potravinářské přídatné látky a zdroje živin přidávané do potravin (EFSA - Panel ANS) mimo jiné i hodnoty naměřené dozorovými orgány členských států unie. Pro sušené meruňky (n=208) jsou zde uváděny naměřené koncentrace celkového SO_2 0,8 – 4402,5 mg.kg^{-1}, přičemž průměrná koncentrace činila 1336,4 mg.kg^{-1}. Námi zjištěné koncentrace 1008 – 3184 mg.kg^{-1}, s průměrnou hodnotou 1798 ± 628,6 mg.kg^{-1} jsou srovnatelné s výše uvedenými údaji. Vyšší průměrná hodnota zjištěná v naší studii jde na vrub menšímu počtu analyzovaných výrobků, kdy se mají možnost na průměru více projevit extrémní hodnoty a také tomu, že ve studii EFSA je hodnota snížena započítáním nižších koncentrací SO_2 v nesířených meruňkách.

Závěr

Z celkového počtu čtrnácti odebraných vzorků (n=14) nevyhovělo legislativnímu limitu (max. 2000 mg.kg^{-1}) celkem 5 výrobků, tedy 35,7 %. Průměrná koncentrace SO_2 činila 1798 ± 628,6 mg.kg^{-1}, což ukazuje na zbytečně vysoké množství SO_2 používaného výrobci, neboť ani u výrobku s nejkratší dobou trvanlivosti se neprojevovaly žádné senzorické

odchylky. V rámci pokusu byl zjišťován i případný vliv oplachu meruněk proudem studené pitné vody po dobu 3 minut, který se však ukázal být statisticky nevýznamným ($p>0,05$). Studii je třeba dále rozšířit o oplach za jiných podmínek (doba, vyšší teplota, dosoušení po oplachu).

Poděkování
Tato studie byla financována Ústavem hygieny a technologie mléka, FVHE – VFU BRNO.

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Využití kvantových teček pro značení alergenů hořčice a sóji v modelových vzorcích

Quantum dots utilization for soy and mustard allergens labelling in model samples

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Summary
Due to the constant increase of patients suffering from food allergies also increases the need for suitable and reliable methods for the specific detection. Among these methods belong IHC fluorescence methods. This work is focused on the usage of QDs in the IHC determination of allergen soybean and mustard in range 0.0001%; 0.001%; 0.01%; 0.1%; 1%; 10% in model samples of chicken hams. The aim was to develop a suitable methodology and confirm the benefits of the usage of QDs as a flurochrom. For this work was selected classic IHC sandwich method with the application of commercially purchased QDs with the bound secondary antibody.

Keywords: *immunohistochemistry; fluorochromes; CdSe / ZnS QDs; fluorescence microscopy; food allergens*

Úvod

V návaznosti na rostoucí veřejný zájem týkající se problematiky alergenních potravin, roste nejen potřeba nových diagnostických metod, ale také roste pozornost kontrolních orgánů v oblasti kontroly potravin a tím pádem i potřeba spolehlivých metod pro průkaz alergenů v potravinách (Van Hengel, 2007). V průběhu posledních let je rozšiřována oblast identifikace potravinových alergenů o nové metody pro jejich stanovení nebo o modifikace či rozšíření stávajících metod. Mezi běžně využívané metody detekce alergenů patří imunohistochemické metody (IHC). Při detekci pomocí IHC metodami jsou pro značení alergenu běžně používány chromogeny či fluorescenční organická barviva (fluorchromy). Kromě tradičních konvenčních fluorochromů jsou v poslední době používány v imunoanalýze také kvantové tečky (QDs) (Yang et al., 2014). Cílem práce bylo ověřit možnost využití QDs v analýze vybraných rostlinných alergenů (sója, hořčice) a ověřit jejich kvantovou stabilitu v průběhu času.

Materiál a metody

Pro účely studie byly zhotoveny modelové vzorky kuřecích šunek z kuřecí prsní svaloviny, 1,5 % kuchyňské soli, 0,5 % polyfosfátů a s přídavkem sójového proteinového koncentrátu ALPHA 8/P (The Solae, CHE) a drceného hořčičného semínka (Alibona, CZE) vkoncentrační řadě (0.0001%; 0.001%; 0.01%; 0.1%; 1%; 10%). Vzorky byly zpracovány technikou parafínových bločků. Pro každý vzorek bylo vyhotoveno 16 řezů na rotačním mikrotomu RM 2255 (Leica, GER). Řezy byly krájeny na tloušťku 5 µm. Postup IHC metody vycházel z manuálů ke komerčně zakoupeným QDs CdSe / ZnS Qdot 525 goat F $(ab')_2$ anti-rabbit IgG conjugate (Invitrogen, JPN) Invitrogen Qdot Conjugates Protocol Hanbook (Invitrogen, JPN, 2007) a Qdot Secondary Antibody Conjugates (Invitrogen, JPN, 2011) a upraven pro aplikaci na vzorky potravin. Vyšetření řezů bylo provedeno při zvětšení 200x ve fluorescenčním mikroskopu Leica DM 3000 (Leica, GER) za použití fluorescenčního filtru I3 s emisí od 510 nm. Snímání zorného pole bylo uskutečněno pomocí kamery Leica DFC 295 (Leica, GER) ve spojení s počítačovým programem Xn View (Pierre E. Gougelet, FRA). Pro určení quatové

stability QDs byly po dobu 3 měsíců průběžně v intervalu 1 týdne opětovně vyšetřovány vzorky obsahující alergen hořčici. Vzorky byly uchovávány v temnu a chladu (6 °C).

Výsledky
Intenzita kontrastu vybraného alergenu ke kuřecí prsní svalovině (koncentrace primární protilátky 1:500) je uvedena v Tabulce 1.

Tabulka 1: Posouzení kontrastu kvantového výtěžku k pozadí.

Koncentrace přidaného aditiva	**Sója**		**Hořčice**	
	alergen	nespecifická fluorescence	alergen	nespecifická fluorescence
10 %	++	-	+++	+
1 %	+	-	+++	+
0,1 %	+	-	++	+
0,01 %	+	-	++	+
0,001 %	+	-	++	+
0,0001 %	-	-	++	+

- žádný kontrast; + slabý kontrast; ++ silnější kontrast; +++ výrazný kontrast mezi alergenem a pozadím

V práci byla sledována také výdrž intenzity světelného signálu u modelových vzorků obsahujících hořčici o koncentraci 10 %. Výsledky zobrazuje Tabulka 2.

Tabulka 2: Kvantová stabilita.

1. měsíc	týden	1.	2.	3.	4.	5.
	kontrast	+++	+++	+++	+++	+++
2. měsíc	týden	1.	2.	3.	4.	5.
	kontrast	+++	+++	+++	+++	+++
3. měsíc	týden	1.	2.	3.	4.	5.
	kontrast	+++	+++	+++	+++	+++

- žádný kontrast; + slabý kontrast; ++ silnější kontrast; +++ výrazný kontrast mezi obsaženou hořčicí a pozadím

Diskuse
QDs jsou anorganické polovodičové fluorescenční nanočástice s velikostí 1-10 nm vykazující širokou excitaci a úzké symetrické emisní spektrum. Již několik publikací uvádí využití QDs v potravinářských vědách, kde byly použity pro detekci patogenních bakterií a proteinů. V současné době je zkoumána možnost aplikace QDs vázaných s polyklonální protilátkou pro detekci alergenů (Bonilla et al., 2016). V rámci našeho výzkumu se tuto metodu podařilo ověřit, přičemž byly dosaženy různé výsledky podle daného druhu alergenu (Tabulka 1). Alergen sója dával u koncentrace 10 % silnější kontrast, u koncentrací 1 %; 0,1 %; 0,01 % slabý kontrast a u koncentrace 0,0001 % žádný kontrast. Hořčice dávala výrazný kontrast oproti pozadí u vzorků obsahujících 10 % a 1 %, u zbylých obsažených koncentrací silnější kontrast, avšak byl zde pozorován efekt autofluorescence. Příčinou autofluorescence byla přítomnost obalových vrstev z drcených semen hořčice. U vzorků s přídavkem sóje se autofluorescence neprojevila z důvodu použití komerčně zakoupeného sójového koncentrátu, který obalové vrstvy neobsahoval. Námi získané výsledky u alergenu sóji byly ve shodě s dřívějšími výsledky Petrášová et al. (2015). V rámci naší studie byla sledována i stabilita QDs, viz. Tabulka 2. Oproti běžným organickým fluorochromům, jež podléhají fotochemickému vysvícení, vykazují QDs mnohem větší stabilitu. Tato vysoká stabilita umožňuje dlouhotrvající studie, archivaci a zpětné přezkoumání pokusů (Mahmoud et al.,

2011). Kvantovou stabilitu po dobu tří měsíců potvrdili také naše výsledky. Kolektiv autorů Speranskaya et al. (2014) taktéž potvrdil vysokou stabilitu QDs a lepší a snadno zjistitelný světelný signál při značení alergenů oproti běžně využívaným fluorochromům. Kvantovou stabilitu je možné také využít v dalších analýzách jako je například sledování lokalizace alergenu v matrici vzorku.

Závěr

Byla potvrzena vhodnost značení vybraných alergenů sóji a hořčice pomocí QDs. Alergen označený QDs dával ve srovnání s pozadím vysoký kontrast u vzorků hořčice a nízký kontrast u sóje. Byla potvrzena kvantová stabilita QDs po dobu 3 měsíců. Alergeny značené QDs lze tedy hodnotit i po několikaměsíčním skladování bez významného zhoršení emisního spektra QDs.

Poděkování

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Určení druhu škrobu pomocí skenovací elektronové mikroskopie
Determination of kinds of starches by scanning electron microscopy

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Summary
The goal of this research is to determine different types of starch with use of scanning electron microscopy (SEM). In food processing industry, starch is used in natural or modified forms. The modification can be either chemically or physically. The most usual way to detect starch and its type (most frequently potato, corn and wheat starch) is by using light microscopy. However, thist method is not always fully reliable in determining by which method was the starch modified. On the other hand the SEM can, based on the knowledge of the specific morphological characteristics, accurately distinguish between the types and methods of starch modification. This can, for example, be used in product control, to determine whether there is not a deceptive indication of the used starch.

Keywords: *modification; physical; chemical; potato starch*

Úvod
Škrob je přirozeně se vyskytující zásobní polysacharid. Vyskytuje se ve formě granulí, které obsahují dva typy polysacharidu: amylopektin a amylózu. (SVEGMARK a kol., 2002). V rostlinách ho můžeme nalézt v hlízách brambor, v kukuřici, v cereáliích (pšenice, ječmen, proso, tapiok), v hrachu či v rýži (ASHOGBON a AKINTAYO, 2014). Tento škrob nazýváme nativní, přirozený. Nativní škrob má v potravinářském průmyslu omezené využití, není příliš stabilní a při použití škrobu ve výrobku může docházet k oddělování vody (SANTANA a MEIRELES, 2014). Z těchto důvodů se nativní škrob modifikuje a to buď fyzikálně anebo chemicky. Modifikace vede k potlačení určitých vlastností a ke zvýraznění vlastností žádoucích. Fyzikální modifikace je levná a bezpečná, zahrnuje mechanické poškození částic (trhlinky, dutinky, promáčknutí, zvětšení či zmenšení částice), které způsobuje vyšší nasákavost škrobu či lepší jeho rozpustnost ve výrobku. Chemická modifikace zahrnuje použití chemikálií pro zlepšení vlastností škrobu. (VASCONCELOS a kol., 2015) Chemicky modifikovaný škrob se ve výrobcích uvádí pod E kódem (Vyhláška 4/2008 Sb.), což může výrobce svádět k neuvedení přesného způsobu modifikace škrobu na obale výrobku.

Škrob, jak nativní, tak i modifikovaný, se využívá potravinářství nejvíce jako zahušťovadlo, stabilizátor či k výrobě želé. Dále se škrobu využívá například či jako pojivo v obalech, při ošetřování kůže (kožedělný průmysl), zvýšení pevnosti papíru či jako plnivo léčiv ve farmaceutickém průmyslu.

Identifikace a průkaz škrobů ve výrobku se provádí více metodami. Nejznámější a nejjednodušší je reakce škrobu s jodovým roztokem, kdy vzniká modré zbarvení. Tato zkouška má pouze orientační charakter, zda škrob ve výrobku je či není. Další metodou je světelná mikroskopie, kdy na základě znalosti morfologie škrobových zrn dokážeme určit i druh škrobu. Pomocí SEM dokážeme určit přesný tvar, velikost a škrobové zrno je lehce rozlišitelné i pro méně zkušeného hodnotitele. Tím, že vidíme povrch zrna, dokážeme i určit, zda byl škrob modifikován chemicky či fyzikálně.

Materiál a metodika

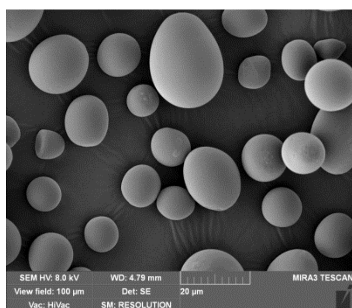
Vzorky škrobu pro elektronový mikroskop byly neinvazivně naneseny na vodivou lepicí pásku, pokoveny 10 µm vrstvou platiny a prohlíženy pod skenovacím elektronovým mikroskopem (MIRA3, Tescan, BRNO) při daném zvětšení odpovídající velikosti škrobového zrna.

Ze sypkých vzorků škrobu byly nejdříve připraveny parafínové bloky a následně řezy o síle 4 µm, které byly umístěny na podložní skla. Barvení pro světelnou mikroskopii bylo použito Lugol- Calleja. Vzorky byly nejdříve odparafinovány xylenem (10 min) a alkohol/éter 2/3 + 1/3 (10 min). Postup barvení byl následující: jádrová červeň (15 min), vodovodní voda (5 min), Lugolův roztok (5 min), oplach v destilované vodě, roztok B Calleja (5 min), oplach v destilované vodě. Následovalo odvodnění oplachem v 96% a 100% ethanolu, projasnění xylenem čistým a xylenem p.a. po 5 minutách.

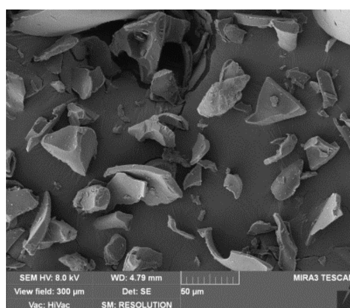
Jako další barvení bylo zvoleno barvení Pas- Calleja. Řezy byly nejdříve odparafinovány xylenem (10 min) a alkohol/éter 2/3 + 1/3 (10 min). Následovalo barvení kys. jodistou (10 min), oplach v redukční lázni a 70 % ethanolu, Schiffovo reagens (15 min), praní v tekoucí vodě (15 min), jádrová červeň (5 min), vodovodní voda (5 min), roztok B Calleja (5 min), oplach v destilované vodě. Následuje odvodnění oplachem v 96% a 100% ethanolu, projasnění xylenem čistým a xylenem p.a. po 5 minutách.

Výsledky a diskuze

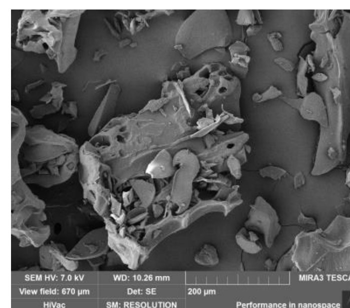
Pro porovnání vzhledu modifikovaných škrobových zrn byla vybrána zrna bramborového škrobu. Jeho zrna jsou v nativním stavu oválného až lasturovitého tvaru se zřetelným rýhováním. Velikost zrn se pohybuje od 10 – 80 µm (OŠŤÁDALOVÁ a POKORNÁ, 2014). Na fotografiích ze světelné mikroskopie lze rozpoznat jednotlivá škrobová zrna, lze vidět, že vzhled modifikovaných zrn je jiný, než nativních. Zrna jsou porušená a mají jiný tvar. Ani se znalostí morfologie však nelze zcela jednoznačně určit, zda byl škrob modifikován fyzikálně či chemicky. Na následujících fotografiích pořízených pomocí SEM můžeme porovnávat vzhled škrobových zrn. Na obr. č. 1 lze vidět neporušené zrna nativního škrobu, na obr. č. 2 lze vidět zrna po fyzikální modifikaci. Zrna jsou mechanicky porušena, lze vidět rozpad stěny zrn. U obr. č. 3 vidíme narušenou stěnu zrna chemikáliemi, po které následoval rozpad zrna. Dále jsou patrné prohlubně a další deformace stěny.



Obr. č. 1: bramborová nativní zrna



Obr. č. 2: fyzikální modifikace bramborového škrobu



Obr. č. 3: chemická modifikace bramborového škrobu

Závěr

Pro orientační stanovení přítomnosti škrobu ve výrobku se hodí jiné způsoby identifikace než SEM, např. světelná mikroskopie či zkouška jodem. Ovšem jen SEM nám dá jasnou odpověď na způsob modifikace škrobu. Díky SEM vidíme rozpad, porušení či jiné deformace stěny škrobového zrna.

Poděkování
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SEKCE 2

Výživa, dietetika hospodářských zvířat a hygiena vegetábilií

Selected Blood Biochemical Indicators of the Dwarf Lop Rabbits in Relation to a Different Diet Composition

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Summary
The aim of this study was to evaluate the effect of different types of the diets on selected biochemical blood indicators in the dwarf rabbits. The study was conducted on a total of 27 young rabbits belong to the Dwarf Lop breed. These rabbits were randomly divided into 3 dietary groups. The control group received a foreign commercial diet used for dwarf rabbits in the course of entire monitored period. The both experimental groups received two specific types of the experimental diets in relation to the age. These two types of both experimental diets contained lupin seeds. We observed the significant changes in the concentration of the albumin ($P<0.01$), glucose ($P<0.05$), urea ($P<0.01$) and inorganic phosphorus ($P<0.01$) among the evaluated groups of rabbits. Furthermore, we found a significant effect of diets on the activities of alkaline phosphatase ($P<0.05$), alanine aminotransferase ($P<0.01$) and aspartate aminotransferase ($P<0.05$) in the blood plasma. Values of blood indicators in all dietary groups in our study were consistent with the physiological values published for healthy rabbits. The found findings indicate that the white lupin seeds can be the suitable component in the diets for the dwarf rabbits. However, further studies will be necessary to perform the diet´s optimization with respect to the dwarf rabbit's health.

Keywords: *dwarf rabbit; nutrition; lupin seeds; clinical chemistry; laboratory values*

Introduction

Recently, the dwarf rabbit rearing was spread and the rabbit become a favorite companion animal for human (González-Redondo and Contreras-Chacón, 2012). With respect to strictly off-production purposes of the dwarf rabbits, a good health state linked by long lifespan should be considered (Proença and Mayer, 2014). The dietary inclusion of the white lupin seeds showed a favorable effect on health state in the meat-type rabbits (Uhlířová *et al.*, 2015). The aim of our study was to evaluate the effect of the different dietary inclusion of a white lupin seeds in diets on the selected biochemical blood indicators of raised Dwarf Lop young rabbits.

Material and Methods

The study was conducted on a total of 27 dwarf rabbits belong to the Dwarf Lop breed. The young rabbits were randomly divided into 3 groups (control group, experimental groups A and B). All of the young raising rabbits were housed and cared under the same husbandry conditions. The control group received a foreign complete commercial diet for dwarf rabbits (Berkel-Futter Light 6008, Coesfeld, Germany) in the course of entire monitored period (CP – crude protein 160.5 g/kg, CFi – crude fibre 173.2 g/kg, CS – crude starch 151.9 g/kg, CFa – crude fat 26.8 g/kg). The experimental diets contained white lupin seeds var. Amiga. Rabbits of the experimental groups were fed two specific subtypes of the diets in relation to their age. The experimental diet A for post-weaning raising rabbits consisted of 100 g white lupin seeds in 1 kg of the diet (CP 164.3 g/kg, CFi 167.7 g/kg, CS 204.0 g/kg, CFa 36.3 g/kg). The experimental diet B consisted of 200 g of white lupine seeds in 1 kg of the diet (CP 187.1 g/kg, CFi 167.7 g/kg, CS 159.0 g/kg, CFa 41.8 g/kg). Rabbit kits with lactating does received the pre-weaning subtypes of the experimental diets until their 7th week of age. Subsequently, the weaned raising rabbits were fed the post-weaning subtypes of the diets from 8th to 15th weeks of age. At the age of 15 weeks, a blood sampling was taken. Blood samples were

centrifuged at the laboratories of the Department of Animal Nutrition and the Department of Animal Husbandry and Animal Hygiene. Basic biochemical indicators (total protein, albumin, glucose, total cholesterol, triacylglycerids, creatinine, urea, alkaline phosphatase - ALP, alanine aminotransferase - ALT, aspartate aminotransferase - AST, calcium and inorganic phosphorus) were determined in the blood plasma using a DPC Konelab 20i Analyzer® (Thermo Fischer Scientific, Finland). One-way variance analysis (ANOVA) was used to determine differences in the evaluated indicators. When ANOVA showed significant differences among the groups, Tukey´s HSD test was used.

Results and Discussion

Values of the blood plasma biochemical indicators are presented in Table 1.

Table 1: Selected biochemical blood indicators in the raising Dwarf Lop rabbits.

Indicator	Units	Dietary group					
		Control (n=9)		Experimental A (n=9)		Experimental B (n=9)	
		x	SEM	x	SEM	x	SEM
Total protein	g/L	55.54	1.20	54.62	1.70	54.88	1.36
Albumin	g/L	35.56^{B}	0.71	$33.03^{A,B}$	0.89	30.84^{A}	0.78
Glucose	mmol/L	$6.20^{a,b}$	0.26	5.72^{a}	0.23	6.93^{b}	0.33
Total cholesterol	mmol/L	1.31	0.06	1.31	0.13	1.49	0.14
Triacylglycerids	mmol/L	1.07	0.17	0.82	0.12	1.23	0.18
Creatinine	µmol/L	94.95	6.49	102.61	7.58	80.00	10.22
Urea	mmol/L	5.94^{A}	0.39	8.73^{B}	0.69	6.34^{A}	0.31
Alkaline phosphatase	µkat/L	$2.61^{a,b}$	0.19	3.02^{b}	0.25	2.23^{a}	0.17
Alanine aminotransferase	µkat/L	1.51^{B}	0.18	0.86^{A}	0.10	0.75^{A}	0.08
Aspartate aminotransferase	µkat/L	0.82^{b}	0.14	0.44^{a}	0.04	$0.53^{a,b}$	0.09
Ca	mmol/L	3.01	0.06	2.88	0.08	3.05	0.04
Inorganic phosphorus	mmol/L	2.05^{B}	0.10	2.02^{B}	0.09	1.55^{A}	0.10

^{a,b}: Means within a row with different superscripts letters differ (P<0.05); ^{a,b}: Mean within a row with different superscripts letters differ (P<0.01); x: Arithmetic Mean; SEM: Standard Error od the Mean

The our results are consistent with physiological laboratory values published for the healthy rabbits (Campbell, 2004; Graham and Mader, 2012). Regarding the basic biochemical panel, we found the effect of the diets on the albumin (P<0.01), glucose (P<0.05), urea (P<0.01) and inorganic phosphorus (P<0.01). As for the urea concentration, we found a slight elevation in both of the experimental groups, while these observed values fall into the stated physiological range. We suppose that this increase in urea level could be caused by generally higher protein content in lupin seeds (Kroupa *et al.*, 2015). Furthermore, within the both of the experimental groups, we found predominantly decreasing tendencies in the enzymes of ALP (P<0.05), ALT (P<0.01) and AST (P<0.05) in their blood plasma.

Conclusion

Our findings showed, that the biochemical indicators in Dwarf Lop breed are affected by the different diets composition. The different diet prescriptions had a statistically significant

effect on the concentration of the albumin, glucose, urea and inorganic phosphorus, and also on activities of the ALP, ALT and AST in the blood plasma. With respect to the good general health state of the monitored raising rabbits, it can be concluded that the white lupin seeds can be suitable component in the diets used for dwarf rabbits. However, further studies will be necessary to perform the optimization of diets with respect to the dwarf rabbit's health.

Acknowledgement
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Production of nutrients green mass by three varieties of white lupine

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Summary
The objective of the study was to compare the production ability of three varieties of white lupins (Amiga, Dieta and Zulika), grown at Nový Jičín university farm own by Veterinary and Pharmaceutical University Brno. Each variety was grown on the area about 10 ha, under identical soil and climate conditions. Sampling of green mass was carried out random selection of 10 samples from the area of 1 m² of each variety. Sampling, were made at the age of 15 weeks of crops when the crops were at the stage of fully developed green pods. Compared production per hectare, of individual varieties tested lupine white grown in the same soil and climatic conditions, we concluded that the highest production potential in the age of 15 weeks showed a variety Zulika, in comparison with the variety Dieta and Amiga. From a nutritional point of view, the variety Zulika can be assess very positive, because it contained the green mass most of nitrogenous substances.

Keywords: *white lupin; Amiga; Dieta; Zulika; production of green mass; chemical analysis*

Introduction

At the beginning of the last century, new, so-called "sweet" lupin varieties low in alkaloids (bitter substances) and high in proteins were developed. That was an impulse for renewed interest in its utilization and lupin became the source of proteins in the nutrition of both humans and animals (Dijkstra et al., 2003). So in the 20th century, lupin became part of modern agriculture and food systems. In Europe, mainly two varieties are grown: *Lupinus luteus* and *L. albus*, while in Australia it is *L. angustifolius* (Cowling et al., 1998). The development of varieties with solid pods and varieties low in alkaloids allowed that these varieties stopped being used as green manure and soiling crops and became legumes grown for seeds. The nutrient composition of lupin is exceptional, it has a high content of proteins and soluble fibre and, unlike cereals, a low content of starch (Petterson et al., 1997). *Lupinus albus, L. angustifolius*, and *L. luteus* have a relatively low content of oil, lupins do not contain anti-nutritional factors such as trypsin inhibitors and saponins. As the source of energy they compete with cereals and as the source of proteins with oil seed meals. The lupin value is enhanced by the capacity to supplement other food components to achieve an overall balance of nutrients (Straková et al., 2006).

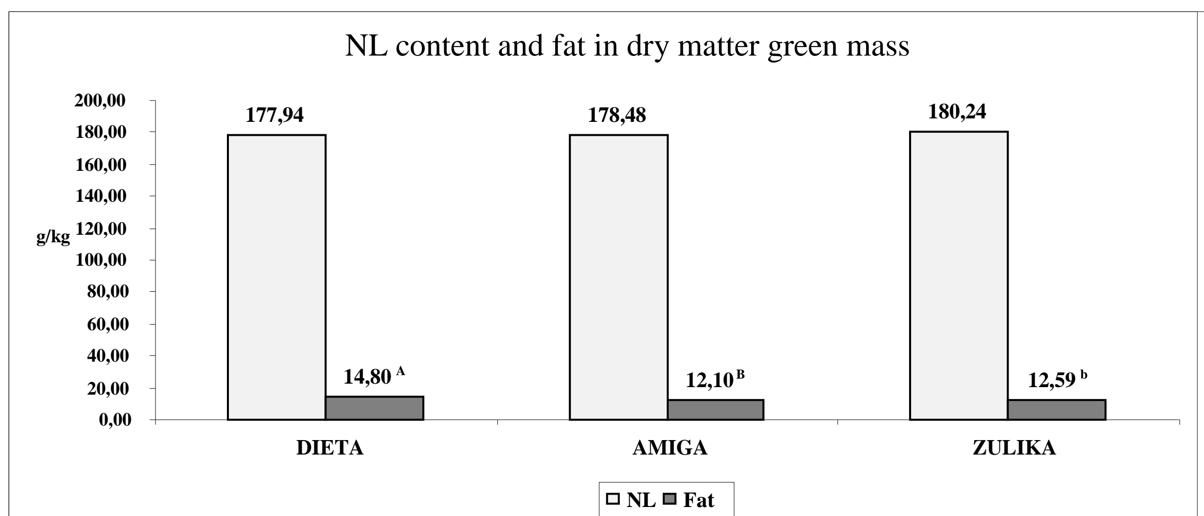
Material and Methods

The objective of the study was to compare the production capabilities of three varieties of white lupine in the same soil and climatic conditions at Nový Jičín university farm in 2015. Three promising varieties was selected for the experiment, variety Dieta, Amiga and Zulika. Each of these varieties was grown on an area of 10 ha. Each variety were sown simultaneously 11. 4. 2015 in the amount of 2 q/ha. Harvest of all three varieties was realized in the period 28. 8. 2015. Sampling of green matter was conducted by random selection of 10 samples of 1 m² area of each variety. Samplings were carried out in the age 15 week of crops, the crops were at the stage of fully developed green pods. In the following period, also did not increase the volume of vegetation, followed by aging and shrinking green pods of green mass. The collected samples of vegetation were subsequently dried and homogenized for analytical analysis. Within chemical analysis were collected in green mass samples these nutrients:

dry matter by drying the sample at 105 ◦C to constant weight, containing nitrogen by the Kjeldahl method Buchi analyzer (produced Centec automatic spol. Ltd.), petroleum grease extraction device ANKOM^{XT10} Fat Analyzer (company OK SERVICE BioPro®), crude fiber, and the individual fractions (ADF, ADL, NDF) device Ankom^{220} Fiber Analyzer (produced by OK SERVICE BioPro®) starch was determined by polarimetry, ash gravimetric method after ashing the sample at 550 °C under specified conditions, brutto energy (BE) calorimetrically AC 500 instrument (LECO), nitrogen-free substances and organic matter were determined by calculation. The results were processed by statistical methods using statistical software Unistat 5.6 for Excel. Were evaluated mean values and their difference multiple comparison using Tukey-HSD test, at a significance level of $P \leq 0.01$ and $p \leq 0.05$. Each indicator is presented of diameter (x) and standard deviation (± SD).

Results and Discussion

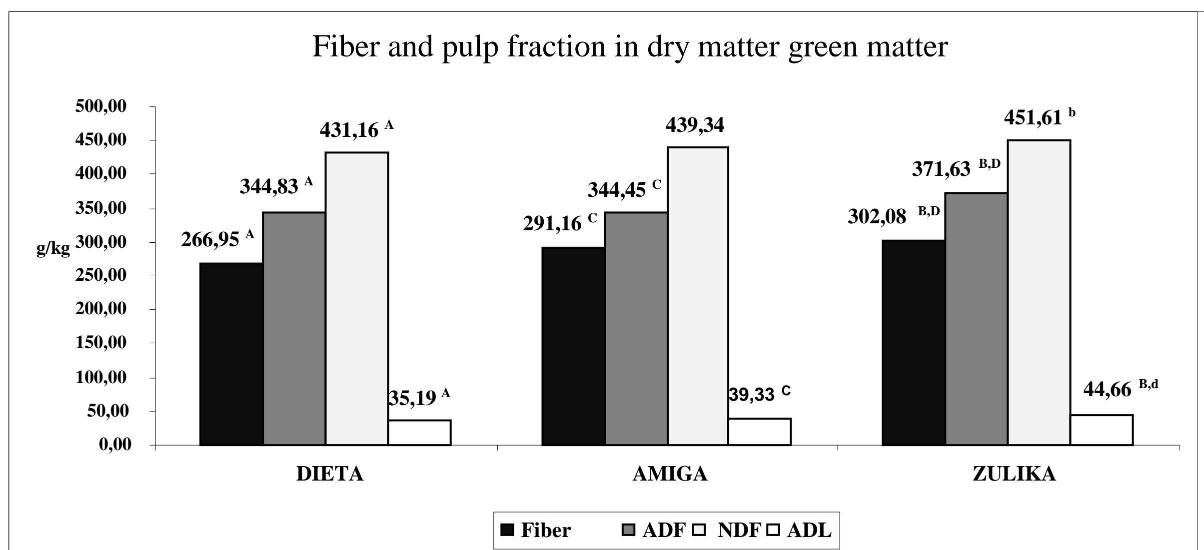
Average values of green matter yields (DM) at the age of 15 weeks crops indicate that productive variety Zulika is 4.23 kg/m^2 (0.63 kg/m^2), Amiga 3.70 kg/m^2 (0.58 kg/m^2) and the lowest production was variety Dieta 3.33 kg/m^2 (0.59 kg/m^2). The production of green mass per 1 m^2 has been proven highly significantly higher average value of the variety Zulika compared with Dieta. In this crops solids relevance is not proven, it is due to differences in water content of the fresh crop. In the present study we were interested not only the quantity of production, but also its quality, evaluated based on the nutrient composition of dry matter green matter in the tested varieties. Graph 1 shows the content NL and fat in dry matter and green mass at the three tested varieties of lupine. Between the average values NL were not statistically significant differences between the varieties, despite the variety Zulika showed the highest content of NL. Conversely, the variety diet was statistically significant $P \leq 0.01$ high fat content compared with the Amiga and Zulika.



Graph 1: The average content of solids and fat in the three varieties tested white lupine (AB, CD highly significant difference between the average values $P \leq 0.01$).

Interestingly, as documented in graph 2, the differences in crude fiber and fiber between factions of the complex. Statistically, the highest average fiber $P \leq 0.01$ was demonstrated in a variety Zulika compared Dieta varieties and Amiga. Similarly, even in the ADF was highly significantly $P \leq 0.01$ the highest average value in the variety Zulika compared Dieta varieties and Amiga. For variety Zulika was determined and the highest average value for NDF, which is significantly $P \leq 0.05$ differed from the average value of the NDF in the diet varieties. Also

Zulika variety proved the highest average value of the ADL, which is highly significantly P ≤ 0.01 different from the average value of the variety ADL Diet and conclusively P ≤ 0.05 variety of diets.



Graph 2: The average content of fiber pulp fractions, and in the three varieties tested white lupine (AB, CD highly significant difference P ≤ 0.01, AB and CD significant difference between the average values P ≤ 0.05).

Conclusion

In terms of production per hectare compared to the individual tested varieties of white lupine grown in the same soil and climatic conditions, we concluded that the highest production potential at 15 weeks of age showed a variety of vegetation Zulika, compared with the variety Dieta and Amiga. Variety Zulika 1 ha produced the most: the green mass of fresh and dry matter, nitrogen substances, fiber and pulp fractions, organic matter, ash and brutto energy. Also from the point of view of nutrition variety Zulika is very positive, because contained in the green mass the most of nitrogenous substances.

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SEKCE 3

Veterinární ekologie

Odpadní vody rezervoárem *E. coli* rezistentní k beta-laktamovým antibiotikům

Wastewater as reservoir of *E. coli* resistant to beta-lactam antibiotics

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Summary
Antibiotic resistant bacteria in aquatic environments pose a great concern for human and animal health. The aim of this study was to examine the presence of Escherichia coli isolates producing extended-spectrum beta-lactamases (ESBL) in municipal and hospital wastewater treatment plants (WWTPs) located in Brno. Samples were collected from the raw sewage and the effluent of WWTPs and cultivated on chromogenic medium with cefotaxime. From each sample 40 presumptive E. coli isolates were examinated for ESBL and AmpC beta-lactamases using a phenotypic assay and polymerase chain reaction. Clonal relationship of the isolates was analysed by pulse field gel electrophoresis. ESBL-producing E. coli isolates were found in all samples except treated hospital wastewater. The most prevalent gene encoding ESBL was bla_{CTX-M}*. Our study shows that processes using in municipal wastewater treatment are inadequate for removing resistant bacteria and points out the risk of their spread into the environment.*

Keywords: *wastewater; antibiotic resistance; E. coli*

Úvod

Beta-laktamová antibiotika patří mezi významná léčiva v boji proti bakteriálním infekcím. Rezistence bakterií k těmto látkám je často zprostředkována plazmidově nesenými geny pro produkci enzymů širokospektrých beta-laktamáz (ESBL). Infekce vyvolané bakteriemi produkující ESBL představují jeden z majoritních problémů veřejného zdraví, jelikož jsou spojeny s vyšší nemocností a úmrtností pacientů.[1] V posledních letech je výskyt rezistentních bakterií v životním prostředí považován za velmi znepokojivý.[2] Čistírny odpadních vod (ČOV) představují místo, kde se do kontaktu dostává odpad z různých zdrojů (např. lidské a zvířecí exkrementy, průmyslové odpady), a zároveň poskytují vhodný prostor pro horizontální přenos genů mezi bakteriemi různého původu.[3] Četné studie dokumentují zvyšující se podíl rezistentních bakterií jak v nepřečištěné odpadní vodě, tak na odtoku z ČOV. Nedostatečné ošetření odpadních vod umožňuje šíření rezistentních bakterií do životního prostředí.[1,3]

Materiál a metody

V říjnu roku 2016 bylo odebráno 5 různých vzorků odpadních vod ze dvou brněnských lokalit. V městské čistírně odpadních vod (ČOV) Brno Modřice se jednalo o surovou odpadní vodu na přítoku do ČOV a o přečištěnou vodu, která se následně vypouští do řeky Svratky. Ve Fakultní nemocnici Brno Bohunice byla odebrána voda na přítoku a odtoku nemocniční ČOV (odpadní voda zejména z infekčních klinik) a odpadní voda z nemocnice vedená kanalizací do ČOV Brno Modřice (tzv. odpadní voda splašková).

Sterilně odebrané vzorky odpadních vod (1,5 l) byly uchovávány v chladu a zpracovány v den odběru. Pro selekci izolátů *E. coli* rezistentní k cefalosporinům byl použit *Brilliance™ E. coli/coliform Selective Agar (Oxoid, UK) s přídavkem cefotaximu* (2 mg/l; Sigma-Aldrich, USA). Z každého vzorku s pozitivním nárůstem bakterií bylo vybráno 40 presumptivních kolonií *E. coli* k následné charakterizaci. Získané izoláty byly druhově identifikovány pomocí

MALDI-TOF hmotnostní spektrofotometrie (MALDI biotyper; Bruker Daltonics, USA). Produkce širokospektrých beta-laktamáz (ESBL) a AmpC beta-laktamáz u izolátů *E. coli* byla určena Mastdiscs™ ID D68C AmpC a ESBL testem (Mast Diagnostics, Velká Británie). Sporné výsledky byly ověřeny double-disk synergy testem. U kmenů s pozitivním ESBL fenotypem byla testována přítomnost genů bla_{CTX-M}, bla_{OXA}, bla_{TEM} a bla_{SHV} kódující klinicky významné beta-laktamázy metodou polymerázové řetězové reakce (PCR).[4] U izolátů s AmpC fenotypem byla provedena PCR detekce genů skupin bla_{MOX}, bla_{CMY}, bla_{DHA}, bla_{ACC}, bla_{ACT} a bla_{FOX}.[4] Epidemiologická příbuznost izolátů *E. coli* byla určena metodou pulzní gelové elektroforézy za použití enzymu XbaI (Takara, Japonsko). Získané makrorestrikční profily byly vyhodnoceny softwarem BioNumerics (verze 6.6, Applied Maths, Belgie).

Pro stanovení podílu *E. coli* a koliformních bakterií rezistentních k cefotaximu vůči celkovému počtu všech izolátu těchto taxonomických skupin byl každý vzorek vody kultivován na výše zmíněném chromogenním médium s přídavkem antibiotika a bez něj.

Výsledky

Izoláty *E. coli* rezistentní k beta-laktamovým antibiotikům byly získány ze všech typů vod kromě přečištěné odpadní vody z ČOV FN Brno (Tabulka 1). ESBL produkce byla zjištěna celkem u 146 (97,3 %; n=150) izolátů *E. coli*, 5 izolátů vykazovalo produkci AmpC beta-laktamáz (3,3 %), u jednoho z těchto kmenů byla zjištěna přítomnost ESBL i AmpC fenotypu (0,7 %). Nejčastěji detekovaným genem u izolátů *E. coli* s produkcí ESBL byl bla_{CTX-M} (99,3 %; n=146). Podrobný rozpis prokázaných fenotypů rezistence a genů rezistence k beta-laktamům v závislosti na typu odpadní vody je uveden v Tabulce 1.

Tabulka 1: Souhrn fenotypu a zastoupení testovaných genů rezistence k beta-laktamovým antibiotikům u izolátů *E. coli* z odpadních vod izolovaných na půdě s cefotaximem.

počty *E. coli* / vzorek	celkem	ESBL	AmpC	ESBL+ AmpC	bla_{CTX-M}	bla_{OXA}	bla_{TEM}	bla_{SHV}	bla_{CMY}	bla_{DHA}
ČOV Modřice přítok	38	37	1	0	37	2	14	0	1	1
ČOV Modřice odtok	34	31	2	1	32	6	9	0	2	0
ČOV FN Brno přítok	39	38	1	0	37	36	26	0	0	0
ČOV FN Brno odtok	0	0	0	0	0	0	0	0	0	0
FN Brno splašková	39	39	0	0	39	27	27	0	0	1

Makrorestrikční profily kmenů vykazovaly podobnost v rozmezí 53 – 100 %. Identický izolát byl nalezen v nemocniční splaškové vodě a v odpadní vodě přitékající na ČOV Modřice. Shodné izoláty byly detekovány i ve vzorcích odpadních vod na přítoku a odtoku ČOV Modřice.

Na odtoku z ČOV Brno byl zjištěn vyšší procentuální podíl cefotaxim-rezistentních *E. coli* a koliformních bakterií vztažený k jejich celkovému počtu než na přítoku do čistírny (viz tabulka 2).

Tabulka 2: Podíl počtu [%] *E. coli* a koliformních bakterií rezistentních k cefotaximu vůči jejich celkovému počtu z odpadních vod.

Vzorek	*E. coli* [%]*	*koliformní bakterie bez E. coli* [%]**
ČOV Modřice přítok	0,25	0,08
ČOV Modřice odtok	0,87	0,30
ČOV FN Bohunice přítok	4,83	16,7
ČOV FN Bohunice odtok	-	-
FN Bohunice splašková	0,67	0,34

*Procentuální zastoupení cefotaxim rezistentních *E. coli* k jejich celkovému počtu získanému na médiu bez antibiotika
**Procentuální zastoupení cefotaxim rezistentních koliformních bakterií k jejich celkovému počtu počtu získanému na médiu bez antibiotika

Diskuse

Zvyšující se prevalence ESBL produkujících bakterií je dokumentována celosvětově.[2] Zastoupení *E. coli* produkující ESBL na odtoku z ČOV Brno činilo 94,1 % (32; n=34), podobný výsledek byl dokumentován ve španělské studii.[5] Z celkového počtu 185 enterobakterií rezistentních k cefalosporinům vykazovalo ESBL produkci 163 izolátů (88,1 %). Vysoký výskyt enterobakterií s ESBL v odpadních vodách a ve vodním prostředí naznačuje dopad lidských aktivit na šíření těchto rezistentních kmenů ze zemědělské produkce, aglomerace a klinického prostředí.[5] V naší i španělské studii byl $\textit{bla}_{\text{CTX-M}}$ nejčastěji detekovaným genem pro produkci ESBL (125; n=185). Ke stejnému závěru dospěli ve studii zabývající se výskytem *E. coli* s ESBL v městských a nemocničních odpadních vodách ve Francii[6]. Autoři této studie zároveň poukazují na statisticky významné zvýšení podílu *E. coli* produkující ESBL na odtoku z ČOV oproti přítoku. Obdobné výsledky jsme obdrželi i v našem výzkumu z ČOV Brno Modřice. Možným vysvětlením je selekční tlak působící během čistících procesů v ČOV a vhodné prostředí kalů pro předávání rezistence k antibiotikům mezi bakteriemi.[1] Nulový výskyt *E. coli* a koliformních bakterií v přečištěné odpadní nemocniční vodě z FN Brno je spojen se zařazením chlórové dezinfekce v čistícím procesu, která účinně eliminuje výše zmíněné bakterie.

Závěr

V odpadních vodách města Brna bylo prokázáno vysoké zastoupení izolátu *E. coli* rezistentních k cefalosporinům, zejména s produkcí ESBL kódovaných geny $\textit{bla}_{\text{CTX-M}}$. Zároveň studie poukazuje na nedostatek čistírenských procesů používaných v městské čističce odpadních vod pro eliminaci bakterií vedoucí k šíření rezistentních bakterií do prostředí.

Poděkování

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SEKCE 5

Veřejné veterinářství a ochrana zvířat

Neinvazivní monitoring stresu u lemurů kata (*Lemur catta*)
Non-invasive monitoring of stress in ring-tailed lemurs (*Lemur catta*)

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Summary
The ring-tailed lemur (Lemur catta) is listed as an endangered species (according to the IUCN Red List) and is suspected of suffering from many stressors that may be reflected by faecal glucocorticoid hormones. There is a number of analyses that can be used to measure concentrations of steroid hormone metabolites in faeces of the animals. Each of them had to be validated for each species before application, because metabolism and excretion of steroids differ significantly between species, and sometimes even between sexes. The aim of this paper is to inform about some possibilities how to validate the most common method used for non-invasive faecal monitoring, the enzyme immunoassay, namely competitive ELISA.

Keywords: *faeces; glucocorticoids; biological validation; competitive ELISA; welfare*

Úvod
Ověření zvolené metody pro daný druh je pro její použití a získání relevantních výsledků velmi důležitou částí neinvazivního monitoringu stresu u zvířat. Bez vhodné metody nebudou výsledky odpovídající a naměřené koncentrace vylučovaných glukokortikoidních hormonů tak nemusí odpovídat realitě (Schatz and Palme, 2001). Pokud je pro určený druh metoda použita poprvé, je nutné ji validovat, neboť zde jsou velké rozdíly v metabolismu a exkreci steroidních hormonů u jednotlivých druhů zvířata (Touma et al., 2004; Palme, 2005). U každé zvolené metody je testována její přesnost, citlivost a zkřížená reaktivita mezi různými metabolity obsaženými ve vzorcích.

Neinvazivní monitoring stresu u lemurů kata
Koncentrace glukokortikoidních hormonů jsou nejčastěji zjišťovány za použití imunoanalytických metod, konkrétně metodami RIA (radioimmunoassay) a EIA (enzymoimmunoassay). Jak je uvedeno výše, je nutné před použitím jakékoliv z nich nejdříve zjistit jaký typ glukokortikoidního hormonu je u daného druhu do výkalů vylučován.

Pro rozlišení jednotlivých metabolitů glukokortikoidních hormonů ve fécés je doporučeno nejdříve analyzovat vzorky pomocí vysokoúčinné kapalinové chromatografie (HPLC), která separuje jednotlivé metabolity prezentované ve fécés (Palme, 2005). Ty budou následně analyzovány pomocí vhodně zvolené imunoanalytické metody.

Součástí ověřování metody by měla být i tzv. farmakologická validace, která by měla demonstrovat, zda je vybraná metoda analýzy schopna rozpoznat změny v koncentracích fekálních glukokortikoidních metabolitů ve srovnání se změnami v hladinách těchto hormonů v krvi (Palme, 2005). Nejčastější a nejužívanější je ovlivnění aktivity adrenokortexu syntetickou formou adrenokortikotropního hormonu (ACTH) ve specialitě Synacthen, což bylo použito pro nespočet studií u různých druhů zvířat (Touma and Palme, 2005).

Někdy je nemožné provést farmakologickou validaci, speciálně pak u zvířat ohrožených. V tomto případě je možné zvolit tzv. biologickou formu validace jako alternativu, při které silný stresový impulz dokáže ovlivnit aktivitu adrenokortexu a zapříčinit tak zvýšení hladin glukokortikoidních hormonů (Touma and Palme, 2005). Například jak uvádí Rangel-Negrín

et al. (2014), je možné za silný stresový impulz považovat odchyt a transport zvířat. Studie Anfossi et al. (2014) zase hovoří o odchytu a imobilizaci zvířete jako o vhodném stresoru k biologické validaci metody.

U lemurů kata bylo prozatím provedeno málo studií zabývajících se neinvazivním monitoringem stresu, tedy analýzou vzorků výkalů pro zjištění koncentrací metabolitů glukokortikoidních hormonů. Doposud provedené hovoří převážně o sledování volně žijících skupin lemurů kata a o vlivu sezónních a vnitroskupinových stresorů. Například Cavigelli (1998) sleduje vliv střídání období sucha s obdobím dešťů, dostupnost potravy, dominanci ve skupině a pozdní gestaci na výši stresové odpovědi u skupiny samic lemurů kata. Vzorky fécés, které byly odebírány, byly analyzovány metodou RIA (radioimmunoassay) a srovnány s hladinami kortizolu v krevním séru. Ve studii Pride (2005) je pozorován vliv velikosti skupiny na hladinu kortizolu a metabolitů glukokortikoidních hormonů ve vzorcích výkalů odebíraných od obou pohlaví. Vzorky byly znovu analyzovány metodou RIA. V jiné studii byl pozorován vliv období, vnitroskupinových aktivit a reprodukční aktivity na hladinu stresových markerů. Byly srovnávány jejich koncentrace mezi vzorky výkalů a krevního séra u obou pohlaví. Vzorky byly analyzovány znovu metodou RIA (Starling et al., 2009). Gould et al. (2005) ve své studii uvádí, že mohou být hladiny glukokortikoidních hormonů u samců lemurů kata ovlivněny obdobím, stabilitou skupiny a interakcemi mezi jedinci při udržování postavení ve skupině. Vzorky fécés odebírané od volně žijících samců byly analyzovány metodou EIA.

Doposud žádná studie nevalidovala metodu ELISA pro stanovení hladin glukokortikoidních hormonů ve vzorcích výkalů u druhu lemur kata (*Lemur catta*). Na Ústavu ochrany zvířat, welfare a etologie (VFU Brno) právě probíhá výzkum v této oblasti, kdy byly od obou pohlaví druhu lemur kata odebírány vzorky fécés po působení stresorů jako jsou odchyt, manipulace a imobilizace, což je dle dosavadních zkušeností považováno za dostatečně silný impulz k vyvolání stresové reakce a zvýšení hladin glukokortikoidních hormonů ve výkalech pozorovaného druhu. Lze tedy tuto studii považovat za biologickou validaci metody. Vzorky byly analyzovány metodou ELISA, pro rozlišení dominantně vylučovaných metabolitů glukokortikoidních hormonů (fCMs) bylo použito šesti různých typů protilátek zacílených na kortizol, kortikosteron a metabolity 5α-pregnane-3β,11β,21-triol-20-one; 11β-hydroxyaetiocholanolone; 11-oxoaetiocholanolone-17-CMO:BSA a 11-oxoaetiocholanolone-3-HS:BSA. Dle dosavadních výsledků je dominantně vylučovaným fekálním metabolitem u lemurů kata 11β-hydroxyaetiocholanolone.

Závěr

Studie zabývající se neinvazivním monitoringem stresu je nadále nutné doplňovat. Do budoucna by bylo vhodné porovnat doposud získané výsledky za použití biologického stresoru s farmakologickou validací, tedy ovlivnění hladin glukokortikoidních hormonů pomocí aplikace ACTH minimálně čtyřem jedincům, dvěma samcům a dvěma samicím.

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Vliv přepravní vzdálenosti na úhyny krůt přepravovaných na jatky
Impact of travel distance on mortality of turkeys transported for slaughter

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Summary
In the period from 2009 to 2014, overall mortality of turkeys transported for slaughter in the Czech Republic was 0.15%. The transport-related mortality differed for different travel distances. The lowest mortality rate was found in turkeys transported for distances up to 50 km (0.02%). The transport-related mortality increased with longer distances traveled. The highest losses were recorded for distances from 201 to 300 km (0.543%).

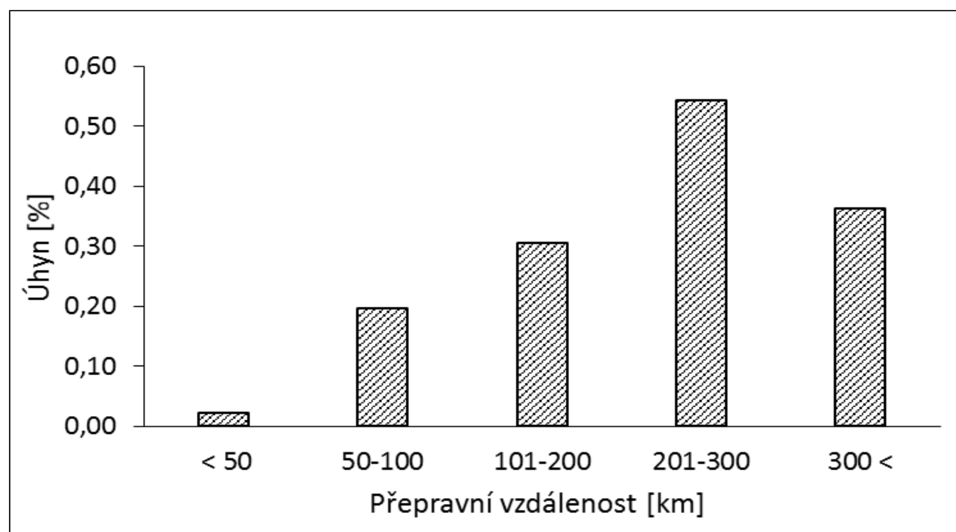
Keywords: *poultry; distance; transport; mortality; death on arrival*

Úvod
Při přepravě zvířat na jatky je nutné dodržovat podmínky pro zachování pohody zvířat. V případě zhoršených podmínek při transportu může docházet v různém rozsahu k působení stresových faktorů, které mohou negativně ovlivnit jedince (Marchewka et al., 2013). Jedním z hlavních ukazatelů úrovně welfare krůt při přepravě je výsledný počet jedinců uhynulých při přepravě nebo v souvislosti s přepravou. Tento údaj zároveň i prezentuje druhý pohled, a to ekonomickou ztrátu, způsobenou úhynem jedinců. Úhyny krůt při přepravě kolísají vlivem rozdílných přepravních podmínek, např. rozdílná přepravní vzdálenost, délka trvání přepravy, teplota okolního prostředí, mikroklima na vozidle, styl jízdy řidiče, vibrace vozidla, hluk, narušení skupin krůt a hladovění (Mitchell and Kettlewell, 1998; Nijdam et al., 2004; Bianchi et al., 2005; Voslářová et al., 2006). Cílem této studie bylo posoudit vliv přepravní vzdálenosti na úhyny krůt v souvislosti s jejich přepravou na jatky.

Materiál a metodika
V období let 2009 - 2014 byl sledován počet krůt přepravených na jatky v České republice a počet krůt uhynulých v souvislosti s touto přepravou pro různé přepravní vzdálenosti. Jednotlivé přepravy byly pro tyto účely rozděleny do kategorií dle přepravní vzdálenosti následovně: do 50 km, 50 – 100 km, 101 – 200 km, 201 – 300 km a více jak 300 km. Výsledky byly statisticky vyhodnoceny pomocí programu Unistat v. 6.5. (Unistat Ltd., GB). Porovnání četnosti úhynů krůt pro jednotlivé intervaly přepravních vzdáleností za celé sledované období 2009-2014 bylo provedeno pomocí 2x2 kontingenční tabulky a χ^2 testu (Zar, 1999).

Výsledky a diskuze
Celková úroveň úhynů krůt přepravovaných na jatky v období od roku 2009 do roku 2014 byla 0,15 %. Graf 1 znázorňuje procento úhynů ve sledovaných intervalech přepravní vzdálenosti. Nejnižší úhyny byly zjištěny při přepravě krůt do 50 km (0,02 %). S rostoucí vzdáleností se zvyšovala úroveň úhynů až na 0,54 % při přepravách krůt na vzdálenost 201 - 300 km. Pokles procenta úhynů krůt jsme zaznamenali při vzdálenost větší než 300 km, ale tento pokles nebyl statisticky významný. K podobným závěrům dospěli v dřívějších studiích také Voslářová et al. (2006, 2007), kteří potvrzují zvyšující se trend mortality krůt s prodlužující se přepravní vzdáleností. Pokles trendu v úhynů krůt při přepravě na vzdálenost více jak 300 km si autoři vysvětlují zajištěním lepších podmínek welfare ptáků vzhledem k očekávané dlouhé přepravě a snížení množství přepravovaných ptáků na tyto vzdálenosti. Obdobně Nijdam et al. (2004) a Večerek et al. (2006) ve své studii u brojlerů zjistili nárůst

mortality při prodlužující se přepravní vzdálenosti. Bianchi et al. (2005) dokázali nižší mortalitu u ptáků přepravovaných v čase do 3,5h v porovnání s přepravou 3,5 – 5h a v intervalu více jak 5h, které se od sebe významné neliší. S prodlužující se dobou přepravy prokázali také zvýšený úbytek hmotnosti jedinců a větší výskyt poranění.



Graf 1: Úhyny krůt při přepravě v závislosti na přepravní vzdálenosti.

Závěr

Welfare krůt při přepravě negativně ovlivňuje prodlužující se přepravní vzdálenost, při které dochází mimo jiné ke zvýšení mortality krůt. Z výsledků vyplývá, že snížení přepravní vzdálenosti a zkrácení nutné doby expozice krůt přepravnímu stresu by mohlo vést k výraznému snížení mortality krůt při přepravě.

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Effects of the travel distance on transport-related mortality in calves

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Summary
The transport-related mortality, which comprises the number of animals that die during transport to a slaughterhouse or shortly after being delivered to a slaughterhouse, is a crucial indicator of animal welfare during transport. The aim of this study was to determine the mortality rate in calves resulting from transport to slaughter in the Czech Republic in the period from 2009 to 2014, and to investigate the effect of travel distance. The total mortality rate of transported calves amounted to 0.296% during the monitored period. The travel distance was proved to have a significant impact on the mortality rate among transported calves. The highest mortality rate (0.480%) was recorded for transport distances of 51–100 km, contrarily, the lowest mortality rate (0.000%) was observed for transport distances over 200 km.

***Keywords**: cattle; death rate; conveyance; journey distance*

Introduction

Shortcomings in the welfare of transported cattle may lead to the increased morbidity and mortality in animals weakened by transport stress, to damage to carcasses with adverse effects on meat quality. It is, therefore, extremely important to ensure the consistent observation of the requirements of animal well-being set out in the valid legislation and to reduce the stress load on transported animals as much as possible.

There is a large number of factors that may have a considerable influence on the welfare of transported animals, such as the age and state of health of the animals, the length of transport, intervals for drinking, feeding and rest, the treatment of the animals during their loading and unloading, the density of animals in the vehicle, the spacing of animals on the bed of the vehicle, the construction and equipping of the vehicle, the way in which the vehicle is driven, the microclimate in the vehicle, etc. The travel distance belongs among the most important of these factors, since the longer the transportation, the longer the action of all present factors capable to affect adversely the welfare of transported animals. Long distance animal transport is more likely to compromise animal welfare and meat quality than shorter journeys (Perez et al. 2002). When monitoring the long-haul transport of cattle in North America, Gonzales et al. (2012) found that calves and culled cattle were more likely to die during the journey compared to feeders and fat cattle. The effect of travel distance was also found in the mortality of calves transported for slaughter in the Czech Republic from 1997-2004, the recorded mortality rate for distances up to 200 km was markedly higher in comparison with distances up to 50 km (Vecerek et al. 2006). What's more, the sensitivity of cattle to travel distance (and generally to transport conditions) differs both in dependence on the category of cattle transported and in dependence on the cattle breed and production type. It is, for this reason, important to consider and define specific circumstances under which transportation is performed when studying effects of travel distance on the welfare of transported animals.

Materials and Methods

The numbers of calves transported to slaughterhouses in the Czech Republic and the numbers of calves that died in connection with this transport were recorded by veterinary inspectors in the years 2009 to 2014. The data comprising figures obtained on all cattle transports conducted in the Czech Republic in the given years were collected in the database of the State Veterinary Administration Information Centre. The data obtained were subsequently exported

into the program Excel for statistical processing. Differences in the mortality rate between individual travel distances (up to 50 km, 51-100 km, 101-200 km, and over 200 km) were calculated from the results obtained for each year and for the entire period studied. The effect of travel distance on the mortality of calves transported to the slaughterhouse was determined on the basis of calculation of the total number of transported calves and the total number of calves that died during a certain travel distance over the whole period studied and calculation of this mortality rate in percent. All the data was analysed with the use of the statistical program Unistat 6.5 (Unistat Ltd., GB). Statistical comparison of mortality rates was performed with the use of a Chi-square test and contingency tables. Fisher's exact test was used if the frequency of the studied characteristic was lower than 5. The significance level was 0.05.

Results and Discussion

In the years 2009 to 2014, we monitored a total of 64 752 calves that were transported to various slaughterhouses in the Czech Republic, of which 192 died. The total mortality rate among the calves associated with transport was 0.297%.

The effect of the travel distance on the mortality of calves transported is given in Table 1. It follows from Table 1 that the vast majority of calves (90 %) were transported for distances not exceeding 100 km. The differences in the mortality of calves depending on the travel distance are shown in Fig. 1. Transported calves showed the highest mortality rate for transport distances of 51 – 100 km (0.480%), followed by transport distances of 101-200 km (0.332%). Surprisingly, the lowest mortality rate among transported calves was recorded for the longest distances (> 200 km), calves exhibited no mortality at all (0.000%). Significant differences were found between distances up to 50 km and 51-100 km (P = 0.000), and also between 101-200 km and over 200 km (P = 0.033).

Table 1: Numbers of calves transported to slaughterhouses and the numbers of calves dying as a result of transport for different distances in the period from 2009 to 2014.

	Travel Distance	<50 km	51-100 km	101-200 km	>200 km
Calves		Number of animals			
	transported	39 968	18 319	5 117	1 348
	died	87	88	17	0

Contrary to a number of previous studies (e.g. Vecerek et al. 2006), we found higher mortality among calves transported over shorter distances compared to longer ones. At the present time, transport conditions seem to be appropriately adapted to the demands of long-haul transport. Provided conditions are good, most healthy and fit farm animals could be exposed to long transport durations without their welfare necessarily being compromised. Generally, it is not transport duration *per se* but associated negative aspects which give rise to particular welfare issues (Miranda-de la Lama 2013). In our study, we recorded the lowest mortality rate among transported calves for the longest transport distances (> 200 km). The reasons could be the increased legislative requirements placed on the long-haul transportation of livestock, which force hauliers to pay increased attention and provide more intensive care to animals transported for longer distances, particularly to those that are supposed to be the most vulnerable, i.e. calves. Furthermore, only animals in better condition are chosen for long transportation during pre-transport selection. The reason for higher death rates over shorter transport may be the fact that breeders as well as hauliers underestimate the importance of ensuring adequate conditions.

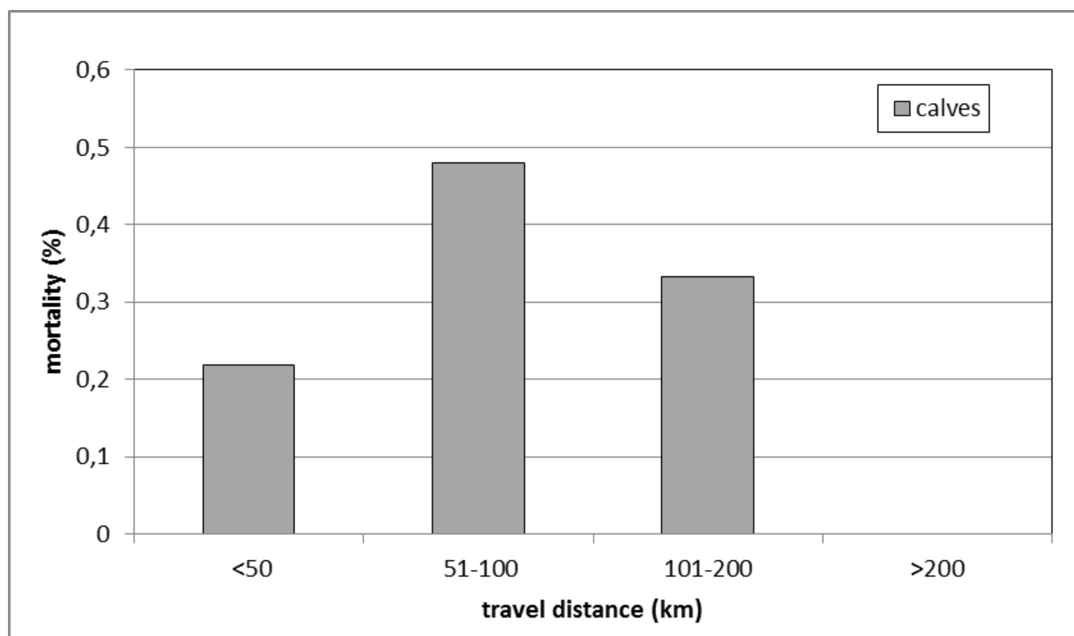

Figure 1: Transport-related mortality of calves in relation to transport distance in the period from 2009 to 2014.

Conclusion

Our results differ from the conclusions of previous studies in terms of the correlation between the mortality rate in transported calves and the travel distance. Our study does not confirm the frequent conclusions that long distance transport compromises the welfare of transported animals: the calves mortality decreased with increasing travel distance. Mortality in calves during long-haul transport can be reduced still further if all the demands of animal welfare during transport are consistently observed. In order to prevent animal mortality during transport, it is necessary to take account of all factors, which can affect animal welfare, and to perform continual checks on the condition of the animals to ensure they are capable of handling transport stress successfully.

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Microchip status and gender of sheltered dogs as factors affecting their length of stay

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Summary
This study is focused on microchip status and gender of sheltered lost and abandoned dogs as factors affecting their length of stay in one municipal Czech shelter, in a city where mandatory microchipping has been introduced. Lost female as well as male dogs with microchip remained significantly shorter (median 0 days) in shelter than lost females or males without microchip (median 1 day). This study didn't recognize neutered individuals. Abandoned males with microchip remained significantly longer (18 days) in the shelter than males without microchip (12 days); abandoned female dogs with microchips had significantly longer (18 days) length of stay than those without microchip (9 days). There was no significant difference in the length of stay of abandoned or lost males and females. Microchipping of dogs is related to faster reunification process, but could hinder adoption process. Better understanding of factors that positively affects reunification or adoption process in sheltered dogs can significantly shorten their length of stay in Czech shelters and help to improve adoption process itself, last but not least improve sheltered dogs welfare.

Keywords: *traceability; sex; identification; stray; dog*

Introduction

Gender of sheltered dogs has been proven to be a significant factor affecting their length of stay. It has been proven that abandoned female dogs have shorter length of stay than males (Patronek et al., 1995; Diesel et al., 2007, Zak et al., 2015). Other factors that may significantly influence the length of stay of sheltered dogs include microchipping of dogs (Fajtó et al., 2015, Zak et al., 2017). Lost pets identified with microchips are more likely to be returned to their original owner than pets without a microchip (Dingman et al., 2014; Fajtó et al., 2015; Lancaster et al., 2015). Better traceability to the original owners means faster reunification of the dog and its original owners and decrease in the numbers of unreclaimed dogs (Lord et al., 2009, Fajtó et al., 2015). However, there may be some problems with the registration of microchips which can lead to dogs being untraceable to their owners, despite dog having a microchip (Lord et al., 2009; Lancaster et al., 2015).

Materials and Methods

Records on a total of 2283 sheltered dogs in a selected municipal shelter within a period of 5 years (from 1 January 2010 to 31 December 2014) were analysed. The shelter is situated in the city, which has employed mandatory microchipping before the studied period (2009). The gender and microchip status of each sheltered dog was checked and recorded by authorized shelter personal only. For the purposes of this study, dogs were divided into two groups: lost (reclaimed) and abandoned (unreclaimed). Furthermore, sheltered dogs were divided into two other groups according to their gender (males / females) and microchip status (microchipped / non-microchipped). Dogs that died or were euthanized during the stay were excluded from the study. The results were analysed using the statistical package Unistat 5.6. (Unistat Ltd., London, UK). Two independent variables were constructed from the shelter´s original information: gender (2 levels: male, female); microchip status (2 levels: microchipped, non-microchipped); the effects of these independent variables on their length of stay (LOS) as a dependent variables was analysed. The LOS of dogs was defined as the number of days from the intake date to the date when the dog was either adopted or returned to its original owner.

The effects of gender and microchip status were analyzed using a Kruskal-Wallis ANOVA and subsequently by a non-parametric multisample median test (Zar 1999) as a post hoc test for pairwise comparisons. We also calculated actual and relative frequencies of lost and abandoned dogs in selected categories according to gender and microchip status and analysed differences among these categories. Frequencies were compared on the basis of a chi-square analysis of contingency tables 2x2 (Zar 1999). A *P*-value < 0.05 was considered as significant.

Results

Of total 2283 dogs sheltered, 979 (42.9%) were abandoned and 1304 (57.1%) were lost dogs. There were total of 1493 (75.4%) male dogs sheltered, with significantly (p < 0.001) more non-microchipped males (52.5%, 784) than microchipped males (47.5%, 709); the rest (34.6%, 790) were female dogs, with significantly (p < 0.001) more non-microchipped females (63.2%, 499) than those microchipped (291, 36.8%). Significantly (p > 0.05) more sheltered males (both lost and abandoned) were microchipped than females. Out of all 1304 lost dogs sheltered, there were significantly (p < 0.05) more microchipped lost males (63.5%, median LOS 0 days) compared to non-microchipped lost males (36.5%, median 1 day) as well as microchipped lost females (56.7%, median 0 days) compared to non-microchipped females (43.3%, median 1 day). There was a significant difference in LOS comparing microchipped lost females to non-microchipped females (p < 0.001), as well as between microchipped males and non-microchipped males (p < 0.001). However, there was no significant difference (p > 0.05) when comparing LOS of lost microchipped males to microchipped females, nor between lost non-microchipped males and non-microchipped females. Out of all 979 abandoned dogs sheltered, there were significantly (p < 0.05) more non-microchipped abandoned males (76.6%, median 12 days) compared to microchipped abandoned males (23.4%, median 18 days), as well as non-microchipped abandoned females (84.1%, median 9 days) compared to microchipped females (15.9%, median 18 days). There was a significant difference in LOS when comparing non-microchipped abandoned females to microchipped females (p < 0.001), and comparing non-microchipped males to microchipped males (p < 0.05). There was a significant difference when comparing LOS of abandoned non-microchipped males to non-microchipped females (p < 0.05), however, there was no significant difference comparing LOS of microchipped males to microchipped females (p > 0.05).

Discussion

This study has shown that microchipped lost dogs (both males and females) stayed in the shelter significantly shorter than those without microchip and proves that such dogs are more likely to be returned to their original owner than dogs without a microchip (Dingman et al., 2014; Fajtó et al., 2015; Lancaster et al., 2015, Zak et al., 2017). As shown in this study, microchipped abandoned females have significantly shorter length of stay than microchipped males. This finding that females are more likely to be adopted can be supported by other studies (Patronek et al., 1995; Diesel et al., 2007, Zak et al., 2015). However, it is also possible that shorter LOS in females can be more or less a result of their lower offer in the shelters. Interestingly, there was no significant difference in the LOS of non-microchipped abandoned males and females, and these dogs stayed in the shelter the same median LOS. This study proves that for some reason microchipped abandoned dogs may not be as preferable among adopters as the ones non-microchipped, since microchipped abandoned females have stayed in the shelter 9 days (males 6 days) longer than the ones without a microchip. This finding suggests that shelter workers might wait longer for original owners of microchipped dogs to be found and reunited with the dog, before they put the dog for

adoption. The fact that these dogs are microchipped can also lead shelter staff to spend more time searching for the owner in various databases or wait until registry operators send them information, whether the original owner was found in the registry or not, compared to dogs that have no microchip and not have to be searched for in microchip registries. Microchipped dogs that stayed in the shelter as unreclaimed could represent abandoned dogs that could have been registered but owner decided to get rid of the dog or couldn't find the dog in the shelter, or shelter was not able to contact the owner, maybe for the reason that dog was not registered at all or the data in the microchip registry were incorrect or not up to date (Lord et al., 2009).

Conclusion

Regardless of the gender, microchipped lost dogs are more likely to be returned to their original owner than dogs without a microchip. No difference in length of stay was found between microchipped lost males and females, as well as between microchipped abandoned males and females, however non-microchipped abandoned females stay in the shelter shorter time than non-microchipped males. Better understanding of factors that positively affects reunification or adoption process in sheltered dogs can significantly shorten their LOS in Czech shelters and help to improve adoption process itself, last but not least improve sheltered dogs welfare.

Acknowledgement

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Comparison of successfully adopted dogs and dogs returned to the shelter

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Summary
Successful rehoming of the shelter dogs is a difficult process and it is unfortunately common that some of them are returned. Moreover, some of them are given back to the shelter several times. There are only a few studies analysing adoptions resulting in the rejection of the newly adopted dog. Information on dogs in three shelters located in Moravia were collected and each dog was individually assessed in terms of its health, behaviour and general appearance. Out of 146 observed dogs, 72 dogs were adopted. Among these, 23 dogs were returned to the shelter. Comparison of a group of successfully adopted dogs and dogs returned to the shelter revealed that dogs successfully adopted were younger ($P < 0.05$) and in better condition ($P < 0.01$) prior to their adoption than dogs rejected by their adopters. No difference ($P > 0.05$) was found in sex ratio and the size of dogs between the two groups.

Keywords: *returned dogs; behaviour problems*

Introduction

Adoption of dogs from shelters is a complex phenomenon including many aspects, e. g. breed type, size, colour of coat, mode of intake, length of stay, health and behaviour of dog. The perception of new owners, their skills, knowledge and responsibility seem to be very important.

In spite of the effort of the shelter staff to find a match to dogs appropriate new owners, some of the adoptions fail.

The European studies analysing adoption success present different accounts of rejected dogs. Diesel et al. (2005) found 14%, Mondelli et al. (2004) found from 13% (females) to 17% (males), Bailey et al. (1997) 12.7% of the owners. These authors present problems with behaviour, health of the owner and moving as the main reasons for giving dog back to the shelter.

The aim of our study was to assess the rejection rate in dogs kept in Czech shelters and to compare characteristics of successfully adopted dogs and dogs returned to the shelter.

Materials and Methods

For the purposes of our study, all dogs kept in the selected shelters in the monitored period (146 dogs in total) were regularly observed on individual level. The three dog shelters selected for the study were located in Moravia. The data about each animal collected from shelter records consisted of gender, age and size. Furthermore, general appearance, body condition, behaviour, movement, eye, skin and fur condition, breathing and digestion were assessed individually by the observer. A scoring system (1 to 5) was used to evaluate selected characteristics of each dog. Number one meant the best, number five meant the worst grade. The individual observation of dogs was performed twice a month from November 2016 to February 2017. Normality of data was checked using the Kolmogorov - Smirnov test (Zar 1999). As data were not distributed normally, the non-parametric Mann – Whitney test was used for testing the differences between adopted and returned dogs.

Results and Discussion

Out of 146 observed dogs, 72 dogs were adopted. Among these, 23 dogs were returned to the shelter during the monitored period. Out of returned dogs, one dog was returned five-times,

another dog four-times and three of them twice. Successful adoption rate was 49.32% and the rate of rejected dogs was 15.75%. The number of returned dogs in our study corresponds with the results of Diesel et al. (2005), Mondelli et al. (2004) and Bailey et al. (1997). Their results showed no statistical differences in the size, breed, sex and health scores of returned and successfully adopted dogs. However, in our study we found statistically significant difference ($P < 0.05$) between age of returned and successfully adopted dogs. Our results suggest that older dogs are more likely to be returned to the shelter. A highly significant difference ($P < 0.01$) was found also in condition of returned and successfully adopted dogs. In general, dogs in better condition were more likely to be successfully adopted. Nevertheless, there were also geriatric dogs or dogs with health problems successfully adopted. For example, the eleven years old female with inoperable cancer of jawbone, sixteen years old Rottweiller female with serious health history or eight years old Labrador which stayed nearly six years in the shelter. As far as the reasons of rejection of the dogs go, the available shelter records specify in most cases aggression followed by disharmony in new homes, health problems of the owners, destructive behaviour, personal problems, separation anxiety and movement. All these statements correspond with results of Wells and Hepper (2000), Mondelli et al. (2004), Diesel et al. (2005).

Marston and Bennet (2003) recommend researching residential circumstances, lifestyle, family composition, previous pet ownership, education and income levels and attempt to formulate a psychological profile of adopters and their expectations. The rejection rate can be decreased by pre-adoption counselling to adjust adopter expectations. Educational efforts would be appropriate, especially information on the basic biology of dogs as well as knowledge that many undesirable behaviour can be modified.

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SEKCE 6

Veterinární toxikologie a toxikologie potravin

The effect of selected tricyclic antidepressants on fish early-life stages

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Summary
Pharmaceuticals are chemicals designed to have a specific mode of action even at low concentrations. Therefore, their presence in water bodies and the effect on non-target animals has been under great discussion, as aquatic organisms are particularly important targets, which are exposed via wastewater residues during their whole life. The aim of this study was to assess the effect of tricyclic antidepressants on early-life stages of common carp (Cyprinus carpio). The embryo-larval toxicity test with amitriptyline, nortriptyline and clomipramine and their mixture at concentrations of 10; 100 and 500 µg/l was started immediately after fertilization according to the OECD guideline 210 and took 30 days. Higher mortality, changes in development as well as antioxidant and biotransformation enzymes activity were observed. Results of this study confirm the idea, that pharmaceutical residues can have considerable effects on aquatic vertebrate.

Keywords: *Cyprinus carpio; amitriptyline; nortriptyline; clomipramine; oxidative stress*

Introduction

Similarly to other groups of pharmaceuticals, psychiatric drugs are not completely metabolized by the human body and are excreted as unchanged parent compound or as metabolites (Heberer, 2002). Consequently, pharmaceuticals enter the environment through the waste water treatments plants effluents. Since are pharmaceuticals designed to have a specific mode of action even at low concentrations, their presence in water bodies and the effect on non-target animals has been under great discussion, because aquatic organisms are particularly important targets, which are exposed via wastewater residues over their whole life (Fent et al., 2006). The aim of this study was to examine the effect of long-term exposure of tricyclic antidepressants amitriptyline, nortriptyline and clomipramine on early- life stages of common carp (*Cyprinus carpio*) and to assess their mixture toxicity, as chemical mixtures occurring in surface bodies can be more toxic than single substances because of chemical interactios (Celander, 2011).

Material and Methods

An embryo-larval toxicity test with tricyclic antidepressants amitriptyline hydrochloride (A), nortriptyline hydrochloride (N) and clomipramine hydrochloride (C) (Sigma Aldrich, Czech Republic; chemical purity ≥ 98%) and their mixture (ANC) was performed on common carp (C*yprinus carpio*) according to OECD guideline 210 (Fish, Early-Life Stage Toxicity Test – OECD, 1992). Tested concentrations were 10 µg/l (low-L), 100 µg/l (medium-M) and 500 µg/l (high-H) of tested substances. The control group was exposed to the dilution water. Hatching and mortality were checked twice a day. Larvae were fed *ad* libitum twice a day with freshly hatched *Artemia salina* from day 5 post fertilization (pf). Embryos, larvae and juveniles were sampled on days 5, 12, 19, 26 and 30 pf (completion of the test). Samples were taken in 4% formalin in order to record developmental stages, morphometric and condition characteristics and morphological anomalies. Furthermore, ten samples were taken from A_L,

N_L, C_L and ANC_L so as to evaluate antioxidant and biotransformation enzymes activity and lipid peroxidation using the TBARS assay (Luschak et al., 2005).

Results and Discussion

Considerable increase in mortality among groups was observed during hatching and start of external feeding.

The phenomena of development retardation appeared among the exposed groups since day 5 post fertilization (dpf), even though hatching speed was stimulated ($p<0.01$) after exposure to all tested compounds compared to the control. Morphometric and condition characteristics were also significantly influenced after the long-term exposure to tested compounds and their mixture. These results are in accordance with the study of Yang et al. (2014), who studied the effect os subletal concentration of amitriptyline (from 1 ng/l to 1 mg/l) in zebrafish (*Danio rerio)* embryos upto 120 hours post fertilization. Moreover, the swimming was affected from day 4 pf, where fish from groups A_H and ANC_H were not swimming, but just floating apathetically and then they sank to the bottom of the vessel (Figure 1). This behaviour was later observed also in A_M, N_H, C_L, C_M, C_H, and in all ANC groups.

Figure 1: Common carp (*C.carpio*) larvae exposed to A_H (right) compared to A_L (left).

Subchronic exposure of tested compounds to *C. carpio* early-life stages caused also changes in antioxidant and biotransformation enzymes activity and elevated lipid peroxidation (see Table 1).

Table 1: Activity of GPx (nmol NADPH/min/ mg protein), GR (nmol NADPH/min/mg protein) and GST (nmol/min/mg protein) in common carp (mean±SEM) and TBARS values (nmol/g) (median) after exposure to tricyclic antidepressants.

	Control	AL	NL	CL	ANCL
GPx	46.08±3.22	53.25±2.00	40.65±2.48	43.65±3.26	32.70±1.63*
GR	6.08±0.37	7.08±0.21*	7.47±0.18**	6.63±0.24	7.15±0.35*
GST	100.16±4.71	105.85±3.63	88.47±3.75	83.84±7.76	87.19±4.32
TBARS	7.6	11.98*	11.06*	8.68*	8.44

* Significant differences between tested groups and the control are indicated by $p<0.05$.
** Significant differences between tested groups and the control are indicated by $p<0.01$.

Conclusion

Based on the results of this study, tricyclic antidepressants have a significant effect on fish early-life stages, as both development and oxidative stress indices were affected by the subchronic exposure.

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Effect of salt bath on biochemical indices of rainbow trout after treatment of proliferative kidney disease

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Summary
The aim of this study was to investigate the effects of NaCl bath on biochemical indices of rainbow trout after treatment of proliferative kidney disease. The experiment included fish which exibited symptoms of proliferative kidney disease. One part of fish was treated with NaCl bath and second one was without treatment. After 14 days of treatment individual blood samples were taken from fish of untreated group (control group) and treated group. Additional blood sampling was carried out after 7 days of regeneration. Biochemical indices were analyzed in plasma. After 14 days of treatment, we found out significant ($p < 0.05$) alterations in most of plasma biochemical indices. Significant decreaces of ALT, AST, LDH and significant elevations in triacylglycerols, glucose, amonnium, calcium and cholesterol were observed in treated group. After seven days of regeneration, we found out decrease of lactate dehydrogenase and elevations in ALP, glucose, amonnium, calcium and cholesterol in treated group.

Keywords: *PKD; Tetracapsuloides bryosalmonae; NaCl bath; biochemical indices; rainbow trout*

Introduction

Proliferative kidney disease (PKD) is an parasitic disease caused by *Tetracapsuloides bryosalmonae* (Bettge et al. 2009). PKD is affecting mainly salmonid species. The main symptom of PKD is a proliferation of the intersticial kidney tissue which named the disease. Other unspecific symptoms are anemia, ascites, exopthalmus and apathy. (Schubiger et al. 2003). Fish which surviving the clinical phase of PKD are able to restore renal strucure. (Schmidt-Posthaus et al. 2012)

Material and Methods

This study was carried out in collaboration with BioFish s.r.o. (Pravíkov, Czech Republic). The company specializes in intensive farming of trouts and previous periods have repeatedly experienced problems with occurrence of PKD and related mortality. The experiment was carried out in recirculation system at Department of Ecology and Diseases of Game, Fish and Bees (University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic). The experiment included fish which exhibited clinical symptoms of PKD. One part of fish was treated with NaCl bath and second one was without treatment. During 14 days, sodium chloride was dosed up to the concentration of 0,81 % in group of treated fish. Mortality in both groups was observed during experiment, however, mortality was significantly lower in group exposed to NaCl treatment. The therapy was stopped after 14 days, but fish response and ability of regeneration were observed for further period of 7 days. After 14 days of treatment, individual blood samples were taken from 15 fish of treated group and 15 fish of untreated group. Additional blood sampling was carried out after 7 days (27 fish from treated group; 22 fish from untreated group). Blood samples were stabilized with heparin sodium, centrifugated and plazma samples were frozen and stored at -85 ° C. Statistical analyses was performed using Unistat 5.6. software. Data were tested for normality (Shapiro-Wilk test) and

differences in measured indices between treated and untreated groups were tested using unpaired t-test a Mann-Whitney test. Significance was accepted at $p < 0.05$.

Results and Discussion

A significant changes were observed in most of monitored biochemical indices. After 14 days of treatment, a significant decrease of ALT, AST and LDH ($p<0.05$) and significant increase in triacylglycerols, glucose, amonnium, calcium and cholesterol ($p<0.05$) were found in treated group. After 7 days of regeneration, a significant decrease of LDH ($p<0.05$) and significant increase in ALP, glucose, amonnium, calcium and cholesterol ($p<0.05$) were found in treated group.

Table 1: Plasma biochemical indices of rainbow trout after NaCl treatment (14 days ($^*p<0.05$) and after regeneration (7 days) ($^*p<0.05$) Data are presented as mean±standard error of mean.

Parameters	Treatment (14 days)		Regeneration (7 days)	
	Fish without treatment	Fish treated with NaCl	Fish without treatment	Fish treated with NaCl
ALT [µkatal/l]	**0.38±0.03**	**0.27±0.04***	0.33±0.02	0.28±0.02
AST [µkatal/l]	**8.22±0.54**	**6.35±0.48***	7.96±0.39	6.81±0.42
LDH [µkatal/l]	**24.38±2.03**	**15.73±3.35***	**19.95±1.44**	**15.80±1.54***
ALP [µkatal/l]	0.84±0.12	1.09±0.18	**0.56±0.05**	**0.88±0.10***
CK [µkatal/l]	51.70±10.27	34.44±5.62	51.46±12.48	54.77±8.97
Triacylglycerols [g/l]	**1.06±0.10**	**1.72±0.18***	1.88±0.21	2.23±0.25
Glucose [mmol/l/]	**3.10±0.15**	**3.78±0.28***	**3.57±0.28**	**5.37±0.28***
Amonnium [µmol/l]	**135.97±6.31**	**162.83±10.92***	**169.84±11.52**	**234.22±18.36***
Calcium [mmol/l/]	**2.22±0.04**	**2.43±0.04***	**2.47±0.04**	**2.62±0.04***
Phosphorus [mmol/l/]	2.67±0.07	2.84±0.26	2.91±0.08	2.91±0.11
Lactate [mmol/l/]	1.65±0.25	1.75±0.35	3.81±0.62	4.90±0.58
Cholesterol [mmol/l/]	**4.44±0.27**	**6.61±0.39***	**4.94±0.18**	**7.28±0.34***
Total protein [g/l]	37.61±2.87	42.84±2.02	45.75±1.11	42.13±2.30
Albumin [g/l]	14.78±1.32	16.82±0.73	18.36±0.58	16.93±0.88

Conclusion

Obtained results confirmed that NaCl bath could be used as a suitable possibility for treatment of PKD.

Acknowledgements

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Choroby volně žijících zvířat a zvířat zoologických zahrad

Antibiotická rezistence aeromonád v kaprovém rybníkářství
Antibiotic resistance of aeromonads in carp fishery

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Summary
To treat diseases caused by Aeromonas spp., antibiotics are used, but bacteria become gradually resistant to it. The aim of this study was to determine antibiotic resistance of aeromonads in carp fishery. Fifty strains of aeromonads were isolated from overall 154 fish coming from three fish breedings. Aeromonads went through identification and determination of their sensitivity to six antimicrobials (oxytetracycline, flumequine, florfenicol, sulphamethoxazole/trimethoprim, enrofloxacin, oxolinic acid). The highest resistance rate was detected for oxytetracycline (40%), highest sensitivity had bacteria to florfenicol (resistance rate only 2%). The results point to aeromonads being grately resistant to oxytetracycline, which is widely therapeutically used for cyprinids. On the contrary, florfenicol, registered for rainbow trout (Oncorhynchus mykiss), could be suitable alternative to this antibiotic.

Keywords: *Aeromonas spp.; antibiotic resistance; carp fishery; oxytetracycline; florfenicol*

Úvod
V chovech kaprovitých ryb se nelze vyhnout bakteriálním infekčním onemocněním, které často vedou k ekonomickým ztrátám. V České republice patří mezi častá bakteriální onemocnění erytrodermatitida kaprů způsobená bakteriemi rodu *Aeromonas* spp. K léčbě infekcí způsobených bakteriálními patogeny jsou využívány antimikrobiální látky (Smith, 2008). Velkým problémem je však narůstající rezistence bakterií vůči antimikrobiálním látkám a selhání terapie (Cizek et al., 2010; Dobiasova et al., 2014).

Cílem této studie bylo stanovit aktuální úroveň fenotypové a genotypové rezistence aeromonád izolovaných z klinických případů erytrodermatitid a septikémií v chovech kaprovitých ryb.

Materiál a metodika
Odběr vzorků a mikrobiologické vyšetření
V období od března do září 2016 jsme celkem vyšetřili 154 ryb (133 jedinců druhu *Cyprinus carpio*, 13 jedinců druhu *Carassius auratus*, 8 jedinců druhu *Silurus glanis*) ze dvou hospodářství a jednoho soukromého rybníka. Po humánním usmrcení bylo provedeno patologicko-anatomické vyšetření, během kterého byl vždy získán stěr z kůže, případně z kožních lézí a tkáň sleziny dle metodiky pro odběr vzorků pro bakteriologické vyšetření (Piačková a kol., 2014). Metodika mikrobiologického vyšetření vzorků probíhala podle standardizovaných protokolů zavedených na pracovišti a uvedených v předchozích studiích (Cizek et al., 2010; Dobiasova et al., 2014).

Získané izoláty byly subkultivovány a identifikovány hmotnostní spektrometrií na zařízení MALDI-TOF Biotyper (BrukerDaltonic, Německo). Identifikované čisté kultury byly uchovány v kryoprotektivním médiu při -70 ^{o}C a postupně byly charakterizovány i jejich fenotypové a genotypové vlastnosti.

Testování rezistence k antimikrobiálním látkám
Diskovou difúzní metodou byla stanovena citlivost vůči 6 vybraným antimikrobiálním látkám schváleným pro potravinové ryby v ČR nebo EU (oxytetracyklin, florfenikol, sulfametoxazol/trimetoprim, flumequin, enrofloxacin a kyselina oxolinová). Dále byla u všech kmenů zjištěna minimální inhibiční koncentrace k ciprofloxacinu (MIC_{cip}) metodou zřeďování v bujonu (Mikrolatest MIC Ciprofloxacin, Erba Lachema, ČR) (CLSI, 2006).

Detekce genů rezistence k antimikrobiálním látkám a detekce genů virulence
Teplotní lyzí byla izolována DNA rezistentních kmenů, která sloužila jako templát pro identifikaci genů rezistence a virulence metodou polymerázové řetězové reakce (PCR). Metodou PCR byl u rezistentních izolátů proveden průkaz genů kódujících rezistenci vůči tetracyklinům (*tetA* až *G*), k sulfonamidům (*sul1* až *3*) a trimetoprimu (*dhfrA17*, *dhfrA1*, *dhfrA12*), k chinolonům vázanou na plazminech (*qnrA* až *D*, *qnrS*, *qepA*, *oqxAB*, *aac(6´)-Ib-cr*) a k amfenikolům (*catA1*, *cmlA* a *floR*). Dále byla PCR provedena detekce genů kódujících integrázy (*intI1* a *intI2*). K vizualizaci produktů PCR byla použita gelová elektroforéza. Izoláty se sníženou citlivostí k ciprofloxacinu ($MIC_{cip} \geq 0,05$ mg/l) byly testovány na přítomnost PMQR genů s následnou sekvenací (Wilkerson et al., 2004; Dolejska et al., 2007).

Všechny izoláty aeromonád byly podrobeny vyšetření na přítomnost 7 vybraných genů virulence (*ahh1*, *asa1*, *act*, *ast*, *ascV*, *eno*, *aexT*) metodou PCR (Martino et al., 2011).

Výsledky
Z prvního hospodářství bylo vyšetřeno 120 ryb, z toho 36 ryb (30 %) bylo kultivačně pozitivních. Z druhého hospodářství bylo vyšetřeno 25 ryb, z toho 6 ryb (24 %) bylo kultivačně pozitivních. Ze soukromého rybníka bylo vyšetřeno 9 ryb a 8 z nich (88,9 %) bylo kultivačně pozitivních.

U 17 % z celkového počtu 154 ryb byly při patologicko-anatomickém vyšetření nalezeny kožní léze ve formě povrchových nekróz až po vředy zasahující do svaloviny. Některé léze byly ve stádiu hojení, což ukazuje na již prodělané infekční onemocnění. U zhruba 8 % vyšetřovaných ryb byla zaznamenána atrofie zadní části plynového měchýře a u necelých 5 % přítomnost meziorgánových srůstů. Ryby byly, až na výjimky, v dobrém výživném stavu. Kultivačním vyšetřením bylo získáno 50 bakteriálních izolátů. Všechny bakteriální izoláty byly získány ze sleziny.

Diskovou difúzní metodou byla fenotypová rezistence izolátů (n=50) prokázána nejčastěji k oxytetracyklinu – u 20 izolátů (40 %), naopak rezistence k florfenikolu jen u 1 izolátu (2 %). K sulfametoxazolu/trimetoprimu byla prokázána rezistence u 14 izolátů (28 %), ke kyselině oxolinové u 12 izolátů (25 %), k enrofloxacinu u 10 izolátů (20 %) a k flumequinu u 7 izolátů (14 %).

Metodou PCR bylo zjištěno, že 18 izolátů (n=50) neslo alespoň jeden gen rezistence. Celkem 18 izolátů (36 %) neslo gen rezistence vůči tetracyklinům (*tetE* u 13 izolátů, *tetA* u 5 izolátů), 3 izoláty (6 %) nesly gen rezistence vůči chinolonům (*qnrS1, qnrS2,* u třetího izolátu se variantu genu *qnrS* zatím nepodařilo určit), 1 izolát nesl geny rezistence k sulfonamidům, včetně potencovaných (*sul2* a *dhfrA1*). Gen *intI1* byl detekován u jednoho izolátu, gen *intI2* nebyl nalezen. Deset izolátů mělo $MIC_{cip} \geq 0,05$ mg/l. Zjištěné hodnoty se u těchto izolátů pohybovaly v rozmezí 0,06 – >4 mg/l.

U jednotlivých izolátů aeromonád se vždy vyskytoval jeden až pět genů virulence. 19 izolátů (38 %) neslo pět ze sedmi testovaných genů virulence. 3 izoláty (6 %) nesly pouze jeden gen virulence. Gen *ast* nebyl detekován u žádného izolátu.

Diskuze

Z výsledků vyplývá, že k oxytetracyklinu, který je v ČR registrován od roku 1997 jako jediné antibiotikum k léčbě bakterióz kaprovitých ryb, byla zjištěna poměrně vysoká rezistence. Tyto výsledky korespondují s již dříve publikovanými studiemi zabývajícími se touto problematikou (Cizek et al., 2010; Dobiasova et al., 2014). Florfenikol, registrovaný v ČR pro pstruha duhového (*Oncorhynchus mykiss*) by mohl být k oxytetracyklinu adekvátní alternativou.

Výsledná data z vyšetření na přítomnost genů virulence poukazují na přítomnost vyššího počtu těchto genů, kterou lze hodnotit jako potenciální vyšší virulenci daných izolátů.

Závěr

Závěrem lze konstatovat, že s ohledem na vysoké hodnoty antibiotické rezistence aeromonád je nutné tuto problematiku i nadále monitorovat. Tato situace vyžaduje rovněž co největší zaměření na využití preventivních opatření v chovech ryb, a tím snížení spotřeby antibiotik.

Poděkování

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The occurrence of protozoan parasites in different wild and domestic mammals in South Africa

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Summary
There is a lack of information available on protozoan parasites in African animals. Here we investigate the occurrence of protozoan parasites in mammals from South Africa. Protozoan parasites were detected in 13 of 56 (23 %) faecal samples. Cryptosporidium spp. and Isospora spp. were detected in 8 (14 %) and 5 (9 %) samples, respectively. Antibodies to T. gondii and N. caninum were not detected by ELISA in any of 32 sera tested. T. gondii was detected by PCR in tissue of five of 243 (2 %) animals; in domestic dog (Canis lupus familiaris), gerbil spp. (Gerbilliscus spp.), greater kudu (Tragelaphus strepsiceros), honey badger (Mellivora capensis) and white-tailed mongoose (Ichneumia albicauda). All tissue samples were negative for N. caninum. The study increases our knowledge on the occurrence of protozoan parasites in populations of wild and domestic animals in South Africa.

Keywords: *Coprology; Cryptosporidium; Isospora; Neospora; PCR; serology; Toxoplasma*

Introduction

Protozoan parasites cause several diseases in animals and humans, especially in developing countries with large numbers of immunosuppressed people (Samie et al., 2014). Despite the obvious economic and health importance of parasitic protozoans there is a lack of information in African countries where wildlife tourism is key components of the economy.

One of the most diarrhoea-causing protozoan parasite *Cryptosporidium* spp. is recognized as key contributors to the morbidity and mortality of infants and immunosuppressed individuals, particularly in developing countries (Pedersen et al., 2014; Samie et al., 2014). The infection with *Isospora* spp. is species-specific and is acquired by the ingestion of sporulated oocysts in contaminated water or food or by ingestion of infected paratenic hosts (Olusegun et al., 2009). Other protozoan parasites *Toxoplasma gondii* and *Neospora caninum* have a world-wide distribution, causing serious illnesses (Dubey et al., 2007). Importance of *T. gondii* in South Africa is mainly because of its zoonotic potential. As South Africa accounts for 17 % of the global HIV burden, such high incidence of immunosuppressive infections puts the human population at a high risk of opportunistic infections such as toxoplasmosis (Hammond-Aryee et al., 2014). *Neospora caninum* is closely related to *T. gondii* and has been implicated mostly as a cause of abortions and reproductive diseases in domestic animals (Njiro et al., 2011).

Material and Methods

Faecal samples, were collected from 56 carnivores and examined using a modified Ziehl-Neelsen staining method and microscopically examined with 100 × magnification with immersion oil for the detection of *Cryptosporidium* spp.

Serum samples were obtained from 32 animals from 3 orders: Artiodactyla (n = 18), Rodents (n = 8) and Perissodactyla (n = 6). Whole blood samples were originally collected into vacutainer tubes, centrifuged, sera extracted and stored at -20 °C until analysis. Sera were

used to detect antibodies to *T. gondii* and *N. caninum* by Enzyme Linked Immuno Sorbent Assay (ELISA kit ID Screen *T. gondii* Indirect Multi-species and ELISA kit ID Screen *N. caninum* Indirect Multi-species, respectively), according to the instructions (IDvet, Grabels, France). Optical densities were measured spectrophotometrically at 450 nm. The ratio of optical densities was calculated as sample/positive control (S/P %), according to the formula: S/P (%) = (OD sample/OD positive control) × 100. Samples with S/P (%) ≥ 50 % were classified as positive.

Brain samples (n = 243) were collected from hunted animals during years 2012 – 2015 or from road killed animals in 5 provinces of South Africa from various domestic and wild animals belonging to Rodentia (n = 198), Carnivora (n = 23), Artiodactyla (n = 14), Primates (n = 5), Macroscelidea (n = 2) and Lagomorpha (n = 1). DNA was isolated using a Jetquick Tissue DNA spin kit (Genomed, Germany) and stored at -20 º C until analysis. Polymerase chain reaction (PCR) with the specific primers of repetitive sequence B1 for the detection of *T. gondii* was performed (Burg et al., 1989). For *N. caninum* detection, the primers Np6plus and Np21plus annealing to the Nc-5 region were used (Yamage et al., 1996). PCR product of *T. gondii* and *N. caninum*, respectively were analyzed on 2% agarose gel.

Results

Protozoan parasites were detected in 23 % (13/56) of faecal samples. Six (11 %) and eight samples (14 %) were positive using flotation and by Zeihl-Neelsen staining, respectively. *Cryptosporidium* spp. and *Isospora* spp. were detected in eight (14 %) and five (9 %) samples, respectively. Mixed infection of *Cryptosporidium* spp. and *Isospora* spp. was recorded in banded mongoose only.

Serum samples were negative for antibodies to both *T. gondii* and *N. caninum*.

T. gondii was detected by PCR in the tissue of five animals, including domestic dog, gerbil, greater kudu, honey badger and white-tailed mongoose. This is the first isolation of *T. gondii* from white-tailed mongoose and honey badger. Unfortunately, complete genotyping of *T. gondii* positive samples was not successful. *N. caninum* was not confirmed in any of the animals.

Discussion

The most important finding, considering the zoonotic importance and public health implications was the detection of oocysts of *Cryptosporidium* spp. in faeces of eight animals (of seven species) from the Limpopo Province of South Africa. Samie et al. (2006) found a high prevalence of *Cryptosporidium* infection in school children (18 %) and hospital patients with diarrheic symptoms (57 %) in the Venda region, Limpopo Province, SA. A later study from the same province showed a prevalence of 27 % for *Cryptosporidium* spp. in HIV/AIDS patients (Samie et al., 2014). The transmission of this parasite among wildlife and domestic animals poses a serious risk of infection to humans, in particular those suffering from immunosuppressive diseases. Therefore, it is important to continue research on this topic to inform public health officials. *Isospora* spp. oocysts were found in faecal samples of five animals (dog, lion, caracal and two banded mongooses) from Limpopo Province.

In South Africa, there is a high incidence of immunosuppressive infections in humans that could lead to clinical manifestation of toxoplasmosis due to a reactivation of chronic infection (Hammond-Aryee et al., 2014). In our study, all tested sera (the majority of which are from wild animals) were negative for *T. gondii* antibodies. *T. gondii* was however isolated from tissue samples in 13 % of carnivores, 8 % bovidae and 0.5 % rodents sampled. Fortunately, the conditions for sporulation and survival of *T. gondii* oocysts are not optimal in the

Limpopo Province due to the dry and hot climate. According to our knowledge, this is the first isolation of *T. gondii* from white-tailed mongoose (*Ichneumia albicauda*) and honey badger (*Mellivora capensis*). All samples (sera and tissues) were negative for *N. caninum*. Nevertheless, it is important to continue sampling in diverse mammals to understand the role of *N. caninum* in the sylvatic cycle.

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Analysis of the full G gene sequence of the viral haemorrhagic septicaemia virus isolate from the Czech Republic

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Summary
Production of salmonids in the high-capacity fish farms is the fastest growing fish-related industry in the Czech Republic. The virological pathogen with the most severe economical impact in these establishments is the viral haemorrhagic septicaemia virus (VHSV). High genetic variability of this RNA virus from the family Rhabdoviridae could be used in molecular epidemiology.

Presented study focuses on a VHS outbreak in the Vysočina region from the autumn of 2016. Clinical signs of the disease and the means of isolation and detection of the virus responsible are described. The genetic analysis of the isolate will be provided, using the full glycoprotein gene sequencing; classifying it into VHSV genotype I class Ia. This Czech isolate is compared with sequences available in the GenBank database, with emphasis on isolates from countries with high fish-trade rates with the Czech Republic, looking for a possible trade-related spread of the virus.

Keywords: *VHSV; glycoprotein; sequencing; Czech Republic*

Introduction

Viral haemorrhagic septicaemia virus (VHSV) is one of the most important viral diseases of fish worldwide. It affects more than 80 salt- and freshwater fish species. The virus causes heavy economical losses in the production of farmed rainbow trout *(Oncorhynchus mykiss)* in the Czech Republic, due to the severity of the clinical outbreaks and costly eradication measures in the affected facilities. The number of VHSV cases in the Czech Republic grew significantly in the last few years.[1]

VHSV is a single-stranded RNA virus from the family *Rhabdoviridae*. It can be genetically divided into four genotypes (I-IV) and further into classes and sublineages. The strains have specific geographic distribution, with the most prevalent class in the freshwaters of continental Europe being class Ia. The differences amongst strains of the virus can by exploited by the means of molecular epidemiology, with the potential to examine the routes of transmission of the pathogen between various outbreak sites. Analysis of the full glycoprotein gene of the virus is the most widely used mean of VHSV characterization.

Materials and Methods

Samples: 30 specimens of rainbow trout were examined. They originated from a fish farm using a recirculation aquaculture system, which is located in the Vysočina region. The fish expressed clinical signs including distended abdomen, exophthalmos and darkening of the skin. Hemorrhages were present in the skin, around the fin bases, in organs and in the muscle tissue.

Virus isolation and identification: Samples from spleen, heart and anterior kidney were pooled, homogenized, centrifuged, mixed with antibiotics and inoculated on a rainbow trout gonad (RTG) cell culture. After the 7-day incubation period cytopathic effect was observed and the supernatant from the cell culture was separated and submitted to RNA extraction using the QIAamp Viral RNA Mini Kit (Qiagen). The presence of VHSV was confirmed using the one-step real-time RT-PCR.[2]

RT-PCR: To amplify the genetic information of the virus, RT-PCR was performed in 50 µl setting using Titan One Tube RT-PCR System (Roche) with 10 µl reaction buffer, 5 µl 100 mM DTT, 1 µl 10 mM dNTP, 1 µl enzyme mix, 5µl of RNA extract, 50 pmol of each of the primers (Table 1) and RNase-free water. Thermal conditions of the reaction were: 50 °C for 30 min, 94 °C for 15 min, 40 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 2 min, followed by single instance of 72 °C for 7 min. Presence of the amplified product was evaluated in a 5 µl sample by aragose gel electrophoresis. The rest of the product was purified using the High Pure PCR Product Purification Kit (Roche).

Table 1: Primers for RT-PCR.[3]

Primer: GB+ (for)	5´- GTCGAAGAAGAGATAGGC – 3´
Primer: GB- (rev)	5´- GTTGGGTCGCCATGTTTCT– 3´

Sequencing: The full glycoprotein gene sequence was obtained using the Sanger sequencing method via the company SEQme (Dobris, Czech Republic), using the primers described in the Table 1.

Analysis: The sequence was edited and aligned using the BioEdit Sequence Alignment Editor v. 7.2.0. (Tom Hall). Sequences for comparison were obtained from the GenBank database.

Results

The length of the obtained glycoprotein gene sequence of the VHSV was 1524 nucleotides. Phylogenetic analysis placed this isolate into genotype I class Ia. Comparison of the isolate with glycoprotein gene sequences from the GeneBank database revealed, that the isolates with closest nucleotide identity of 94.49 % belonged to genogroup Pol-I. These isolates were described by Reichert et al. and originated from the south of Poland.[4]

Discussion

Sequencing of the full glycoprotein gene of the VHSV has become the most universally used method of analyzing isolates of this virus, yet there are currently no published results of such analysis from the Czech Republic. Results of this study support the theory, that most of the European VHSV isolates belong to the genotype Ia. The fact, that this Czech isolate is closely related to VHSV isolates from southern Poland - a region with close geographical and fish-trade relationship with the Czech Republic - shows, that fish-trade plays an important role in the transmission of this disease. Future sequencing of other VHSV isolates from the Czech Republic could give insight on VHSV transmission in this region.

Conclusions

Full length glycoprotein gene sequence of VHSV isolate was successfully obtained and analyzed. Isolate of the virus, that previously caused an outbreak of the disease in a fish-farm in the Vysočina region, was placed in the genotype Ia, with closest nucleotide identity to VHSV strains isolated from southern Poland.

Acknowledgements

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In vitro reproductive toxicity of Cd, Hg and Pb to fallow deer (*Dama dama*): a model study of Sertoli cells and ovariocytes

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Summary
Cell culture models are useful tools in toxicological studies of environmental pollutants. In vitro assessment of cadmium-induced toxicity, in comparison to mercury & lead, to isolated Sertoli cells and ovariocytes derived from both male and female fallow deer (Dama dama), is intended in this study to illustrate the potential effects of heavy metals exposure on selected wildlife population.

Keywords: *heavy metals; cytotoxicity; reproductive health; fallow deer*

Introduction

Heavy metals accumulation in game animals' tissues is described in several studies (KUITERS, A.T.1996, HOLM, J.1993, KALAS JA. 1994). Accumulation levels may vary according to animal-related factors (species, sex, age and exposed organ sensitivity); whereas a tendency to increase concentration of metals within aged animals is reported. Females show higher metal concentration than males; this contrast between sexes could be interpreted as a result of differences in metabolic rate, reproductive and hormonal status of the animal (BURGER, 2007, GARCÍA, 2011). Sensitivity of particular organs to metal accumulation and toxicity is observed in liver, kidney, muscles and gonads (MARIA E. ARIZA, 1999), the reproductive organs appear to be more sensitive to metal toxicity; particularly cadmium chloride; and this could be due to their dense vascular structure; whereas the vascular system is considered a major target of cadmium toxicity (PROZIALECK WC.et al. 2008). In vivo studies on rat testicular cells (Sertloi and germ cells) have evidenced the role of lower doses of cadmium in mediating endocrine disruption at hormonal level, and cell necrosis at ultra-structural level (PIASEK, M. 1999). In vivo injection of cadmium chloride in mouse ovary has demonstrated that cadmium changes the ultrastructure of ovarian cells tissue, and affects follicles maturation and corpus luteum degradation (WANG, YING, et al. 2015) whilst In vitro; it affects the steroidogenesis process in different stages of female reproductive cycle (PIASEK, M. 1999).

Material and Methods

Cells isolation and culture

Sertoli cells were isolated from testicular tissue following a protocol of two enzymatic digestion steps; 1mg/ml collagenase II and 1mg/ml trypsin 1:25, and DNaseI 5µg/ml in DMEM: F12. Cells were cultured in complete DMEM: F12 using 5µg/ml DSA-Lectin coated tissue culture flasks supplemented with 5mg/ml bovine serum albumin in DPBS. Ovariocytes were obtained by primary culture of ovarian tissue fragments in DMEM:F12 supplemented with fetal bovine serum10% and penicillin-streptomycin 1%. Cells were allowed to grow in controlled culture environment in 37°C & 5% CO_2.

Cytotoxicity assay

Cytotoxicity was determined through assessment of Lactate Dehydrogenase activity, using Roche Cytotoxicity Detection Kit^{PLUS}{LDH}. Cells were cultured in 96 wells plates in density of $1x10^4$ cells per well for 24h before metals treatment. The culture media was then replaced with fresh pre-prepared assay media with five different concentrations; 15µM, 30µM, 60µM, 125µM, 250µM cadmium chloride, mercuric methylene chloride and lead chloride

respectively for another 24h. LDH activity was determined at 490 nm using microplate reader.

Results and Discussion

The degree and severity of toxicity varied according to metal and cells type *figs.* (1, 2); both gonads appear to be sensitive to $CdCl_2$ exposure *fig.* (3), with notable dose-dependent pattern in case of Sertoli cells. Absence of this pattern in ovariocytes may suggest the occurrence of; either a robust adverse mechanism organized by these cells or an earlier and acute cellular damage. Lower doses of methylene mercuric chloride >1&<60µM caused significantly higher cytotoxic effect *fig.* (4); which could allow to set a threshold dose of > 1 & $\ll 15$µM (Sertoli cells) and ≤ 1 µM (ovariocytes) for mercury-induced toxicity; however ovariocytes seem to be extremely sensitive to $MeHgCl_2$ toxicity.

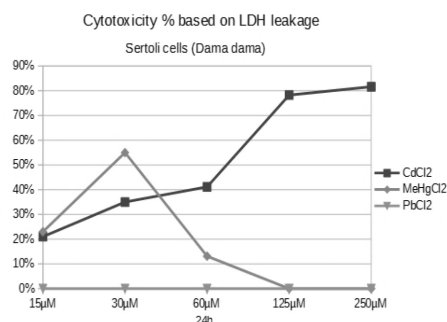

Figure 1: LDH leakage from Sertoli cells.

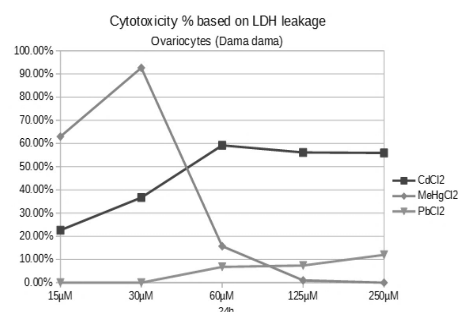

Figure 2: LDH leakage from ovariocytes.

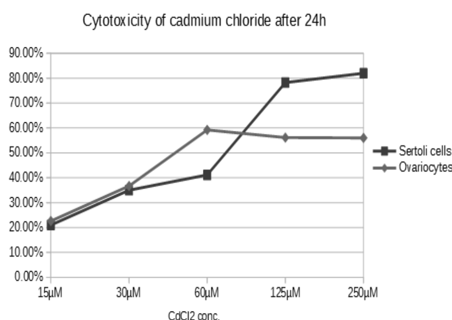

Figure 3: $CdCl_2$ cytotoxicity after 24h.

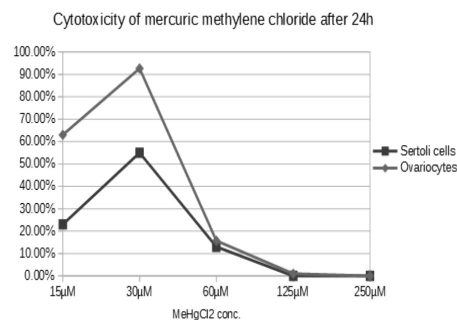

Figure 4: $MeHgCl_2$ cytotoxicity after 24h.

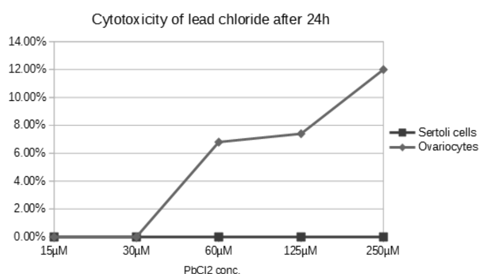

Figure 5: $PbCl_2$ cytotoxicity after 24h.

*Results for 1 µM and > 250 µM metals treatment are not provided.

Treatment with lead chloride has shown mild cytotoxic effect to ovariocytes starting from 30µM, with trend to increase cytotoxicity only at comparatively higher doses >250 µM in both cells type *fig.* (5); this may suggest that $PbCl_2$ is less able to induce cytotoxic effect; due

to its intracellular ability to bind and function at other's microelements sites. Cellular morphological deformity was also detected with initial observation under inverted microscope; thus the occurrence of cellular organelles damage is tentatively evident.

Conclusion

The results of this study have proved that, $CdCl_2$ and $MeHgCl_2$ are potent to arouse fallow deer's Sertoli cells and ovariocytes disrupt, therefore further assessment of metals toxicity mechanism, and the ability of the respective cells to handle exposure is required.

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Endoparasitic infections in Central European bats

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Summary
Aim of the study was assessment of prevalence of trypanosomes in European bats. There was a high prevalence of Trypanosoma dionisii B in Noctule bats from the Czech Republic. This species of trypanosomas was previously desribed only in Great Britain and it is known, that this trypanosoma is closely related to Trypanosoma cruzi from South America.

Keywords: *bats; parasites; trypanosoma*

Introduction

Parasitism is one of the life strategies, when parasite lives at the expense of its host. Relationship between host and parasite is a result of long time coevolution and is characterized by certain balance between host and parasite. Thus, there is not a surprise that mild parasitic infections in wildlife are quite common and mostly have very low health impact. On the other hand, parasitic infections can have some minor effect on reproduction and fitness of individuals (Merino et al., 2000).

Bats are very specific group of mammals considering their special way of living, physiological adaptation and their pathogens. They are known as reservoirs of lots of viruses (Calisher et al., 2006), but recently we suppose they are also original hosts of trypanosomas subgenus *Schizotrypanum* (Hamilton et al., 2012a), where also belongs *Trypanosoma cruzi*, the causative agent of Chagas disease in South America. Subgenus *Schizotrypanum* includes mostly species specific for bats, only *Trypanosoma cruzi* evolved the ability to infect other mammalian species. There is a theory that this relationship between subgenus *Schizotrypanum* and bats started long time before the break-up of supercontinent and this theory is supported by phylogenetic analysis (Hamilton et al., 2012a). Hamilton et al. (2012b) made a survey of blood samples of bats from Great Britain with use of 18S rDNA analysis and found out that *Trypanosoma dionisii* B isolated from blood sample from *Nyctalus noctula* is phylogenetically closely related to isolates of *T. dionisii* from South America than to *T. dionisii* A from *Pipistrellus pipistrellus* from Great Britain (Hamilton et al., 2012b). Since this study concerning trypanosomas in European bats is one of only few published, we decided to realize analysis of blood samples from *Nyctalus noctula* from Czech Republic.

Materials and Methods

In terms of this project were collected and processed blood samples from Noctule bats, that were at the beginning of hibernation rescued and held in captivity, some of sampled bats were caught into nets by chiropterologists from Institute of Vertebrate Biology AV ČR during scheduled catches (permission AOPK ČR č.j. 01662/MK/2012 S/00775/MK/2012, č.j. 866/JS/2012). The blood sample was obtained from *v. saphena* and puncture was treated with tissue glue to prevent unwanted blood loss. Before releasing bats were supplemented with liquids and energy by perorally applicated glucose solution. As a part of the project biochemical and hematological parameters were processed right in field using mobile hematological and biochemical analyser VetScan and iSTAT (Abaxis, USA).

Part of blood sample was obtained for DNA isolation, subsequent nested PCR trypanosoma-specific analysis and sequencing as well as blood smears were made for analysis of

differential count of leukocytes. In seven handicapped females confirmed positive for trypanosomas via blood smears we monitored parasitemia progress during gravidity and lactation and we also analysed blood samples from their offspring. Blood samples were collected from the total of 90 *Nyctalus noctula* individuals.

Results

In terms of this study we obtained blood samples from adult 79 Noctule bats and from 11 juveniles of trypanosoma-positive females. Nested PCR prevalence of trypanosoma in blood from 79 adult animals during year was 43,59%, during hibernation it was 42,85% and in normothermic animals caught during swarming the prevalence of trypanosoma was 50%. Based on sequencing trypanosomas in all positive sampled Noctule bats were identified as *Trypanosoma dionisii* B. Handicapped females were sampled repeatedly during late hibernation, gravidity, lactation and weaning of juveniles. Quantity of trypanosomes fluctuated during the whole period of sampling and in some individuals after negative result of blood sample subsequent sample was positive again. All samples from juveniles of trypanosoma-positive females were negative on PCR. As a part of this study we analysed hematological and biochemical parameters in Noctule bats hibernating in captivity. There were statistically significant differences in total protein and globulin levels between trypanosoma-positive and trypanosoma-negative individuals.

Conclusion and Discussion

In terms of this study we confirmed presence of *T. dionisii* B (formerly described only in UK) in European mainland for the first time. This isolate, based on phylogenetic analysis, is closely related to isolates from South America (Hamilton et al., 2012b). We diagnosed this trypanosoma in Noctule bats as well as Hamilton and colleagues did (Hamilton et al., 2012b). There was relatively high prevalence of trypanosomas in blood samples we collected from individuals at several localities and there were no significant changes in blood parameters in comparison to trypanosoma-negative animals. The only difference was higher level of globulins as well as total protein in trypanosoma-positive animals. This is the same finding as what Herrera et al. (2002) has seen in coati (*Nasua nasua*) with experimental trypanosomiasis. Contrary to this study, there was neither anemia nor hepatic damage in bats we sampled (Herrera et al., 2002). This finding together with high prevalence and low parasitemia suggest certain level of tolerance as a result of long term co-evolution of this trypanosoma and bat species (Best et al., 2008). This co-evolution theory is also supported by absence of hematological and biochemical changes in positive animals. Surprisingly, all blood samples collected from trypanosoma-positive female juveniles were negative, thus we can suspect, that there is not a vertical transmission route of infection in this trypanosoma species. Level of pathogenicity in this species is questionable, because on one hand this species is able to invade live cells and multiplicate inside of them (Oliveira et al., 2009), on the other hand long term survival without health issues was observed in Great Britain bats infected by this blood parasite (Hamilton et al., 2012b). By the way, we observed 7 positive females with persistent infection during 8 month period and we haven´t seen any health problems in these animals.

Acknowledgment

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Posouzení zdravotního stavu dravců a sov v záchranných centrech: biochemická a parazitologická studie

Health assessment of birds of prey in rescue centres: biochemical and parasitological study

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Summary
Wild birds are continually exposed to many anthropogenic and natural stressors in their habitats, including pathogens. Animal rescue centres in the Czech Republic provide aspects of care to abandoned, neglected, and injured animals. Birds are frequent patients, and it is important to assess their health status. Optimal investigation includes haematological and biochemical parameters and parasitological examinations. The objective of this study was to evaluate biochemical parameters and parasitological examinations of members of families Accipitridae, Strigidae and Falconidae.

Keywords: *Accipitridae; Strigidae; Falconidae; pathogens*

Úvod

Volně žijící ptáci jsou neustále vystaveni mnohým antropogenním a přirozeným stresorům. Kombinovaná expozice je běžným problémem v znečištěném prostředí, kde organismy musí čelit více stresorům, včetně patogenů (Sagerup et al., 2009). V České republice vzrůstá snaha o záchranu poraněných volně žijících zvířat. V záchranných stanicích tak představují dravci a sovy významnou část pacientů. Zpětné vypuštění zvířat do volné přírody je primárním cílem záchranných stanic, a proto je důležité správně posoudit aktuální zdravotní stav zvířat při příjmu. Vyšetření by optimálně mělo zahrnovat kombinaci hematologického, biochemického a parazitologického vyšetření (Atkinson, 2008; Tully, 2000).

Cílem projektu bylo posouzení zdravotního stavu zvířat držených v zajetí, a to pomocí stanovení biochemických parametrů a parazitologického vyšetření dravců a sov.

Materiál a metody

Vzorky krve pro hematologické a biochemické vyšetření a také vzorky trusu pro parazitologické vyšetření byly získány v rámci spolupráce se záchrannými stanicemi pro poraněné živočichy v České republice. Krev byla zvířatům odebírána z *vena cutanea ulnaris* nebo z *vena jugularis*. Biochemické hodnoty krve byly zpracovány přenosným biochemickým analyzátorem VetScan iSTAT 1 (Abaxis, USA). Pro parazitologické vyšetření byla využita flotační metoda a celkově bylo vyšetřeno 82 zvířat. Pohlaví u druhů, které nebylo možno určit na základe pohlavního dimorfismu bylo stanoveno s využitím PCR metody.

Výsledky

Hodnoty biochemických parametrů byly rozděleny do dvou skupin – klinicky zdravá a klinicky nemocná zvířata. Celkově bylo vyšetřeno 82 zvířat a zvířata byla rozdělena do souborů dle čeledí (*Accipitridae, Strigidae* a *Falconidae*). Z čeledi *Accipitridae* bylo vyšetřeno 21 jedinců, z toho klinicky 6 nemocných. Z čeledi *Strigidae* bylo vyšetřeno 20 jedinců, z toho klinicky 4 nemocní. Z čeledi *Falconidae* bylo vyšetřeno 41 zvířat, z toho klinicky nemocní byli 4 jedinci. Tabulka č. 1 ukazuje porovnání biochemických hodnot mezi klinicky zdravými a klinicky nemocnými zvířaty u čeledi *Falconidae*. Parazitologickému vyšetření bylo podrobeno 82 zvířat, z toho bylo pozitivních 31 jedinců, tj. 25,42 % z celkového počtu. Zastoupené rody parazitů byly *Eimeria, Capillaria, Porrocaecum* a druh

Syngamus trachea. U šesti jedinců, z čeledí *Accipitridae* a *Falconidae* byla prokázána kombinovaná invaze více druhy parazitů.

Tabulka 1: Porovnání biochemických hodnot mezi klinicky zdravými a klinicky nemocnými zvířaty u čeledi *Falconidae* (n = 41). Šedá pole v tabulce – hodnoty mimo rozsah klinicky zdravých zvířat.

Parametr	**klinicky zdravá zvířata** (n = 37)	**klinicky nemocná zvířata** (n = 4)			
	střední hodnota ± SO	***Falco tinnunculus* I.**	***Falco tinnunculus* II.**	***Falco tinnunculus* III.**	***Falco rusticolus x Falco cherrug* IV.**
AST(µkat/L)	0,73 - 1,53	1,1	35,1	3	1,6
CK (µkat/L)	6,65 - 17,62	52,8	0	54,3	5,3
UA (µmol/L)	142,9 - 505,91	743	190	805	216
GLU(mmol/L)	15,12 - 19,67	11,4	16	12,3	15
CA (mmol/L)	2,05 - 2,43	1,57	1,98	1,57	2,43
P (mmol/L)	0,38 - 1,27	0,78	0,93	0,31	1,47
TP(g/L)	26,67 - 35,49	12	29	19	41
ALB (g/L)	17,35 - 29,1	9	10	14	0
GLOB (g/L)	2,11 - 11,11	0	0	0	0
Na (mmol/L)	145,68 - 152,32	121	117	128	147
K (mmol/L)	3,4 - 4,8	4,8	22	3,1	2,3
Cl (mmol/L)	112,95 - 120,24	97	96	100	106

Diskuse

Znalost normálních hodnot krevních parametrů dravých ptáků je nezbytným předpokladem posouzení jejich zdravotního stavu (Lumeij, 2007; Tully, 2000). I když dostupnost těchto znalostí má ve vědecké literatuře rostoucí trend, až moderní přístroje, které mají malé nároky na objem krve, umožňují relativně efektivně krevní parametry měřit. Celosvětovým problémem dravých ptáků ve volné přírodě i v zajetí je expozice a otrava olovem (Pikula et al., 2013). Kromě toxikologického vyšetření na koncentraci olova v krvi i ve tkáních je vyšetření krve (hematokrit, hemoglobin) prvním indikátorem otravy, který umožňuje rychle nasadit patřičnou léčbu.

Závěr

Porovnáním biochemických hodnot byl zjištěn statisticky významný rozdíl mezi souborem klinicky zdravých a klinicky nemocných jedinců u většiny sledovaných parametrů. Zejména jsme zjistili rozdíly v hodnotách albuminu, globulinu, kreatinkinázy, chloridů a hemoglobinu. Lze také předpokládat, že pokud bude do budoucna navýšen počet zdravých zvířat, dojde ke zpřesnění referenčního rozmezí. Druhy, u kterých nebylo možné určit pohlaví pomocí standardních neinvazivních postupů, bylo provedeno určení pohlaví pomocí metody PCR. Jako nejvhodnější se jeví použití primerů 2550F/2718R podle Fridolfsson and Ellegren (1999).

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