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**O1**

### Transcutaneous ultrasonography in horses with (acute) colic

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**Introduction:** Abdominal ultrasonography is a safe and useful diagnostic modality to evaluate gastrointestinal organs of horses with acute or chronic colic. It enables the clinician to image regions inaccessible by means of rectal palpation. This presentation aims to give an overview over the findings of ultrasonography of the equine abdomen with a focus on the adult horse with acute colic.

**Technique:** Preparation with alcohol on short hair can be sufficient to obtain diagnostic images, but especially with winter coat and long hair, image quality can be greatly improved by clipping the area of interest prior to ultrasonography. The clipped skin should be washed with water, dried and lubricated using sufficient amounts of ultrasound contact gel.

In order to penetrate as far into the abdominal cavity as possible, low frequency transducers should be used (2 to 3.5 MHz). The area of interest should be scanned in two perpendicular planes using a convex transducer. More superficial structures or neonatal abdomens can be imaged using higher frequency probes.

**Gastrointestinal tract:** The gastrointestinal tract is most important in colic horses, but also in patients suffering from weight loss or exercise intolerance it should be evaluated thoroughly. In acute colic cases the ventral abdomen, ventral flank regions, the duodenum, stomach and the nephrosplenic space can be imaged in only a few minutes. Hence, ultrasonography can assist the clinician in making the decision for surgical therapy. If chronic colic or weight loss are the reasons for the exam the whole of the abdomen should be evaluated with equal thorough care.

**Stomach:** The stomach is located 5-10 cm medial to the skin at the left side of the thoracic wall. This

depth depends on the state of gastric filling. Gastric imaging normally should not extend caudal to ICR 15, but is individually very variable. In the normal adult horse the stomach can be imaged over three to five intercostal spaces between the eight and 15<sup>th</sup> intercostal space. In fed ponies the stomach may be seen in more intercostal spaces (up to eight in three ponies 24 hours postgastroscopy). Gastric wall thickness, contents and distention can be evaluated ultrasonographically. A wall thickness of more than 7-8 mm can hint towards gastric ulceration and warrants gastroscopic evaluation. Masses can be due to squamous cell carcinoma or other neoplasms. In horses with gastric neoplasia transcutaneous ultrasound could also reveal omental masses between the gastric wall and the spleen in selected cases. In horses with small intestinal ileus and subsequent secondary dilatation of the stomach, a fluid-filled dilated stomach can be visualized in the stomach window.

**Intestinal wall:** The measurement of intestinal walls is possible in different parts of the intestinal tract. Five layers of the thickened intestinal wall can be evaluated using high frequency probes: the innermost hyperechogenic borderline between intestinal lumen and mucosa, the hypoechoic mucosal layer, hyperechoic submucosa, hypoechoic muscle layer and hyperechoic border between serosa and peritoneal cavity. Oedema, infiltrative bowel disease and strangulating intestinal lesions can lead to a thickening of the intestinal wall. In strangulating small intestinal lesions usually the steno-stenotic segment of the small intestine will get thickened.

**Small intestine:** Small intestinal distention, not necessarily palpable per rectum, can be found using ultrasonography. Non oedematous distended small intestine showing motility is often found in horses showing colic, but is not essentially caused by primary small intestinal lesions. Immobile, dilated small intestinal loops are an indication for surgical intervention. Small intestinal strangulating obstructions were shown to be diagnosed more reliably by ultrasonography than by rectal palpation. Primary small intestinal lesions often show an increase in wall thickness (> 3 mm), are distended and lack motility. Small intestinal entrapment

into the epiploic foramen could be diagnosed earlier with the use of ultrasound in horses showing only low-grade colic and horses with inconclusive rectal findings. Distended or thickened small intestine can be imaged predominantly in the ventral abdominal regions. Klohn et al. (1996) even found a 100% sensitivity, specificity, positive and negative predictive value for small intestinal strangulating obstructions if immotile abnormal small intestine was detected ultrasonographically. Other reasons of increased small intestinal thickness can be: infiltrative bowel disease, peritonitis, intraabdominal abscessation, salmonellosis, lawsonia intracellularis infection or alimentary lymphosarcoma. The duodenum cannot be assessed by other means than ultrasonography (except for its most oral part which can be imaged endoscopically). The locations to image the duodenum are ventral to the caudal pole of the right kidney at the 16<sup>th</sup>-17<sup>th</sup> intercostal space on the right flank and in the 12<sup>th</sup>-15<sup>th</sup> intercostal spaces between the liver and the right dorsal colon. The duodenal wall thickness measures 3-4 mm. Its contents consist of chyme or fluid and, sometimes, small amounts of gas.

**Large intestine:** The large intestine can be identified on the basis of its sacculated appearance and the hyperechogenic luminal contents. The right dorsal colon does not show a sacculated appearance but rather a smooth curving surface on the right side (between the 10<sup>th</sup> and 14<sup>th</sup> intercostal spaces) adjacent to the liver. The right dorsal colonic wall thickness ranges from 2.2-5.9 mm in healthy horses. Intestinal motility can readily be imaged. The caecum can be identified due to its dorsoventrally oriented taenial bands.

Impactions due to ingestion and accumulation of sand have been diagnosed ultrasonographically. Typical hyperechoic granular echoes casting acoustic shadows can be imaged in the most ventral abdomen. Furthermore the motility of the affected colon is reduced.

The intestinal wall in cases of colon torsion gets oedematous with a loss of its normal layering. Pease et al. (2004) defined a colonic wall thickness of >9 mm in the ventral abdomen as suggestive of colon torsion with a sensitivity of 67% and a specificity of 100%. Other identifiable diseases are intussusceptions, commonly involving the caecum or right ventral colon, large colon displacements and right dorsal colitis. In the healthy horse, the colonic mesenteric vessels cannot be imaged in transcutaneous ultrasonography. In the case of right dorsal displacement of the large colon or a 180° colon volvulus, though, these vessels can be followed transversely at the right abdominal body wall.

**Nephrosplenic space:** The spleen can be imaged from the left abdominal wall. It usually extends from the 8<sup>th</sup> intercostal space (cranial pole) to the left flank region (caudal pole). The parenchyma should be homogenous and moderately echogenic (hyperechoic compared to the liver parenchyma) with a smooth rounded dorsal border.

The nephrosplenic space can be imaged dorsally in the left 17<sup>th</sup> intercostal space. In cases of left dorsal displacement of the large colon (LDDLC), the left kidney cannot be imaged dorsomedial to the spleen,

the spleen is displaced ventrally and a horizontal hyperechoic borderline between the dorsal image of the spleen and the entrapped bowel can be visualized. Ultrasound is a useful complementing diagnostic aid to diagnose an LDDLC, but false positive and false negative diagnoses occur. Therefore, ultrasonographic findings should always be interpreted together with the findings of transrectal palpation.

**Conclusion:** Ultrasonography of the equine abdomen is a well-tolerated, non-invasive technique, which in experienced hands can provide information about large parts of the gastrointestinal tract and inner organs. It can complement the clinical examination and other diagnostic techniques and help to decide towards medical or surgical colic treatment as well as help to identify intraabdominal masses and their nature. As with any other diagnostic method, ultrasonographic findings should always be considered critically with respect to the other clinical and clinicopathological data and other diagnostic procedures of the patient.

## O2

### Myxomatous mitral valve disease – epidemiology and prognostic factors

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**Introduction:** Myxomatous mitral valve disease (MMVD) is the most common cardiovascular disease that affects primarily small breed and aged dogs, with an exception of Cavalier King Charles Spaniels that can be affected at an early age. Myxomatous mitral valve disease is an important cause of morbidity and mortality of the affected population. Odds ratios (OR) for MMVD were calculated in affected breeds. Survival was analyzed with Kaplan-Meier analysis.

**Material and methods:** Medical records of 9238 dogs were reviewed. Among them 559 dogs (6%) were found with confirmed cardiac diseases, out of which 489 dogs (87%) had acquired heart diseases. Within this latest group 337 dogs (68%) had myxomatous mitral valve disease (MMVD). There were 2.95 times more males than females affected with MMVD. The mean age within the MMVD group was 10 years. Tricuspid valvular disease was represented in 9.8% of dogs and mitral valve prolapse in 12.5 % of cases. The five most commonly represented breeds were as follows: miniature and medium poodle (120 dogs), mixed breed (64 dogs), English Cocker Spaniel (35 dogs), Pekingese (15 dogs) and Doberman pinscher (10 dogs). The most commonly presented breeds OR were as follows: miniature and medium poodle 6.8 ( $p < 0,001$ ), English Cocker Spaniel 1.7 ( $p = 0,003$ ), mixed breed 0.7 ( $p = 0,012$ ), Pekingese 3.4 ( $p = 0,000$ ) and Doberman Pinscher 1.2 (NS). Cavalier King Charles Spaniel had an OR of 7.5 ( $p = 0,012$ ).

**Results:** Median survival in the group of MMVD ( $n = 109$ ) was 614 days. We compared the length of survival in different breeds. There was a significant

difference in survival curves between mixed breed dogs (n = 21; median survival 353 days) and other dogs of various breeds (n = 8; median survival 697 days); the same was observed comparing mixed breed dogs with Poodles (n = 27; median survival 872 days) and Cocker Spaniels (n = 14; median survival 780 days). However in all three comparisons 20% of dogs had the same survival without significant difference.

**Conclusion:** From this we could conclude that either mixed breed dogs are given less care or their survival is simply worse. Comparison between survival curves of other more commonly represented breeds showed no significant differences. Dogs that had atrial fibrillation had significantly shorter survival (n= 7; median 195 days) than dogs without this arrhythmia (n=102; median survival 632 days). Dogs with atrial fibrillation, atrial premature beats and ventricular premature complexes together had significantly shorter survival time (median survival 219 days) than dogs without these arrhythmias (median survival 634 days).

### O3

#### **Preliminary studies on the prevalence of specific antibodies against *Mycoplasma bovis* in calves**

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**Introduction:** Most of the *Mycoplasma* microorganisms have a secondary role in the appearance of infection in cattle. *Mycoplasma bovis* (*M. bovis*) is an exception; this agent has a primary role in the occurrence of the disease. It is proven that *M. bovis* is frequently a causative agent for pneumonia, mastitis and arthritis in cattle. Mucositis of the respiratory tract is a primary site for the colonisation of *M. bovis* in cattle. Respiratory tract mucosa and mammary gland are the most important locations for the maintenance and secretion of *M. bovis*, which can last for several months even. Stress factors such as transportation, entrance into the feeding object, coldness, etc. are related to the nasal secretion of *M. bovis*. Animals with chronic infection and no clinical symptoms, are occasionally shedding *M. bovis* and they are very important for the epidemiology of the infection. The diagnostic procedure is based on clinical symptoms and detection of causative agent, regardless if the infection is found in individual animals or in the whole herd. The most used indirect method is the ELISA test.

**Material and methods:** During a three year period, blood serum samples from calves were collected, examined and analyzed. The samples were taken from animals originating and living on the territory of Serbia, in different regions. Calves were from 7 cattle farms, big and small, and they were of Holstein - Friesian and Simmental

breed. The total number of 4782 samples was collected and examined. The blood sera are analyzed with indirect ELISA test, Biovet Inc. *Mycoplasma bovis* Antibody Test Kit Bovichek® *M. bovis*.

**Results:** In total, from 4782 blood serum samples, positive findings were detected in 237 animals, which means that in 4, 96 % of the total population of the calves examined, specific antibodies against *Mycoplasma bovis* were found.

**Conclusion:** Obtained results show that *M. bovis* is present in cattle herds and it is necessary to continue the research of the epizootical aspect and the role of *M. bovis* in the occurrence of health problems in cattle population. Considering the possible economical losses involved, more detailed research should be taken into consideration.

### O4

#### **Examination of ampicillin resistant *Enterococci* (VRE) isolated from canine and feline fecal swabs**

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**Introduction:** Antimicrobial resistance is an important problem in companion animals, because of the difficulty to treat infections and potential zoonotic transmission. *Enterococci* species, which are normal inhabitants of the gut flora of healthy animals and humans, began to be recognized as an important pathogen in both human and veterinary medicine due to the acquired resistance profiles. The aim of the study is to examine the diversity of ampicillin resistance enterococci species, their antimicrobial susceptibility profiles and to examine virulence related genes in those isolates in cats and dogs.

**Material and methods:** For this purpose, rectal swabs from companion animals were collected and processed for ampicillin resistant enterococci isolation. Following the identification of the isolates, antimicrobial susceptibility of the isolates was determined according to the Clinical and Laboratory Standards Institute (CLSI) standards. Finally, presence of virulence related genes such as *ace*, *gel E*, *efa A*, *AS* and *esp* were examined by PCR.

**Results:** One hundred fifty seven swab samples (86 canine and 71 feline) were examined. VRE were isolated from 18 canine and 17 feline samples. Antimicrobial susceptibility results were varying among the isolates.

**Conclusion:** The VRE isolation rate among pet animals was 22.3 %. Screening of antimicrobial resistant enterococci among companion animals would be useful to detect any emerging antimicrobial resistance problem related to public health.

**O5****Biochemical parameters of brown Swiss heifers in late pregnancy and puerperium**

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**Introduction:** Brown Swiss cattle are a breed with high genetic merit for milk producing. They originated from the slopes of the Alps in Switzerland. According to literature data they are resistant to high environmental temperature and other climatic changes that may cause dairy cows health problems.

**Material and methods:** Sixteen Brown Swiss heifers, which were imported in high pregnancy stage, were involved in the study. Their calving started at the end of May with the duration of the whole of June, with high environmental temperatures. Diet was adequate for the Brown Swiss heifers in the late stage of lactation. After calving they produced approximately 3l milk per day, according to the data of veterinarian. Blood samples were taken by puncture of the *vene jugularis* in the same herd of Brown Swiss heifers, but from two different categories. First group (n=8) included heifers in late gestation, immediately before calving, and the second group (n=8) involved heifers immediately after calving, when clinical signs of metabolic diseases appeared. After spontaneous blood coagulation, serum was used for biochemical analysis by semiautomatic photometer STAT FAX 3300, USA. Biochemical parameters that included glucose, total protein, albumin, calcium, phosphorus, urea, creatinine, triglycerides, cholesterol, magnesium, AST and BHBA were determined by following the instructions of the manufacturer Human Germany. Estradiol and cortisol concentrations were determined by ELISA, on spectrophotometer BDSL Immunoscan Plus, USA, by the manufacturer's instructions.

**Results:** Glucose and total protein concentrations between the two categories of Brown Swiss heifers, late pregnant ( $3.82 \pm 0.40$  mmol/L;  $72.37 \pm 9.67$  g/L, respectively) and puerperal ( $3.46 \pm 1.44$  mmol/L;  $74.16 \pm 6.06$  g/L, respectively) didn't show any statistical differences, because of balanced and sufficient alimentary energy nutrients. Albumin concentration was significantly higher ( $p < 0.05$ ) in late pregnant ( $31.85 \pm 11.37$  g/L) than puerperal Brown Swiss heifers ( $19.33 \pm 5.20$  g/L). Mild hypoalbuminemia in puerperal cows was observed probably due to inability of the liver for albumin synthesis. Serum concentrations of calcium, phosphorus, magnesium, as well as urea and creatinine in Brown Swiss heifers in late pregnancy ( $2.48 \pm 0.27$  mmol/L;  $3.06 \pm 0.28$  mmol/L;  $0.95 \pm 0.13$  mmol/L; as well as  $3.74 \pm 1.69$

mmol/L;  $118.95 \pm 24.43$   $\mu$ mol/L respectively) did not show significant difference from puerperal Brown Swiss heifers ( $2.50 \pm 0.10$  mmol/L;  $3.07 \pm 0.67$  mmol/L;  $0.81 \pm 0.15$  mmol/L; as well as  $2.49 \pm 1.23$  mmol/L;  $108.95 \pm 12.82$   $\mu$ mol/L respectively) although hyperphosphatemia appeared in both groups. Significantly lower ( $p < 0.05$ ) concentration of triglycerides in puerperal Brown Swiss heifers ( $0.09 \pm 0.04$  mmol/L) compared to heifers in late pregnancy ( $0.27 \pm 0.09$  mmol/L) occurs probably as a result of reesterification of nonesterified fatty acids in triglycerides and their retention in the hepatocytes causing hepatic lipidosis and serious liver impairment. Cholesterol serum concentration follows the tremendous metabolic changes that occur in the two different stages. Cholesterinemia in puerperal cows ( $1.24 \pm 0.32$  mmol/L) was significantly lower ( $p < 0.05$ ) than in late pregnant Brown Swiss heifers ( $3.01 \pm 1.06$  mmol/L), probably due to insufficient liver capacity for cholesterol synthesis and its elimination in the blood stream. Ketonemia within the physiological ranges in both groups and there was no significant difference between late pregnant Brown Swiss heifers ( $0.34 \pm 0.13$  mmol/L) and puerperal heifers ( $0.48 \pm 0.20$  mmol/L). Nonesterified fatty acids released by lipomobilisation were not oxidized in ketone bodies, because of shift metabolic pathway in liver lipogenesis. Serious liver failure was concomitant with statistically significant increase ( $p < 0.05$ ) of AST concentration in puerperal Brown Swiss heifers ( $197.48 \pm 128.51$  U/L), contrary to AST concentration in late pregnant heifers ( $80.05 \pm 14.60$  U/L). High concentration of estradiol ( $378.50 \pm 114.88$  pg/ml) in Brown Swiss heifers in the late gestation has a favorable effect on hepatic lipogenesis; although the concentration of estradiol after calving was significantly lower ( $10.37 \pm 8.74$  pg/ml). Cortisolemia was significantly higher ( $p < 0.05$ ) in puerperal Brown Swiss heifers ( $41.08 \pm 30.82$  ng/ml) compared with the cortisol concentration before calving ( $10.50 \pm 4.19$  ng/ml). Hypercortisolemia in puerperal cows was an indicator of inappropriate postcalving adaptation.

**Conclusion:** According to obtained results for certain biochemical and hormonal parameters, the Brown Swiss heifers in our study were not able to realize their genetic merit as high producing dairy cows due to the inability of metabolic pathways to overcome the critical period and to achieve successful adaptation to stress syndrome.

**O6****Serology testing of rabies vaccination efficacy in pet animals in R. Macedonia in the framework of non-commercial movement of pet animals in the EU**

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**Introduction:** According to the EU Legislation, the Republic of Macedonia is not listed in Part C of Annex

II of EC Regulation No. 998/2003 that lays down the requirements applicable to the non-commercial movement of pet animals. Therefore, when the pet animals originate from rabies-infected third countries (such as R. Macedonia) and enter the E.U., they must be tested for presence of neutralizing rabies antibodies. The test has to be done at least 30 days post vaccination, and the travel/import can be approved 3 months after the blood sampling. Starting from 1<sup>st</sup> of January 2013, the Laboratory for Rabies at the Veterinary Institute - Faculty of Veterinary Medicine in Skopje, is an approved laboratory for serological tests to monitor the effectiveness of rabies vaccination (*Official Journal of the European Union*, 6.12.2012: COMMISSION IMPLEMENTING DECISION of 4 December 2012).

**Material and methods:** The survey is conducted on 203 dog sera and 34 cat sera submitted in the Laboratory for Rabies for neutralizing antibody titration in order to monitor the effectiveness of rabies vaccination in the framework of the non-commercial movement of pet animals in the EU. The tested samples originated from animals vaccinated with 5 different vaccines (Rabikal, Rabisin, Defensor, Canvac – R and Biocan). The neutralizing antibody titration was conducted with the Fluorescent Virus Neutralization test (FAVN) as prescribed by the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2013, Chapter 2.1.13. Rabies and the Standard Operating Procedure of the European Union Reference Laboratory for Rabies Serology (ANSES Nancy Laboratory for Rabies and Wildlife).

**Results:** One hundred and eighty eight dog sera (92.6%) and 31 cat sera (97.1%) had satisfactory titer above the threshold value (0.5 IU/ml). Fifteen dog sera (7.3%) and 1 cat serum (2.9%) did not have the necessary protective titer.

**Conclusion:** The results from FAVN test indicate that there is a high success rate of the rabies vaccination.

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## O7

### Differentiation diagnosis of *Fasciola hepatica* from different animal hosts by PCR

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**Introduction:** Molecular techniques are utilized for the diagnosis of parasitic diseases and identification of parasites, for the development of specific antigens for serological tests and studying immune response in the patients.

**Material and methods:** Random amplified polymorphic DNA-PCR technique was used to differentiate *Fasciola*

*hepatica* DNA from sheep and goat. *Fasciola hepatica* was obtained from sheep and goats. These parasites were washed several times with pH 7.4 phosphate-buffered saline and phenol extraction/ethanol precipitation method was employed in the genomic DNA extraction. In order to optimize the PCR conditions, five primers were selected to amplify the DNA of each *F. Hepatica*. Both sheep and goat products were amplified in different numbers and sizes by RAPD-PCR technique.

**Results:** The results show that different primers gave different bands (fragments) and allowed to identify genetic variations of *F. hepatica*. These bands were species-specific to *F. hepatica* from goat and sheep.

**Conclusion:** Thus the RAPD-PCR method can be useful for the differentiatonal diagnosis of *F. hepatica* of sheep and goat.

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## O8

### Clinical efficacies of inactive *Moraxella bovis* autovaccine in a farm with infectious keratokonjunctivitis

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**Introduction:** Infectious bovine keratoconjunctivitis (IBK) is a highly contagious ocular disease of cattle caused by *Moraxella bovis* and *M. boviculi*. There are 7 serogroup/serotype of *M. bovis*. IBK is usually treated by some antibiotics. Prevention is also conducted by fly control using insectisits.

**Material and methods:** Six ocular samples from infected animals with suspected IBK were cultured on different media and 4 *M. bovis* were isolated. A selected *M. bovis* isolate was cultured in Todd-Hewith with 20 % horse sera at 37°C for 48 h. The culture centrifuged and pellet was inactivated by BEI and 0.5 % formalin. Inactivated bacterine was homogenized with Montanid IMS 3012. After sterility and safety tests, all animals were vaccinated by IM injection and conjunctivally spray.

**Results:** The population of the farm was 1075 cattle. The vaccine efficacies were calculated according to state of the infected and healthy/new cases before and after vaccination. Before vaccination 236 infected animals were treated by antibiotics, while after vaccination, 35 animals were treated with antibiotic. The clinical efficacy of the vaccine was determined to be 6,74-fold and the vaccine protects cattle by 85%.

**Conclusion:** There are numerous *M. bovis* vaccination studies. Most of them report that the efficacy of the *Moraxella bovis* vaccine is low. Briefly, we may be successful in our study, because our vaccine candidate is autovaccine.

**O9****Haematological and coagulation profiles during severe tropical *Theileriosis* in cattle**  
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**Introduction:** Tropical theileriosis is a tick-borne haemoprotzoan disease caused by *Theileria annulata* and transmitted by *Hyalomma spp.*, is one of the most devastating blood parasites affecting cattle. The prevalence, morbidity, and mortality of tropical theileriosis are considerably high. It is estimated that 250 million cattle in many countries, including the southern Mediterranean countries, Turkey, India, and China, are at risk from the disease, which causes serious economic loss through bovine mortality and lost productivity.

**Material and methods:** Blood samples were analysed from 46 adult (19 males and 27 females) Holstein cattle suffering from severe theileriosis that were in the progressive stage of the disease. The age of all animals used in this study ranged from 1.5 to 3 years. A total of 46 clinically healthy Holstein adult (25 males and 21 females) cattle from tick-free farms were used as a control group. They were free of any external, blood, and internal parasites. Blood smears were prepared from the ear tip of animals showing poor general health, cachexia, enlarged nodes, and a rise in body temperature (> 39°C).

For the haematological analysis, approximately 4 ml blood samples were taken from the jugular vein with a syringe containing EDTA. Haematological parameters were determined immediately. For coagulation parameters, blood samples (9 vol.) were collected in 3.8% sodium citrate (1 vol.). The blood collection tubes were kept on ice for no longer than 2h after blood withdrawal to avoid denaturation of proteins. Platelet poor plasma was obtained by centrifugation at 1600 xg for 20 min at 4°C and was stored at 70°C until analysed.

**Results:** Haematological analysis indicated significant decreases in red blood cell count, haematocrit value, haemoglobin amount, mean corpuscular haemoglobin concentration, and white blood cell, lymphocyte, neutrophil, monocyte, eosinophil, and basophil counts. On the other hand, significant increases were seen in mean corpuscular volume and marked reticulocytosis in infected animals compared to the animals in the control (uninfected) group. In the coagulation profile, activated partial thromboplastin time and prothrombin time were significantly prolonged, and platelet counts were significantly less in the infected group compared to those in the control group. Fibrinogen concentration was slightly higher in the infected group than that in the control group.

**Conclusion:** These observations revealed that *T. annulata* infection in cattle is associated with marked changes in the haematological and coagulation parameters.

**O10****Two different protocols for pharmacologically-induced ejaculation in *Equus asinus***

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**Introduction:** Semen collection in male donkeys is very important not only for artificial insemination of jennies or mares, but also for diagnosis and prevention of venereal diseases. Often it can be a problem to train them to mount the phantom, so pharmacologically-induced ejaculation could be one of the possible methods of semen collection.

**Material and methods:** Ten mature male donkeys (3 – 10 years) were used in this study. Two different treatment protocols were used in each of the ten male donkeys. The interval between treatments was at least 24 hours. The first protocol involved the intramuscular application of *Detomidine chloridum* in a dose of 0,02 mg/kg. If ejaculation was not achieved, another intramuscular application of *Detomidine chloridum* was repeated after 15 minutes in a half dose 0,01 mg/kg. The second protocol involved a single intravenous application of *Xylazine* (Xylazin Riemsler 20mg/ml, Bioveta a. s., ČR) in a dose of 0,66 mg/kg. After application of the drugs, the animals were left without any restriction and observed the whole time from administration to complete recovery from sedation.

**Results:** For each animal reproduction history, age, current farming methods and also the response to each of the two treatment protocols was judged. After intramuscular detomidine administration in 2 cases of 10 trials (20 %), ejaculation was observed. After intravenous xylazine administration none of the 10 male donkeys ejaculated. Any reliance between age, reproduction history or farming method was not found.

**Conclusion:** Our results suggest poor success rates of pharmacological-induced ejaculation in donkeys using xylazine and detomidine protocols as published for use in horses. We hypothesize that animal handling and environmental stress likely contributed to the low success rate of these protocols that were originally studied in compliant horses in quiet, controlled environmental conditions.

**O11****Radioprotective effect of montelukast sodium against hepatic radioiodine (<sup>131</sup>I) toxicity: a histopathological investigation in the rat model**

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**Introduction:** Radioactive iodine (<sup>131</sup>I) therapy is widely used in the treatment of hyperthyroidism due to Graves' disease or active nodules and ablation, recurrences or metastases of differentiated thyroid cancer in cats, dogs and humans. Montelukast sodium (Cysteinyl leukotriene receptor-1 antagonist) is also a well-known antioxidant drug. This study aimed to evaluate the histopathological changes in rat livers in the third month following <sup>131</sup>I treatment and the radioprotective effect of Montelukast sodium (ML) against <sup>131</sup>I-related liver damage.

**Material and methods:** Thirty female Wistar Albino rats were randomly divided into three groups: as control group (n=10), untreated rats; second (RAI) group (n=10), oral radioiodine (111 MBq) administrated group, and third (ML) group (n=10), oral radioiodine and ML administrated group. In the third group, ML administration was started 3 days before and ended 10 days after RAI administration. In the third month of radioiodine (RAI) administration, the animals were decapitated and the livers were removed for histopathological examination. The histopathologic data were evaluated comparatively by using the Mann Whitney U test and Fisher's Exact Test Differences were determined in all the parameters in terms of intensity between the control and <sup>131</sup>I groups, which showed the harmful effects of <sup>131</sup>I.

**Results:** In the comparison of the <sup>131</sup>I and ML groups, hyperemia was determined respectively 80% to 40%, the presence of inflammatory cells 70% to 60% and capsule thickening 70% to 40%. Montelukast sodium was observed to have a protective effect especially on hyperemia and capsule thickening.

**Conclusion:** According to the study results, radioactive iodine (<sup>131</sup>I) treatment seems to cause morphological damage to the rat liver, and Montelukast sodium effectively protects the liver against damage.

**O12****Risk management strategies in case of outbreak of Crimean Congo hemorrhagic fever (CCHF) and other vector-borne diseases (VBD): a comparative case study of R. Macedonia, Turkey and Germany**

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**Introduction:** Vector-borne diseases (VBD) are of increasing global Public Health interest given ongoing changes in the environment, global trade and worldwide travel. Improved concepts of adequate risk management together with meaningful risk communication strategies are badly needed to enhance risk governance. Findings of a comparative study, conducted within the EU-funded project 'Biology and control of vector-borne infections in Europe. EDENext (www.edenext.eu), on the risk management strategies dealing with the CCHF in R. Macedonia and Turkey and with the Hantavirus in Germany show considerable differences in organizational structures and risk communications patterns. The risk governance structures on VBDs in each of the three countries will be described and conclusions drawn for the best practices in risk management and risk communication, which are becoming of increasing interest for Public and Veterinary Health.

**Material and methods:** The findings are based on qualitative, explorative data acquired by in-depth interviews with experts in risk management and communication strategies on CCHF and other VBDs in the R. Macedonia, Turkey and Germany.

**Results:** The presence of CCHF and VBD situation is different in the three selected countries: Whereas CCHF is considered as endemic in some areas in Turkey, the situation in the R. Macedonia is indefinite as only recently serological positive ruminants on CCHF were detected as well as the main vector *Hyalomma marginatum* in several regions in the Eastern part. Macedonian PH institutions are dealing effectively though identically with all hemorrhagic fevers, not distinguishing different pathogens. In Germany no CCHF exists, but the Hantavirus is considered endemic in several parts. The risk management strategies and risk communication patterns by Public Health authorities vary in these three countries, influenced by the presence or absence of different VBDs such as CCHF and the Hantavirus. Despite all the differences in organisational risk governance structures in R. Macedonia, Turkey and Germany, we found in all three countries that Public Health measures are based on the principle of scientific risk assessment with the goal to reduce the public health risk successfully.

**Conclusion:** The presence of different VBDs, the different organizational units in each of the countries, a precise definition of the endemic regions, as well as differences in risk perception and risk cultures have to be taken into

account when undertaking meaningful public health activities. A comparison of the risk management structures of the three countries shows the need for cooperation and the potential for mutual learning in regard to effectively dealing with VBDs.

### O13

#### Equine neurology – how to end up with a neuro-anatomical diagnosis

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**Introduction:** Equine neurology can be a challenge for the practitioner. The aim of the workshop is to find an easy to follow structure to identify neurologic lesions and localize them according to the neuroanatomy of the horse.

**Neurologic examination:** The clinical neurologic examination aims at assessing whether there is a neurologic disease and where it is located. Information gathered from the history and general clinical examination can help to exclude non-neurologic diseases from the list of differential diagnosis. For the clinical neurologic examination itself it is important to follow a complete and structured protocol. At first the patient should be observed from a distance to assess the mental state, behavior and posture of the horse.

**Mentation:** The brainstem with its formation reticularis and the ascending reticular activating system are the primary site responsible for the patient's consciousness. Lack of consciousness (graded in depression, somnolence, stupor, or even coma) is therefore commonly due to brainstem lesions, but can also be signs of cerebral dysfunction.

**Behaviour:** The behavior is mainly driven by cerebral function. To determine the behavior, historical information about changes in the horse's behavior are as important as the observation of the patient at the time of examination. Anxiety or aggressive behavior can be hard to deuten. Compulsive stall walking, head pressing, and seizures are obvious signs of cerebral involvement in the disease process.

**Posture:** Already the patient's posture can hint towards a neuroanatomical location. A head tilt can for example regularly be found in association with a vestibular syndrome; a head turn, though, is rather an indication of a unilateral cerebral lesion. Lowering of the head and neck could be due to a somnolence or stupor (i.e. forebrain lesion), but also an expression of generalized weakness (i.e. lower motor neuron disease).

**Cranial nerve function:** Cranial nerve (CN) function tests can help to determine the site of the lesion even more. With the exception of CN I, CN II and CN XI, the cranial nerves originate from the brainstem. To test the olfactory nerve the horse is presented with a smelly substance (e.g. ethanol or herbs) and the reaction is observed. Gentle touch of the finger around the eyes induces a palpebral

reflex (CN V and CN VII). This test is followed by a menace response (CN II and CN VII). The position of the eye (strabismus, nystagmus?), the pupils, and the size of the pupils are noted. To characterize nystagmus, the head is moved from one side to the other and changes in eyeball position are observed. The swinging light test assesses the pupillary response by shining a bright torchlight into each eye from an arm's distance. If a deficit is observed, the dazzle response should be evaluated. Commonly, horses with cerebellar lesions display a negative menace response despite normal eyesight (positive dazzle response). Facial sensory innervation (CN V) can be tested using blunt hemostats. These can also be used to stimulate the nasal septum. A reduced response implies either a lesion of CN V or cerebral perception. The more caudal parts of the CNS are evaluated at rest and tested for spinal reflexes. Neck, trunk, and limbs are assessed for muscle symmetry, differences in surface temperature, sweating and muscle tone.

**Spinal reflexes:** Spinal reflex tests help to examine the function of specific spinal cord segments and their associated nerve roots. The slap test (thoracolararyngeal response), cervicofacial reflex, flexor reflex, cutaneous trunci reflex and the anal reflex can be tested in most patients using blunt hemostats. During testing of the anal reflex, tail tone and perineal sensation can be tested at the same time.

**Pain perception:** To test a horse's pain perception a nociceptive stimulus using a forceps or needle is applied to the neck, trunk or limbs. After conscious perception the horse will react with an avoidance behavior.

**Gait assessment:** The assessment of the gait is a central component of the neurologic examination. It aims at the identification of the limbs showing gait abnormalities and should be performed on a hard surface, with the horse walked in a straight line and thereafter with the horse walking in serpentine and circles. The horse should rather be walked than observed at the trot because an ataxia is seen much easier in slower movements. The horse should be observed from all sides. If there is doubt about the gait characteristics walking the horse on soft ground, above obstacles or in small circles can exacerbate the ataxia and make it easier to detect. If the horse is diminished of eyesight compensation (e.g. walking with elevated head or blindfolding) an existing ataxia will also be more pronounced. In cases of vestibular disease, blindfolding of the horse can worsen the ataxia and the head tilt. An ataxia of all four limbs can be due to a lesion of the cervical spine or an intracranial lesion. If the lesion is located in the thoracolumbar spinal cord, only the hind limbs are affected by ataxia.

**Proprioceptive testing:** The evaluation of the horse's proprioception is done by pulling the tail at rest and with the horse walking in a straight line. Pushing the standing horse to one side and backing of the horse are also useful tests to observe proprioceptive deficits. Trotting the horse or leading the horse in tight circles and performing an abrupt stop is more useful to detect proprioceptive deficits than manual placement of the limbs in non-physiologic postures.



**Conclusion:** At the end of the neurologic examination all findings are summarized and it is assessed whether there is a neurologic lesion. Secondly, the neuroanatomic location of that lesion is determined. Further diagnostics are usually necessary to find an etiologic diagnosis for the individual patient. These diagnostics are beyond the scope of this workshop and include CSF analysis, radiology, computed tomography, magnetic resonance imaging, myelography and serology among others.

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#### O14

### Basic principles of ECG readings in small animals

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Electrocardiography (ECG) represents an important diagnostic tool in the veterinary practice. ECG enables evaluation of the electrophysiology of the heart disorders, as well as noncardiac disorders (electrolyte balance, metabolic disturbances). Finally, ECG is a very useful tool for monitoring the efficiency of cardiac therapy.

Beside the usefulness of the ECG as a screening method for majority of the heart diseases, it is important to note its limitations. Namely, ECG records only a short period of heart activity and for that reason it is most effective in detecting only continuous arrhythmias. Abnormalities that occur predominantly at night or in stressfully situations may be missed on routine examination. In cases where longer monitoring of heart function is required, Holter monitor should be used.

Current veterinary practices have proactive approach in the early diagnosis and treatment of heart diseases to improve quality and length of life. ECGs can be helpful in identifying heart disorders much earlier in the course of the disease, before the appearance of the clinical signs, increasing the scope of therapeutic interventions and preventing or delaying progression of the heart diseases. Regardless of the method of acquisition, the value of an ECG lies in its interpretation. ECG provides only two pieces of information with any degree of reliability: the heart rate and heart rhythm. Other information including an assessment of chamber sizes, axis shift and fluid accumulation is more or less inferred and therefore less dependable.

The following paper will describe the principles of ECG diagnosis, heart rate calculation, and rhythm analyses including information considering arrhythmias as abnormalities in impulse formation or disorders in impulse conduction. Based on our clinical cases this presentation will include some of the heart disorders seen in veterinary practice.

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#### O15

### Echocardiography – what does it tell me?

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Echocardiography is a method of assessment of cardiac structure and function with diagnostic ultrasound. Echocardiography started to become a universal clinical and research tool in veterinary medicine in the early 80's. Echocardiography added tremendously to the understanding of cardiac physiologic and pathologic conditions. Morphology of different structures such as valves, chordae tendinae, papillary muscles, free wall and septum of all cardiac chambers can be visualized and quantified. Systolic and diastolic function can be analyzed and assessed. Furthermore, Doppler echocardiography helps assessing movement of blood and its velocity through which a pressure gradient across valves can be calculated. The latest helps to quantify severity of the stenosis, pressure in the pulmonary or systemic vasculature, etc. Color flow mapping enables visualizing abnormal flow (i.e. regurgitant, shunting) through valves or defects.

In this basic overview, a systematic approach to the patient will be discussed. Standard views will be shown as well as common congenital and acquired disease features will be commented. Quantification of different pathologic conditions will be discussed and a hands-on workshop will be provided to enable participants to experience and understand the standard views and acquire basic measurements.

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#### O16

### Molecular epidemiology: a new approach to food safety

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In the past decades, campylobacteriosis, salmonellosis and Verotoxigenic *Escherichia coli* (VTEC) infection have largely replaced tuberculosis, brucellosis and parasitic diseases as the most common sources of foodborne zoonoses in humans in the EU. This shift in food safety hazard has laid the grounds to push forward a more risk- and food chain-based approach in Europe. This has translated into looking at different possible interventions not only at a pre-harvest (increasing biosecurity and detection of latent infection in livestock and making use of vaccination programs for more unconventional infections at the farm), but also at post-harvest level. In recent years, the European Food Safety Authority (EFSA) carried out an evaluation of meat inspection of the main food producing animals in the public health context and the outcome of this risk assessment is one of the main drivers of the implementation of the modernisation of meat inspection that has already started in some Member States. At the same time, to support and inform intervention strategies to reduce the risk to humans from food poisoning is important to quantify how much infection comes from each source.

This is where molecular epidemiology plays a key role to help identifying the “strains” of pathogens that can be found in animals, food or environment and matching them with the strains that are responsible for human infection. Matching strains between infection sources and human cases is used to evaluate the contributions of different sources to human infection. The application of molecular epidemiology techniques in outbreak detection and investigation, attribution modelling and the integration of the resulting data in risk assessment and management has the potential to encourage a holistic and structured approach to the threats posed by foodborne pathogens along the food chain.

### O17

#### Antimicrobial resistance of *Staphylococcus aureus* isolated from cow's udder in Serbia

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**Introduction:** *Staphylococcus aureus* is often present in the cow's udder and may cause the occurrence of subclinical and clinical mastitis. Due to the ability to synthesize thermo stable staphylococcal enterotoxins, it is a common cause of food poisoning at humans.

**Material and methods:** On 46 dairy farms in central Serbia, cows with abnormal secretion were identified using the California mastitis test. From two large dairy farms in Vojvodina samples were taken from udder quarters with clinical mastitis. From these samples, a total of 75 strains of *Staphylococcus aureus* were isolated and confirmed by both standard microbiological methods for isolation and identification and API Staph test, as well as by detection of *nuc* gene using the PCR method. 62 isolates of *Staphylococcus aureus* were isolated from udder quarters suffering subclinical mastitis and 13 isolates were isolated from udder quarters suffering clinical mastitis. The PCR method was used for determining the presence of a gene responsible for methicillin resistance in strains of *Staphylococcus aureus*. Susceptibility to antimicrobial drugs was determined by the disk diffusion method of Kirby - Bauer for amoxicillin, amoxicillin/clavulanic acid, ampicillin, bacitracin, neomycin, novobiocin, penicillin, tetracycline, trimethoprim and trimethoprim/sulfamethoxazole.

**Results:** All 75 strains of *Staphylococcus aureus* were sensitive to amoxicillin/clavulanic acid, novobiocin, tetracycline and trimethoprim/ sulfamethoxazole. 67 strains (89.33%) were resistant to bacitracin, 13 (17.33%)

to penicillin, 4 (5.33%) to ampicillin and 3 (4.00%) to amoxicillin. Most of the isolates were resistant to one antimicrobial drug, 46 (74.19%) out of 62 isolates originating from cows with subclinical mastitis and 7 (53.85%) out of 13 isolates originating from cows with clinical mastitis. The two most resistant isolates were resistant to 4 out of 10 antimicrobials originating from cows with clinical mastitis. All *Staphylococcus aureus* isolates originating from cows with clinical mastitis were resistant at least to one antimicrobial drug. However, there were 8 (12.90%) isolates originating from cows with subclinical mastitis that showed no resistance at all. The presence of the gene for methicillin resistance was detected in 1 (1.33%) out of 75 isolates of *Staphylococcus aureus* originating from the cow's udder with clinical mastitis.

**Conclusion:** Isolates of *Staphylococcus aureus* originating from cow's udder with subclinical mastitis were resistant to fewer antimicrobials than isolates of *Staphylococcus aureus* originating from cow's udder with clinical mastitis on the basis of susceptibility to 10 antimicrobials. Data on the nucleotide sequences of isolates of *Staphylococcus aureus* were deposited in GenBank and assigned the accession numbers of KJ023978 to KJ024046.

### O18

#### Genetic relatedness of oxytetracycline resistant foodborne *Staphylococcus aureus* strains

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**Introduction:** Antibiotics have been used for decades as growth promoters in animal nutrition and for prevention and treatment of bacterial diseases in animal husbandry and aquaculture. Oxytetracycline is one of the two tetracyclines approved by the USFDA for usage in food-producing animals.

**Material and methods:** In this study, 124 foodborne *Staphylococcus aureus* strains were examined for oxytetracycline (30 mg) susceptibility using agar disk diffusion assay according to CLSI protocol. Genetic relatedness of oxytetracycline resistant strains isolated from different food was determined by Pulsed Field Gel Electrophoresis (PFGE) using *Sma*I restriction enzyme.

**Results:** Agar disk diffusion results indicated that 18 (14.5%) *S. aureus* strains were resistant to oxytetracycline with no inhibition zone. The similarity among PFGE band patterns was evaluated using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) similarity coefficient. Analysis of PFGE band patterns using the software and obtained dendrogram revealed that three groups of *S. aureus* strains showed 100% homology with indistinguishable band patterns, but the other strains showed 58.1-92.3% homology. Some strains that were closely related (>80% homology) were isolated from raw milk and dairy products in different cities.

**Conclusion:** Oxytetracycline was not included in CLSI for staphylococci; however, due to inappropriate usage of this antibiotic in treatment and feed of food animals, oxytetracycline resistant food strains can survive and transfer to human. In order to prevent growth of antimicrobial resistant bacteria, these antimicrobials should be used in an appropriate dosage in food animals.

## O19

### Identification and antibiotic susceptibility of coagulase negative staphylococci isolated from dairy cows with subclinical mastitis

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**Introduction:** This study was aimed to identify coagulase negative staphylococci (CNS) isolated from bovine milk samples with subclinical mastitis and to determine susceptibility of microorganisms to antibiotics.

**Material and methods:** Totally, 286 CNS isolates from milk samples were identified according to colony morphology, haemolysis and biochemical properties.

**Results:** Out of the isolates, 40 (13.98%) were identified as *S. simulans*, 31 (10.83%) *S. epidermidis*, 54 (18.88%) *S. chromogenes*, 31 (10.83%) *S. xylosus*, 22 (7.69%) *S. caprae*, 19 (6.64%) *S. warneri*, 18 (6.29%) *S. haemolyticus*, 13 (4.54%) *S. cheiferi* subsp. *coagulans*, 10 (3.49%) *S. cohnii* subsp. *urealyticus*, 10 (3.49%) *S. saprophyticus*, 6 (2.09%) *S. gallinarum*, 7 (2.44%) *S. hominis*, 4 (1.39%) *S. hyicus*, 2 (0.69%) *S. cheiferi* subsp. *cheiferi*, 9 (3.14%) *S. lentus* and 2 (0.69%) *S. sciuri*. The susceptibilities of CNS were determined to be variable to different antimicrobial agents.

**Conclusion:** This study has shown that identification of CNS isolated from dairy cows with subclinical mastitis should be strictly performed.

## O20

### The evaluation of anti-Campylobacter activity of plant extracts/pure compounds of essential oils with native microflora

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**Introduction:** *Campylobacter jejuni* is a Gram negative bacterium that is a major cause of acute gastro-intestinal

diseases in humans. Poultry products are the main reservoir for gastro intestinal diseases in humans and no effective control measures are available at the present time, there is, therefore, a need for the control of *Campylobacter* colonisation in poultry. The present study aimed to determine the anti-*Campylobacter* activity of plant extracts, essential oils in previously developed and validated *in vitro* fermentation system which provides a suitable environment for the proper functioning of bacterial species.

**Material and methods:** Plant extracts (*Acacia decurrens*, *Acacia saligna*, *Eucalyptus occidentalis*, *Eremophila glabra* and *Kennedia prorepens*), *Leptospermum* oil and cineole were tested at three different concentrations (1.5, 3 and 4 mg/mL) and two different concentrations (0.1 and 0.5%), respectively. Thymol was also tested at two different concentrations 0.015 and 0.007%. Anaerobic techniques were applied during all experiments and all cultures were incubated at 39 °C. The number of *C. jejuni* was counted at 24 h of incubation by plating onto CCDA agar plates. The head space gas pressure in each tubes were recorded at 0, 4, 12, 20, 28, 36, 42, and 48 h of incubation. VFAs (acetate, propionate, n-butyrate, iso-butyrate, iso-valerate and n-valerate) were determined by GC. The production of methane in headspace gases was also analysed.

**Results:** The number of *C. jejuni* was not influenced by plant extracts at concentrations of 1.5 and 3 mg/mL, but colony numbers were under detection limits at concentration 4 mg/mL of plant extracts while there were  $4.1 \pm 0.1 \log_{10}$  cfu/mL in control tubes. Addition of plant extracts except *E. occidentalis* did not alter the gas production at a concentration of 1.5 mg/mL. The extracts reduced the gas production at concentration 3 and 4 mg/mL when compared to control. No changes in pH values were observed. Methane production at the end of incubation decreased ( $P < 0.05$ ) when *E. occidentalis* extract was used compared to control. No viable *C. jejuni* were obtained from the treatments including *Leptospermum* oil at 0.1 and 0.05%, cineole at 0.1% and thymol at 0.015%. *Leptospermum* oil and cineole (0.1%) resulted in significantly less end point gas production. The addition of *Leptospermum* oil and thymol (0.015%) resulted in significantly less total VFAs concentration. However cineole did not affect the total VFAs concentration while it significantly reduced the end point gas production. *Leptospermum* oil and cineole at concentration of 0.1% had significant reduction in methane production when compared to control.

**Conclusion:** These results suggest that EOs/compounds used in this study have bacteriocidal activity towards *C. jejuni* at concentrations tested. It also seems that *in vitro* fermentation assay followed by testing of activity against *C. jejuni* permitted to select the best candidate agents for *in vivo* experiment.

**O21****Antimicrobial resistance in bacteria associated with livestock: a situation in Slovenia**

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**Introduction:** Antimicrobial resistance has become a major problem in human and veterinary medicine as a consequence of the intensive use and misuse of antimicrobials. In food-producing animals, multi-drug resistant bacteria (MDRB) represent a serious problem. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are spreading worldwide and based on their epidemiological and genetic characteristics, different types of MRSA may be distinguished. Recently, a new type Livestock-associated (LA) MRSA (multilocus sequence type ST398), has emerged. In the last decade, occurrence in animals of *Escherichia coli* with transferable genes conferring production of extended-spectrum beta-lactamases (ESBL) or AmpC has been increasingly reported. Furthermore, enterococci are also recognized as feared pathogens that can be challenging to treat. Their importance in human medicine is mainly due to nosocomial infections caused by high-level aminoglycoside resistant enterococci (HLAR) and vancomycin-resistant enterococci (VRE). In this paper, we present the importance of MDRB and their prevalence in food-producing animals in Slovenia.

**Material and methods:** The investigation on the spread of MDRB from clinically healthy animals (nasal swabs, skin, milk, faeces), meat and meat products (pork, poultry), milk and environmental samples (dust, swabs from surfaces in slaughterhouses) was conducted during the period of 2008 - 2014. Bacteriological examinations were performed by standard procedure with selective method on liquid and solid media. The bacteria were additionally phenotypically tested for their susceptibility to different antimicrobial groups and genotypic characterisation by PCR method was also performed.

**Results:** In livestock, MRSA was found only in pigs. LA-MRSA strains harbouring *mecA* gene were isolated from nasal swabs, pork and dust samples. The isolates exhibited *spa* types t011, t108, t034, t685, t451, t943, t1245. A high proportion of ESBL/AmpC producing *E. coli* strains in retail poultry meat samples was established. Molecular typing revealed the presence of *bla* genes belonging to CTX-M-1 group and genes encoding AmpC beta-lactamases CIT. High-level aminoglycoside resistant enterococci were isolated from pork and poultry meat.

**Conclusion:** The presence of MDRB in production animals may serve as a potential reservoir for both resistant bacteria and their resistance genes and can be transferred from animals to humans. Findings of resistant bacteria and their potential zoonotic transmission, especially along the food chain, have emerged as a major concern in human healthcare. Retail meat might be an

important vehicle for the community-wide dissemination of MDRB. However, bacterial pathogens of animal and human origin are becoming increasingly resistant to most frontline antimicrobials, including expanded-spectrum cephalosporins, aminoglycosides, and fluoroquinolones.

**O22****Staphylococcus aureus and production of enterotoxins in cheese and staphylococcus food poisoning**

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**Introduction:** *Staphylococcus aureus* is an important pathogen due to the combination of toxin-mediated virulence, invasiveness and antibiotic resistance. The ability of *S. aureus* strains to produce one or more staphylococcus enterotoxins (SEs) in food products is linked to staphylococcal food poisoning outbreaks (SFPO) with contaminated food products. Many cases of staphylococcal enterotoxicosis remain unreported owing to the rapid course and similarity to other food-borne intoxications, but they are still one of the major causes for gastroenteritis from consumption of contaminated food.

**Material and methods:** In a period of nine months 700 samples of different types of cheese were examined for presence and enumeration of *St. aureus* according to ISO 6888. The strains were then examined for their ability to produce enterotoxins with SET RPLA (SEA, SEB, SEC and SED) detection kit (OXOID, UK). In the two cases SFP, various food samples were examined (bread, meat, cheese and cakes) but after the identification of the possible contamination of the cheese, from the two intoxication cases we examined a total of 4 cheese samples.

**Results:** *St. aureus* was detected in 43 samples (6,2%) of the 700 examined cheese with numbers from 20 to 8000 CFU/g. SEs were detected in 8 (18,6) of the strains, SEA and SEB in one strain each (2,3%), SED in two strains (4,6%) and SEC in 4 strains (9,3%). Two strains showed production of two types of enterotoxins (SEB and SEC; and SEC and SED). In the first case of the SFP, SEB and SEC were detected in the two samples of cheese, while in the second case one cheese sample showed positive reaction for SEB, and the other for SEC and SED.

**Conclusion:** The study showed presence of contaminated cheese with SEs, and two cases of food poisoning, even though the numbers of *St. aureus* were below the limit for detection of enterotoxins given in the Book of Rules for microbiological criteria Of.gaz. No. 100/2008. This points to the need for better sanitation and hygiene practices, as well as the need for further monitoring of the situation with SEs in order to have safer food and avoid SFP outbreaks.

**O23****Microbiological quality of minced meat and red meat preparations consumed in Antalya**  
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**Introduction:** In this study, ribeye, silverside, knuckle, eyeround, striploin, topside, rump, tenderloin and neck preparations were taken separately during twelve months, both from markets and a meat processing unit of company which is active in Antalya.

**Material and methods:** Thirty samples were taken from each of the meat pieces, making it a total of 540 samples used in this research. In addition, 60 minced beef samples were taken from different markets. All samples were examined and evaluated statistically from the point of view of the total aerobic mesophilic bacteria, coliform bacteria, *E. coli* and coagulase positive *Staphylococcus*.

**Results:** Significant differences were observed in the total aerobic mesophilic bacteria and coliform bacteria number of meat preparations which were taken from the meat processing unit ( $p < 0.001$ ). The total mesophilic bacteria count of meat samples was determined as 3.19-3.65  $\log_{10}$  cfu/g. The coliform bacteria counts of samples were in the range from 2.79  $\log_{10}$  cfu/g to 3.22  $\log_{10}$  cfu/g and the highest value belonged to the eyeround and the lowest value was in the silverside. Significant differences were determined between the *E. coli* and coagulase positive *Staphylococcus* counts of meat preparations taken from the meat processing unit ( $p < 0.001$ ). The *E. coli* number of the meat preparations was determined between 2.47-2.67  $\log_{10}$  cfu/g. It was found that sample's coagulase positive *Staphylococcus* count ranged from 2.52  $\log_{10}$  cfu/g to 2.83  $\log_{10}$  cfu/g. Statistical differences were determined in the number of the total aerobic mesophilic microorganisms, coliform bacteria, *E. coli* and coagulase positive *Staphylococcus* numbers of samples which were taken from markets ( $P < 0.001$ ). The total aerobic mesophilic bacteria number of meat preparations taken from markets was determined to be between 3.49-4.18  $\log_{10}$  cfu/g. Coliform bacteria numbers of the samples were determined to be between 2.80-3.71  $\log_{10}$  cfu/g. The lowest value was detected in the tenderloin as 2.80  $\log_{10}$  cfu/g and the highest value in the rump as 3.71  $\log_{10}$  cfu/g. *E. coli* growth was not determined in the knuckle, eyeround, silverside and tender loin taken from the markets and coagulase positive *Staphylococcus* growth was not determined in the samples of knuckle, eyeround, silverside and tenderloin. *E. coli* number of other meat samples was determined as 1.72-2.60  $\log_{10}$  cfu/g and coagulase positive *Staphylococcus* number was determined between 2.60-3.17  $\log_{10}$  cfu/g.

**Conclusion:** As a result, microbial flora of red meat samples and minced beef which are sold to the consumer in Antalya have a higher quality.

**O24****Prevalence of *Campylobacter* spp. in broilers at slaughter**

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**Introduction:** *Campylobacter* spp. is a leading cause of foodborne diarrhea. The most common source of infection is contaminated food, mainly poultry meat. Numerous reports in many parts of the world have shown these organisms to be most prevalent in chickens, with the caeca, colon and cloaca of the birds as the main sites of colonization. The aim of this study was to determine the prevalence of *Campylobacter* spp. in poultry carcasses at slaughter using caecal samples.

**Material and methods:** In this study 166 samples of caeca from broilers were tested for *Campylobacter* spp. prevalence. Upon evisceration, the caeca of each sampled bird were carefully removed from the rest of the intestinal tract, and placed in a sterile plastic bag. The bags were sealed, transported in a cool box packed with ice to the laboratory and cultured within four hours. The samples were enriched in Preston selective broth and incubated under microaerobic conditions on 42°C for 24 hours. The enrichment was streaked on modified charcoal cefoperazone deoxycholate agar (mCCDA) and incubated under microaerobic conditions at 37°C for 48 h. Isolates were identified using standard parameters including Gram staining, oxidase and catalase testing, temperature tolerance, morphology, indoxyl acetate test, hippurate hydrolysis, growth and production of H<sub>2</sub>S on triple sugar iron agar.

**Results:** *Campylobacter* spp. was detected in 61.44% of the samples. A total of 102 isolates were recovered, with *C. jejuni* being the predominant species with prevalence of 40.9%. *C. coli* showed prevalence of 15.8%. *C. lari* 3.61% and *C. upsaliensis* 1.8%.

**Conclusion:** Our findings indicate a high prevalence of *Campylobacter* spp. and this is evidence that the process has a limited effect on the final products. This may represent a health risk to consumers, if proper cooking practices are not employed. The levels and frequencies of *Campylobacter* spp. found during this study appear to be similar to those reported elsewhere in the world.

**O25****Change in fatty acid composition of chilled ram semen in relation to spermatozoa motile activity**

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**Introduction:** Protoplasmic membrane of spermatozoa is directly involved in their motility and biological integrity. The proportion of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) changes in the course of ejaculation and manipulation in *in-vitro* environment along with the production of ATP which is necessary for the sperm velocity. It is debated that SFA and MUFA are not significantly affected by spermatozoa metabolism, whereas n-3 PUFA are most susceptible to lipid peroxidation processes. Our aim was to investigate the change of these fatty acid groups according to the spermatozoa motile activity.

**Material and methods:** Four rams at age of 1.5-4 years were assigned for this study, and ejaculates were collected by artificial vagina method, twice a week during the non-breeding season. CASA system (Hamilton Thorne) was used for assessment of spermatozoa motile activity and according to its software cut-off criteria for total motility/progressive motility ( $\geq 70\%/ \geq 40\%$ ), ejaculates (n=45) were grouped in the following order: Group 1, n=9 (85.55%  $\pm$  8.75/46.77%  $\pm$  6.82); Group 2, n=12 (78.16%  $\pm$  8.11/27.75%  $\pm$  9.51); and Group 3, n=24 (49.04%  $\pm$  16.32/19.29%  $\pm$  9.98). Ejaculates were then stored at 4°C for 2 hours and were processed in gas chromatographer (Model: GC-FID, Agilent 7890A; Method: AOAC996.06, modified FVMS SOP587) to acquire the fatty acid ratio (mean  $\pm$  SD %).

**Results:** Spermatozoa motile activity was positively confirmed by straightness and linearity indexes which were significantly higher in favor of the first versus the second group (80.11%  $\pm$  7.6 vs. 72%  $\pm$  6.7 and 51.55%  $\pm$  15.1 vs. 42.08%  $\pm$  8.04,  $P < 0.05$ , respectively). MUFA had the highest significant proportion in the first group in relation to the second and third group (18.32%  $\pm$  9.98; 7.73%  $\pm$  10.51; 6.53%  $\pm$  9.03;  $P = 0.01$ , respectively). SFA and PUFA means did not differ significantly between group one, two and three (45.15%  $\pm$  15.1 vs. 54.27  $\pm$  16.49 vs. 44.22%  $\pm$  10.84 and 36.51%  $\pm$  10.52 vs. 37.97%  $\pm$  15.08 vs. 45.15%  $\pm$  10.76, respectively).

**Conclusion:** In conclusion to this study, it can be stated that ejaculates with high motile activity of spermatozoa contain higher proportions of MUFA in relation to PUFA. This is due to the peroxidative processes that normally occur in *in-vitro* conditions which cause desaturation of PUFA thus lowering their concentration in the ejaculates.

**O26****In vitro effect of oxytocin and prostaglandin on stallion sperm quality**

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Prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) and oxytocin have been used to improve reproductive performance in many mammalian species, including humans. The goal of the present work was to determine how the addition of PGF<sub>2α</sub> and oxytocin affects stallion sperm quality. Seven different treatments were evaluated: three with only PGF<sub>2α</sub> and three oxytocin treatments. All these substances were added to 16 ejaculates from 16 healthy, 15-22 aged ( $n = 16$ ), and each ejaculate was considered as a replicate. In vitro addition of oxytocin to semen did not show improvement in any quality parameters measured. Supplementation with 40  $\mu$ g PGF<sub>2α</sub> caused a statistically significant increase ( $P < 0.05$ ) in the motility of diluted semen. We concluded that addition of small amounts of PGF<sub>2α</sub> to older stallion semen samples may help to maintain sperm motility. Further research might assess effects of PGF<sub>2α</sub> on fertility. In vivo mare fertility trials on the inclusion of PGF<sub>2α</sub> in semen extenders are therefore warranted.

**O27****Possibility for inducing follicular growth in dairy cows diagnosed with inactive-static ovaries using two different treatment methods – a field trial**

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**Introduction:** Anestrus is common during the postpartum period in high-producing dairy cows. Static ovaries stand as one of the major ovarian dysfunctions in early postpartum period, causing a significant reproductive problem in the dairy industry. The aim of the present study was to compare the ovarian response in cows diagnosed with static ovaries, more than 60 days postpartum using two different treatment (GnRH and eCG) methods.

**Material and methods:** From a total of 298 ultrasonographic examined cows, 58 acyclic cows (no CL, follicles < 8mm, P4 < 0.5ng/ml) were identified and randomly divided into three groups: GnRH (Group 1, n=23), eCG (Group 2 n=23) and Controls (n=12), and allocated thereafter, into subgroups according to the applied doses of GnRH (100 $\mu$ g; 250 $\mu$ g); eCG (750 IU;

1000 IU) whilst control group cows were left untreated. Daily follicular growth rate and treatment respond interval were estimated in all cows based on repeated ultrasound examinations, whereas blood serum P4 sampling was done twice on d0 (start of the experiment) and on d7 after ovulation.

**Results:** Resumption of cyclic activity occurred in 55.17% (32/58) of the treated cows, 56.52% in Group 1; 60.86% in Group 2 and 41.66% in the control group. Overall, the follicular growth rate was not different between the trials group cows (GnRH, eCG). However, the cows from the latter two groups significantly differ from the cows in the control group ( $p < 0.05$ ). eCG or GnRH treated cows responded significantly faster  $6.85 \pm 0.2$  and  $7.84 \pm 0.2$  days, respectively, in comparison to the control group cows ( $17 \pm 0.7$  days,  $p < 0.001$ ). Treatment with single dose of GnRH or eCG caused resumption of follicular growth and ovulation following formation of CL without significances in P4 concentrations on day 7 after ovulation ( $p > 0.05$ ). Cows in Group 2 have significantly higher incidence of multiple ovulations than cows in Group 1 ( $p < 0.05$ ). eCG treatment resulted in a faster response and higher ovulation rate compared to GnRH treatment.

**Conclusion:** Therefore both treatments have shown acceptable results in static ovaries treatment.

## O28

### Early short term water restriction upon liver function and morphology in broilers

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**Introduction:** In broiler production, water, as one of the most important and yet often overlooked nutrients, must be provided within few hours after hatch. During the transportation and accommodation in the hatcheries in the first 24-48 hours post hatch, neonatal chicks are exposed to feed and water restriction, which gives the assumption that important physiological changes do occur in their organism. The objective of the present study was to analyze changes in liver function and morphology in broilers subjected to water restriction as a stress factor.

**Material and methods:** Total of 90 day-old broiler chicks were randomly divided into three groups. One group considered as control received feed and water ad libitum for seven days; the second group considered as experimental group 1, received feed ad libitum for 7 days and was restricted from water starting from day 1 to day 3, and the third group, considered as experimental group 2, received feed ad libitum for seven days and was restricted from water starting from day 5 until day 7 after hatch. For the analyses of serum activity of aspartat aminotransferase and alanine aminotransferase, blood samples were collected by cardiac puncture on day 3, 5

and 7. Tissue samples for organ morphology analyses, were taken after euthanasia by total ether anesthesia.

**Results:** Patomorphological changes such as icteric liver and prominent gallbladder were observed in both experimental groups, being much more outlined in the experimental group 1. Histological liver analyses showed passive hyperemia and increased hepatocyte vacuoles in experimental group 1, whereas in experimental group 2, only enlarged vacuoles were observed. The activities of aspartat aminotransferase and alanin aminotransferase as indicators of liver function were measured in serum in all three groups at day 1, 3, 5 and 7. Compared to the control group, in both experimental groups, there was considerable increase of both enzymes serum activity during the 7 day trial period.

**Conclusion:** The transfer from in ovo condition into postembryonic life, where neonates are constantly exposed to different kinds of stressors, influences chicks development and is surely followed by many changes, as a result to the adaptation in the new environment. Water restriction as one of the unavoidable stress factor in the first hours after hatch, makes changes in chick's homeostasis, followed by changes in different organ morphology and in many biochemical and hematological parameters.

*\*The experimental protocol was approved by the Local Ethical Committee in conformity with the recommendation provided in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS no. 123, Appendix A).*

## O29

### Maturation rate of cattle oocytes of different quality

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**Introduction:** Today, the auxiliary reproductive techniques are frequently used in the improvement of livestock and embryo production. However, the in vivo embryo reproduction rate is low in many laboratories. This is likely caused by the problems during maturation. This study was conducted to analyze the relation between maturation success, which is the most important phase of in vitro embryo production in cattle, with oocyte quality.

**Material and methods:** With this purpose, 1235 cattle oocytes, of which 420 are A, 400 are B and 415 are C quality, were matured for 24 hours in the maturation medium which consisted of tissue culture medium (TCM 199) commercial solution with 3 mg/ml bovine serum albumine (BSA Frak. V) and 100 ng/ml epidermal growth factor (EGF) added. The degrees of effect of oocytes of different qualities (A, B and C quality) on the maturation rates in ICSI applications were evaluated using chi square test in the SPSS.

**Results:** Maturation rates were 94,28% in A quality oocytes, 62,75% in B quality oocytes and 0% in C quality oocytes according to full cumulus expansion criterion, and 89,52%, 55,75%, 1,45% respectively according to primary pole cell excretion criterion.

**Conclusion:** The granulosa and theca cells help the oocyte to supply its needs during the meiotic rest period and to gain the ability to develop during the maturation period. The granulosa cells and the oocyte are called cumulus oocyte complex. The cumulus cells play a key role due to the fact that they contain growth factors, hormones and they have a regulating role in RNA synthesis. As a result, it was found that the maturation rate decreased as the oocyte quality decreased.

### O30

#### Analysis of the demographic data on lost and found dogs in Belgrade, Serbia

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**Introduction:** Few published papers relate to lost dogs and methods used to find them. The same problem persists in Belgrade (Serbia) where the institution of responsible dog-owner relationship is not well established and supervised. The main consequence of this lack is the great population of free roaming dogs. The initial objective of the study was to examine how exterior, sexual and behaviour characteristics of lost dogs is associated with the success rate to be found.

**Material and methods:** Demographic data (breed, age, gender, sex status, size, hair color, visible marks, microchip, behavior) of 246 lost and 81 found dogs in the period of five consecutive years (January, 2009 to January 2014) were sampled, systematized and analyzed from data bases of four organizations for animal protection in Belgrade. Results are given as absolute values and proportions. Statistical analysis (95% confidence intervals - 95% CI and Chi-square test -  $\chi^2$ ) were performed using online statistical software GraphPad QuickCalcs.

**Results:** There were 246 (100%) lost dogs in Belgrade whose owners gave detailed data on them to animal protection organizations. Eighty-one of them were found. The success rate for found dog was 33% (95%CI 27-39). Success rate to be found was higher for lost dogs with special visible marks (30%), microchipped, neutered and young dogs (28%), friendly dogs (27%) small to medium dogs (26%), dogs with light hair coat (26%) and for purebred dogs (23%). Chi-square test disclosed that gender ( $\chi^2=18.328$ ), sex status ( $\chi^2=91.699$ ), hair color ( $\chi^2=51.425$ ), special visible marks ( $\chi^2=99.973$ ) and

behaviour ( $\chi^2=33.151$ ) could be considered as extremely statistical significant ( $P<0.0001$ ) characteristics of lost dogs directly associated with their success rate to be found.

**Conclusion:** The number of lost dogs in Belgrade is extremely high compared to some developed countries, pointing to the persistence of many possible problems associated to dog-owner relationship including lack of education. The number of found dogs is not so high. There are a lot of irresponsible dog owners in Belgrade who allow to their dogs to roam freely and numerous stray dogs on Belgrade's streets. All of these can be considered as reasons for failure in finding lost dogs. However, obtained results from the study suggested that lost purebred, female, neutered, young, small to medium, microchipped, friendly dogs and dogs with light hair and special marks possess higher chance to be found in Belgrade.

### O31

#### The approach to a problem herd with neonatal calf diarrhea

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**Introduction:** Calf diarrhea causes substantial economic losses in cattle herds worldwide. Neonatal calves are particularly sensitive to infections with enteropathogens. The four most important infectious causes worldwide of neonatal calf diarrhea are *Escherichia coli*, rota- and coronavirus, and *Cryptosporidium parvum*. The latest techniques for both therapeutic and preventive measures useful for the cattle practitioner to combat neonatal calf diarrhea will be discussed, with emphasis on fluid therapy and colostrum management. Also, results of a recent study focusing on prevention against the main infectious causes of neonatal calf diarrhea will be presented.

**Material and methods:** Dairy herds (n = 24) with a high percentage of neonatal calves scouring (> 10%) were included and calves were sampled for the presence of the four mentioned enteropathogens. To decrease the diarrhea problems among the neonatal calves, a standard protocol was tested on 13 herds where both *C. parvum* and either *E. coli* or rota- or coronavirus were identified as being involved in the diarrhea problems. This protocol consisted of 2 points of action: preventive vaccination of the dams against *E. coli*, rota- and coronavirus, and preventive administration of halofuginone lactate to newborn calves. To evaluate the colostrum management of the participating herds, the passive transfer status of the calves was measured both before the onset and at the end of the study.

**Results:** The average percentage of calves suffering from neonatal diarrhea (39.7% vs. 14.3%,  $P < 0.01$ ) and the average percentage of fecal samples positive for *C. parvum* (34% vs. 11%,  $P < 0.05$ ) significantly differed between control herds and trial herds after implementation



of the protocol. However, no significant differences were observed in the percentage of calves excreting *E. coli*, rotavirus and coronavirus between the control and trial herds, both before and at the end of the trial.

**Conclusion:** In herds where the colostrum management suffices, after a thorough diagnosis of rotavirus, coronavirus, *E. coli* or *C.parvum*, a range of easy to implement preventive measures, such as dam vaccination and metaphylactic halofuginone lactate administration can be successful in the prevention of calf diarrhoea. The approach of a problem herd with NCD as described above is simple and can easily be implemented by any practitioner when confronted with NCD problem herds.

### O32

#### Use of modified Calgary-Cambridge guides in bovine consultations

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The Calgary-Cambridge Guides (CCG) delineate a flexible set of clinical communication skills useful for all types consultations initially developed for human medicine and expanded recently for use in veterinary medicine. Bovine practice has become more complex coupled with higher client expectations. Additionally, there are two types of bovine consultations; the individual beast consultation and the herd-level consultant role. It is essential for positive patient and client outcomes that the bovine practitioner seamlessly integrates clinical skills and knowledge with effective communication skills.

The aim of the modified CCG is to provide a set of skills to facilitate a relationship-centred approach to bovine consultations, both at the individual animal and population level. They are described split into seven (plus one) key components 1) Preparation, 2) Initiating the Session 3) Gathering Information, 4) Physical examination 5) Explanation and Planning, 6) Closing the Consultation, 7) Providing Structure, and 8) Building the Relationship. The CCG enable the bovine practitioner to facilitate interacting with that particular client at the time of the consultation, without relying on pre-conceived biases of the client's stereotype (e.g. [1] reclusive traditionalists, [2] pro-activists, [3] wait-and-see-ers and [4] do-it-yourselfers). It is likely that the majority of bovine practitioners do use most of the skills recommended by the modified CCG. These skills are often gained through experience. However, they may not use the skills intentionally and with purpose for a specific communication goal or outcome.

In conclusion, bovine practitioners can improve their communication skills using the set of skills as recommended by the modified CCG. They allow the practitioner to gain insight into the client's understanding of the problem, including underlying aetiology, epidemiology and pathophysiology. The modified CCG also provide opportunity to understand client's expectations regarding the outcome, motivation and willingness to change; all factors associated with increased adherence.

### O33

#### Postural behaviour in gilts: rubber mats versus concrete flooring

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An inappropriate type of flooring may restrict movement and characteristic behaviour display in pigs, if animals feel unsafe on moving and changing posture. In this study, postural behaviour was compared between gilts accommodated in service unit with different types of flooring. The study was conducted in all seasons during four 28-day production cycles, including 10 gilts *per* cycle, equally divided into a control and an experimental group. Control gilts were accommodated in gestation stalls with concrete floor, whereas in the experimental group the floor was covered with rubber mat. Postural behaviour of gilts was observed 4 times *per* cycle for 4 hours. Study results showed that during the cold season control gilts spent more time standing and lying sternally, whereas experimental gilts were mostly lying, predominantly laterally ( $P < 0.05$  all). There were no significant between-group differences according to the time the gilts spent sitting or the frequency of changing posture. However, experimental gilts spent significantly less time changing posture ( $P < 0.05$ ), except for changing sternal to other recorded positions and sitting to sternal position. Accordingly, rubber mats may improve the lying comfort in gilts; however, when using rubber mats, the house thermal conditions should be taken in consideration.

### O34

#### Grazing in dairy farms with milking robots and grazing costs

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The use of milking robots is becoming very widely spread. Using robots usually is difficult or not possible at all when the grazing period starts. There might be many reasons, but they are not yet well researched in Finland.

There are around 600 milking robots all over Finland and it is increasing rapidly by around 80 milking robots per year. The main challenges are how to arrange grazing in farms with milking robots and what are the costs during grazing in robot milking farms?

The study took place in 60 dairy farms with robot milking systems and we interviewed farmers in Northern Ostrobothnia, Northern Savo and Central Finland. The study was made during all of the are grazing period over the six-month cattle grazing. All of the animals were allowed to graze freely. Heifers and cows on dry period graze and get the same indoor diet. Grazing share in interviewed farms is small 25%. Pasture farm used for milking cows on average, is in total of 4.7 hectares, or an average of 0.075 ha / cow. Cows get out in May and grazing ends no later than September. Grazing increases the cost because of investments for materials used for fencing, drinking water, and the organizing of the animals during the grazing period, as well as labor cost for milking. On the other hand the barn working hours will raise the same amount of savings so the grazing related labor or material costs should not be more in it, but the picture becomes different.

### O35

#### Investigation of the antigen recognition and presentation capacity of pecteneal hyalocytes in chicken (*Gallus Gallus Domesticus*)

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**Introduction:** In many animal species, a group of cells, referred to as hyalocytes, are found peripheral to the vitreous body. It is suggested that hyalocytes are involved in phagocytosis. The histochemical properties of these cells and results obtained with cell culture research are in support of the hypothesis that hyalocytes are a subculture of macrophages. The pecten oculi is the only structure of the avascular avian eye, which is heavily vascularized and contains pigment granules. This structure extends from the point of entry of the optic nerve into the retina to the vitreous body. The pecten oculi presents three different types of cells. These are endothelial cells, melanocytes and hyalocytes. This study was aimed at demonstrating the antigen recognition and presentation capacity of the pecteneal hyalocytes in the eye of the chicken during the different stages of embryonic development and in adulthood.

**Material and methods:** In this study, 48 fertilized eggs were used for the evaluation of the embryonic development stages while 12 six-week-old Ross 308 chickens were used for the evaluation of the adult stage. The trial groups established for the evaluation of the

embryonic development stages were assigned according to the Hamburger-Hamilton scale. Pecten oculi samples taken from these animals were fixed in 10% neutral formol. In order to determine the antigen recognition and presentation capacity of pecteneal hyalocytes, the tissue samples obtained from both the embryos and adult animals were treated with monoclonal antibodies against MHC-II, TLR2/CD282 and TLR4, as well as with RCA-1, WGA, and SNA lectins. Furthermore, the developmental stage of the pecteneal hyalocytes was detected by Masson's triple staining.

**Results:** In this study, the pecteneal hyalocytes were first demonstrated to be at HH stages 30-34, and were confirmed to display the same characteristics from their first appearance to adulthood. Chicken pecteneal hyalocytes were observed to react positively for monoclonal antibodies against TLR2 and TLR4, and to react negatively for monoclonal antibodies against MHC-II. Furthermore, the hyalocytes produced positive results for RCA-I, WGA and SNA lectins. Data obtained in this study showed that pecteneal hyalocytes adherent to the pecten oculi in the domestic fowl were capable of recognizing antigens, but were incapable of presenting them.

**Conclusion:** This study contributes to understanding the role of these cells, which are assumed to be of monocyte/macrophage lineage and the functions of which are not fully known in the immune response.

### SS1

#### The awareness questionnaire: attention to brucellosis among internship students in veterinary faculties in Turkey

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Brucellosis is an endemic disease in the animal population in Turkey and other Mediterranean countries, and has a major public health importance. Therefore, it is considered as one of the most important zoonoses in the region as well as all over the world. This survey was conducted among

internship students in veterinary faculties in Turkey with the aim of identifying the awareness of the students' about brucellosis.

The questionnaire was applied to 320 internship students from 6 different veterinary faculties in Turkey. The questionnaire consisted of two parts. In the first part, gender, age, university, experiences of living / working on a farm, contact with suspected animals, suffering from brucellosis, their career plans on farm animals were asked to students. The second part consisted of 12 Likert type (1-5 scale) questions about brucellosis. Non parametric Mann-Whitney U and Kruskal Wallis tests were used to analyze data. Cronbach's alpha ( $\alpha=0,849$ ) indicated a high degree of internal consistency.

Gender and ages of the students had no influence on the response to questions. Career plan and suffering from brucellosis had only significant influence on knowledge about vaccination. It has been observed that the students who contacted with suspected animals had higher awareness of brucellosis. Students, who had experiences living in a farm, had higher awareness about transmission, and protection & control of the disease. The results of the survey also indicate that there were significant differences among the faculties in terms of brucellosis awareness.

## SS2

### Unsaturated fatty acid content in corn flakes

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**Introduction:** Content of fatty acids in food play a very important role in human health. Apart from meat (pork, beef, poultry) and meat products, milk, dairy product and some plants such as palm fruit, coconut and palm kernel oil, which contains higher percentage of saturated fatty acids, olive oil and many grains (barley, corn, rice, oats, wheat), nuts, legumes, plant oils contain more unsaturated fatty acid. The aim of this study was determination of unsaturated fatty acids in corn flakes and describing the importance of unsaturated fatty acid for human health.

**Material and methods:** Fifty samples from corn flakes were collected from the local stores in Skopje and were analysed. For determination of fatty acid contents we used the AOAC Official Method 996.06. Analyses were performed with gas chromatograph (7890 GC system) with flame ionization detector and the calculation of results was made with Chemstation software.

**Results:** The analyses of the results showed that out of the total fatty acids in corn flakes 71.05% were unsaturated

fatty acids. The linoleic fatty acid (C18:2n6c) was the primary fatty acid and its content in the corn flakes was 47.84% (from 40.22 to 50.94%). Oleic acid (18:1n9c) and  $\gamma$ -linolenic acid (18:3n6) were presented with 21.02% (from 17.28% to 25.12%) and 1.39% (from 0.72 to 2.57%) respectively. The concentration of polyunsaturated fatty acids was higher than unsaturated fatty acids.

**Conclusion:** Unsaturated fatty acids are important for human health and the beneficial effects have been shown for the secondary hearth diseases, hypertension, diabetes type 2, ulcerative colitis, renal diseases, rheumatoid arthritis and chronic obstructive pulmonary disease. They are used in prevention of cancer, cardiovascular diseases, autoimmune diseases, inflammatory diseases and depression. Linoleic acid, which is the primary fatty acid in corn flakes, is considered as the most effective supplement in decreasing bad cholesterol, protector of stroke, reducer of blood pressure and platelet aggregation; it also plays an important role in brain function and normal growth and development. The linoleic acid is the precursor of omega-6 fatty acid. From our study we can conclude that corn flakes are a very good source of unsaturated fatty acids and may be used as food rich in unsaturated fatty acids.

## SS3

### The influence of transport on the within flock prevalence of *Salmonella* spp. in laying hens

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**Introduction:** Laying hens eggs are a food most commonly connected with *Salmonella* outbreaks. Therefore there is a national monitoring program for the presence of *Salmonella* in the laying hens flocks. This monitoring plan focuses on the presence of the *Salmonella* in the faeces, and it doesn't determine the prevalence of *Salmonella* within the flock. However the literature states the possibility of the *Salmonella* presence in the laying hens without being shedded in the faeces until there is some kind of stress. The goal of this study was to determine *Salmonella* prevalence within flock, prior and after the transport.

**Material and methods:** A *Salmonella* free flock of laying hens scheduled for depopulation in two weeks was selected for our investigation. Ten pooled samples of 150 g of faeces and 2 pooled dust samples were taken from the farm. Furthermore, 100 samples of cloacal swabs were taken from 100 randomly selected laying hens from the flock in the farm. At the slaughterhouse 50 cloacal swabs were sampled from 50 hens before the slaughtering, and during the evisceration caeca from 50 hens were sampled. All samples were analyzed using ISO 6579:2002

Amd1:2007 method, for the detection of *Salmonella* in animal and environmental samples from primary production. Serotyping of *Salmonella*-isolates was done according to the Kauffmann-White scheme.

**Results:** All the samples taken from the farm (faeces, dust and cloacal swabs) were found to be negative for *Salmonella* spp. As for the slaughterhouse, 4 (8%) of the cloacal swabs, and 8 (16%) of caeca samples were found to be positive for *Salmonella* spp. All of the isolates were confirmed as *Salmonella* *Enteritidis*.

**Conclusion:** Our results lead to suspicion that if the flock is a carrier of *Salmonella* it doesn't necessary shed *Salmonella* in the environment. The official monitoring method, with pool samples of faeces and dust from the farm is not always able to detect the *Salmonella* within the flock unless the hens are shading it. The occurrence of *Salmonella* positive cloacal swabs samples from the slaughterhouse indicates that the introduction of transport stress can provoke *Salmonella* shedding in some of the laying hens that were *Salmonella* carriers. However this study was conducted to only one flock of laying hens, and more flocks should be examined in order to get to a definite conclusion.

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#### SS4

##### Study of aflatoxin contamination in corn and bread in R. Macedonia

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**Introduction:** Aflatoxins (AFT) are poisonous substances which are classified in Group 1 carcinogenic agents to humans by IARC. AFT can occur naturally in food commodities (maize, corn, rice, cottonseed, spices) as a result of fungal contamination in hot and humid environments. In the food, toxin contamination can remain during manufacturing and long after fungi have stopped being biologically active. AFB1, AFB2, AFG1 and AFG2 are the major AFTs of which, AFB1 is the most dominant and potent. In respect of EU legislations, maximum residue level (MRL) for corn and cereals is 5 µg/kg and 4 µg/kg, respectively. In this study our aim was to investigate presence of AFT and searching for correlation between AFTs concentration in corn and commercial bread.

**Material and methods:** The data of food analysis were collected from the laboratory records which declared samples of corn and bread brought from different regions of Macedonia. Corn (n=34) and bread (n=53) results, were appointed in Group 1 and Group 2, respectively. The second group was separated in two subgroups: Group 2-1 (n=32) consisting of white and Group 2-2 (n=21) consisting of bran cornbread results. The extraction and

purification procedures were performed according to AOAC Official method (991.31) with limit of detection (LOD) of 0,005 µg/kg. For statistical analysis, we used Fisher's analysis of variance test, whereas Tukey post-hock test was used for detection of significance in the differences between the groups.

**Results:** According to the LOD threshold, twenty samples from Group 1, 2-1 and 2-2 were detected in the range of 0,15-109,79 µg/kg (n=17; 50%; n=1; 3,12% and n=2; 9,5%, respectively). Only Group 1 samples had AFT concentrations above MRL (n=7; 20,5%) in the range of 15,95-109,79 µg/kg. None of the samples in Group 2 were marked as positive. The analysis of variance between the means of Groups 1, 2-1 and 2-2 (10,8 µg/kg ±25,18; 0,004 µg/kg ±0,0265 and 0,064 µg/kg ±0,026) showed statistically significant difference (F=4,81; P= 0,01). The Tukey post-hock test revealed that the relations of Group 1 vs. Group 2-1 and Group 1 vs. Group 2-2 were statistically significant (P=0,01 and P= 0,04).

**Conclusion:** We found that corn, bran cornbread and white bread had presence of AFT, with levels above MRL for the corn samples only. Nevertheless, we could not find levels of AFT above MRL in the bran cornbread which is produced by this grain. We suggest that the absence of dangerous AFT levels in bran cornbread could be explained with use of corn with another geographic origin or corn from import.

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#### SS5

##### Importance of personnel hygiene in food production facilities

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The ready-to-eat food industry has an important role in the cities and towns of many developing countries. It feeds millions of people daily with a wide variety of foods that are relatively cheap and easily accessible. Poor personnel hygiene is a leading cause of foodborne poisoning and illness. Since the hands touch all parts of the body, a vast array of miscellaneous objects, other people, domestic animals and food, it follows that a diverse microbial flora can exist on the hands. Hand transfer has been identified as a significant mode of transmission for bacteria from person to person, from person to surface and from surface to person (zigzag fashion) and from person to food. Therefore, the hygienic status of personnel hygiene should be routinely controlled in food production facilities. Especially, the number of total mesophilic bacteria, *Esherichia coli* and *Staphylococcus aureus* are very important in the samples taken from personnel hands, regarding food poisoning risk and public health. As a

result, it is concluded that making personnel conscious of reduction of the bacteria found on their hands at high levels, controlling hygiene by carrying out up-to-date HACCP and GMP applications, as well as proper hygiene conditions contribute to a great extent to the possibility of taking the problem under control.

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## SS6

### **Rearing Anatolian buffaloes; from the traditional till today**

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Anatolian Buffalo is the achieved reared mostly in our country. Buffaloes are important animal species for our country. The most demanded traditional milk sweets and famous “Turkish Kaimak” are produced specially from buffalo milk.

Buffalo rearing has scored a slower development and modernization compared to dairy cattle rearing in our country. Generally buffaloes are reared in family type systems. In this system the buffaloes are kept in tied stalls, sent to grazing as a herd and fed with low quality foods. The herds include males also and the buffaloes male naturally. It is impossible to employ in this system scientific reproductive and feeding systems. To achieve genetical improvement, controlled mating, artificial insemination, oestrus handling, production controls, health control and national feeding programs are all necessary. All these applications are missing in the rearing system. Diseases like Brucellosis and Tuberculosis are especially serious challenges to public health. Recently, to overcome these problems modern buffalo farms are being established in our country where scientific feeding, production and record systems are implemented. The aim of these farms is high producing, genetically improved healthy buffaloes. In these farms the animals consume appropriate rations prepared by animal nutritionists according to their production and needs.

The buffaloes are kept in hygienic free stalls instead of being tied in old type stables. Reproduction is controlled by specialized veterinarians routinely and scientific reproductive procedures are employed.

The Anatolian Buffaloes which are the producers of traditional Turkish dishes can be preserved and improved by Scientific Nutrition Programmes; Appropriate Management Conditions or Scientific Procedures and Applications.

## SS7

### **Influence of certain factors on career choices of veterinary students at Istanbul University**

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In this study, the influence of factors such as gender, the year of study, educational achievement level (WGPA grade), the city where s/he was born, taking care of an animal, the family income level and where s/he grew up (big city, urban, rural - village) were investigated with regards to the choice of post graduation choice of work among the students who are studying at the Faculty of Veterinary Medicine, Istanbul University.

Taking veterinary medicine as a field of study, the survey included and discussed the following: small animals (cats, dogs) medicine, farm animals (cattle, sheep, goats) medicine, horse medicine, poultry industry, feed industry, pharmaceutical industry, food control sector, public veterinary medicine and academic research. Within the scope of study, the students of the Faculty of Veterinary Medicine were also questioned about the expectations related to post graduation monthly income and working conditions (daily working hours, working time, etc.).

In this study, pre-prepared questionnaire, was distributed to 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> year students and than asked to complete the questionnaire voluntarily. For each factor, frequency and rate of sub-groups we utilized MS-Excel and SPSS 13.0 software, while the importance of differences between subgroups was analyzed with Chi-square test.

The results showed that, in the process of school education, choosing a course teaching in accordance with careers expectations of veterinary students, may be more appropriated.

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## SS8

### **Usage of helminths as alternative therapy for auto-immune diseases**

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In recent years, with developing technologies and industrialization in most countries, a distinct increase has been seen in auto-immune diseases. Especially, hygiene and health-care concepts were considered as a common

lifestyle, bowel diseases, multiple sclerosis, and some allergic infections which have become important public health problems.

These health-care measures reduced the parasitic diseases in industrialization countries. But in some epidemiological studies have indicated that especially auto-immune diseases are rare where cestodes infections are abundant. In mice models, it is shown that helminthic infections eliminate some auto-immune diseases such as multiple sclerosis, type 1 diabetes, asthma, gastrointestinal diseases and some other allergic diseases.

In this review, we put forward new and interesting therapeutic perspectives, for the prevention of allergic and autoimmune diseases.

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### SS9

#### Effects and importance of student research in veterinary education: a model of Istanbul University

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During your studies, being part of a scientific research and learning scientific terminology are very important for the development of students. Istanbul University Faculty of Veterinary Medicine takes an active role in student research, which is funded from both internal and external sources.

The student Scientific Research Club organizes international an veterinary medicine student scientific research congress. The 16<sup>th</sup> Congress was held on 8-10 of May 2014 in Istanbul with 150 international student participants from 16 countries and 550 students from 21 national faculties. This year, the competition entitled "The Creative Dreams Project for Future Scientists" took place during the congress for the first time. Members of the scientific research club are always pleased to welcome students from all over the world.

Turkey's the first student journal of veterinary medicine called "Zoom" is also published and edited by the students. The journal publishes research articles, case reports and reviews from the veterinary students who are winners of the diagnosis competition for students.

The Research fund of Istanbul University and the Scientific and Technological Research Council of Turkey supply financial support for student research as well as private foundations. Furthermore, the Health Culture and Sport Department of the Istanbul University funds our students participation in scientific congresses and educational travels. The innovative research and development projects of students are supported by The Technology Transfer Center of the Istanbul University. I.U. Veterinary Faculty IVSA club hosts veterinary students from 40 different

countries every year who take part in scientific, cultural and internship programmes, while the entrepreneurship club supports creative and innovative students.

*VEtistanbul Group* which is a formation of scientific, academic and social cooperation was established under the coordination of while the Istanbul University Faculty of Veterinary Medicine in 2013. The aims of the group are to improve academic and cultural relationship, and to enable sharing of educational and research opportunities among the partner Veterinary Education Establishments not only for scholars, but also for the students.

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### SS10

#### Influence of the reproductive status on the development of mammary tumors in bitches

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**Introduction:** Canine mammary tumors are the most frequent neoplasms in bitches, mostly affecting the older patients. They represent approximately 42% of all tumors and 82% of those arising in the female reproductive organs. Approximately 50% of the mammary tumors are malignant with high percentage of mortality (if not treated in time). The aim of this study is to analyze the relationship between tumor incidence and dog's age, heat cycles and sterilization in patients with mammary gland tumors.

**Material and methods:** During a period of two years, 23 canine patients of different breeds, have been submitted to medical and surgical treatment of various types of tumors at the University Veterinary Hospital at the Faculty of Veterinary Medicine in Skopje. All patients were subjected to thorough clinical as well as laboratory examination. Cytology samples were obtained by FNA prior to surgery and the final diagnosis was confirmed by histopathology.

**Results:** In all patients admitted in our clinic, mammary tumors were diagnosed in 43.5% (10 out of 23) of all presented cases with neoplasia. Nine of ten patients subjected in this study were diagnosed as adenocarcinoma and only one patient as benign adenoma. The reproductive history has shown that seven out of nine patients (77.8%) with malignant mammary tumors have never whelped; only one of them was treated with synthetic progestagens and none of them were sterilized. Malignant adenocarcinoma was diagnosed in all nine patients using

cytological and histopathological diagnostic. The only treatment was surgical removal of the tumors (simple or radical mastectomy) and/or ovariohysterectomy.

**Conclusion:** The results of this study have shown that older bitches that have never whelped are more predisposed to mammary tumors. The influence of the ovarian hormones on the mammary gland tissue during different stages of development is a risk factor for occurrence of mammary gland tumors. Based on our findings and the conclusions of other authors, it can be concluded that the risk of developing a mammary tumor multiplies with the increase of the number of estrous cycles, thus early ovariohysterectomy before the first oestrus cycle is recommended as the most efficient preventive measure.

## SS11

### The advantages of the immunohistochemical method in the diagnosis of the circovirus diseases in pigs

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**Introduction:** The circovirus diseases are caused by PCV2 (Porcine circovirus type 2) which is a small, spherical nonenveloped virus with a single stranded DNA genome and is spread throughout the pig industry worldwide. The most significant of the circovirus diseases is the PMWS (post-weaning multisystemic wasting syndrome) which has great impact on the pig production. There is a great discussion between scientists concerning the diagnosis of the circovirus diseases in pigs.

**Material and methods:** In this study we compared the immunohistochemical and the histopathological method in the diagnosis of PCV2 infection in pigs. Thirty pigs from two to five months old with previous clinical diagnosis of the Post-weaning multisystemic wasting syndrome were examined. Necropsy was performed on all pigs and tissue samples for histopathology and immunohistochemical diagnostics were collected in 10% buffered formalin, dehydrated, embedded in paraffin wax and sectioned at 3-4  $\mu\text{m}$ .

**Results:** The histopathological lesions were mainly expressed in the form of lymphocyte depletion and necrosis in the cortex and the paracortex of the lymph nodes, as well as the presence of giant cells in the same areas. The immunohistochemical method revealed the presence of the PCV2 antigen in most of the examined pigs. The presence of the antigen is found in all the lymphoid tissues specifically in the necrotic areas of the lymph follicles, and is less present in the giant cells and the mononucleated phagocytes of the lymph follicles.

**Conclusion:** The immunohistochemical method allowed us to simultaneously observe the tissue changes, distribution of the virus antigen in the tissue and to recognize the pathogenesis of the disease.

## SS12

### Semen quality of Ovchepolean Pramenka rams during non-breeding season

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**Introduction:** Ovchepolean Pramenka is an indigenous breed of sheep in the Republic of Macedonia whose breeding season lasts from the beginning of summer till late autumn. While there is no ovulation in the ewe during in anoestrus, spermatogenesis and sexual behavior in the ram never stop, although some decline in semen quality is reported. The aim of this study was to investigate the quality of semen during the off-breeding season and the influence of age on fertility parameters.

**Material and methods:** Four Ovchepolean Pramenka rams, at the age of 15 months (n=2) and 48 months (n=2), were included in this research. The rams were housed at the premises of the Faculty of Veterinary Medicine, fed ad libitum with good quality alfalfa and concentrate with free access to fresh water. 92 semen samples were collected, twice a week, using artificial vagina method, in the period from March to May. Each ram had two successive mounts in interval of 15 minutes. Initial evaluation (volume, density, wave motility, concentration) of the semen samples was done within 10 minutes from the collection. Afterwards, the total volume of each ejaculate was diluted with pre-warmed Tris-Citrate-Glucose extender in 1:1 ratio. Total (tMOT) and progressive motility (pMOT) were assessed by CASA system (TOX IVOS, Hamilton Thorne Research) both in fresh semen (FS) and semen equilibrated at 5°C for 2 hours (ES).

**Results:** The ejaculate volume (ml) in young and adult rams ranged from 0.3–1.0 (0.7 $\pm$ 0.03) and 0.2–1.1 (0.6 $\pm$  0.03) respectively. The values of sperm concentration ( $\times 10^9$ ) were 0.99-3.28 (2.14 $\pm$ 0.11) in young rams vs 0.74-4.47 (2.21 $\pm$ 0.15) in adult rams. The difference in volume and sperm concentration between young and adult rams was not statistically significant. However, the values of tMOT(%) and pMOT(%) in FS (83.29 $\pm$ 2.13 and 42 $\pm$ 2.14) compared to the values in ES (65.53 $\pm$ 2.17 and 32.22 $\pm$ 1.78) in all rams had statistically high significance (p<0.0001 and p<0.001 respectively).

**Conclusion:** Compared to previous studies, slight seasonal effect (lower volume and concentration) on ram semen quality was recorded in this research. No statistically significant differences regarding semen volume, sperm concentration, total and progressive motility between young and adult rams was detected. This leads to the conclusion that young, as well as adult rams, can be efficiently used for reproduction in the non-breeding season.

**P1****The influence of diets on the developing of gingivitis and periodontitis in dogs**Haxhi Allmuca<sup>1\*</sup>, Jani Mavromati<sup>1</sup>,  
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**Introduction:** Today, dogs are fed on homemade and commercial diets. Recently, there has been a trend of commercial diets. These diets have different influence on the developing of plaques and calculus on teeth surface and gingival margins and, as a result on gingivitis and periodontitis of dogs.

**Material and methods:** The aim of this study was to compare dogs fed on homemade, commercial and mix (homemade+commercial) diets on the development of plaques and calculus, and as a result, gingivitis and periodontitis. 24 dogs, four to six years old, were used for this study. We divided them in three groups, eight dogs for each one. The dogs of the first group were fed with homemade diets, the dogs of second the group were fed with commercial dry food (Pedigree + Top line) and, the dogs of the third group were fed with homemade + commercial food. The study lasted for two years. All the dogs which were included in the study were checked for plaques and calculus deposits and eventually gingivitis and periodontitis, every three months.

**Results:** After two years, in the first, second and third group plaques and calculus were observed at 100%, 62.5% and 87.5% of dogs, respectively. Gingivitis was developed 62.5%, 37.5% and 62.5% in the three groups, respectively. Periodontitis did not develop in all the dogs.

**Conclusion:** According to the results, plaques and calculus were developed more in the dogs fed with homemade diets and less in the dogs fed with commercial diets. Gingivitis were developed less in the group fed with commercial diets and the same in the groups fed with homemade and mixed diet.

**P2****Effectiveness of tooth brushing to the cases of gingivitis and periodontitis of dogs**Haxhi Allmuca<sup>1\*</sup>, Brunilda Mazari<sup>2</sup><sup>1</sup>Faculty of Veterinary Medicine, AUT, Tirana, Albania<sup>2</sup>Small Animal Veterinary Clinic, Tirana, Albania

**Introduction:** Oral and dental diseases generally cause distress and debilitating pain to the affected animal. Prevention is always preferable to treatment and many oral and dental conditions are responsive to preventive measures. Teeth brushing is an effective way of removing plaques and calculus which get accumulated on the surface of the tooth and gingival margins. The plaques and calculus is the main cause of gingivitis and periodontitis of dogs.

**Material and methods:** The aim of this study was to compare the incidence of plaques and calculus of the teeth and gingival margins and, as a result gingivitis and periodontitis of the dogs which are submitted to teeth brushing and the dogs which are not submitted to mouth washing. 18 dogs of the breed Maltese, Yorkshire and German Spitz, from four to six years old were used for this study. The dogs were divided in three groups of six dogs each. The dogs of the first group were subjected to daily teeth brushing, the dogs of the second group were subjected to teeth brushing once a week and the dogs of the third group were not subjected to mouth wash.

**Results:** After one year, we noticed that the dogs of the first group did not develop plaques and calculus and, as a result no clinical signs of gingivitis and periodontitis; the second group, 67% of the dogs developed the plaques on the teeth surfaces, but not calculus; whereas the third group, 84% of the dogs developed plaques and calculus on the teeth surfaces and on the gingival margins. Two dogs of the third group or 33% developed gingivitis as well. No difference was noticed, despite of the breeds.

**Conclusion:** Based on our observation, daily teeth brushing is the most effective way to control plaques and calculus in teeth and, to control gingivitis and periodontitis too. Teeth brushing once a week is not very effective. High incidence of plaques and calculus accompanied with gingivitis (one case) was observed in the dogs which were did not submitted to mouthwash. The success of teeth brushing depends on the cooperation with the dog's owner.

**P3****Resistance of *Salmonella* isolated from affected and dead animals, antibiotic sensitivity testing and selection of highly-effective preparation**Levan Makaradze<sup>1\*</sup>, Zakaria Chekurishvili<sup>1</sup>,  
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**Introduction:** This study concerns the sensitivity testing of salmonella isolated from dead piglets against antibacterial preparations widely-used in medicine and veterinary practice. Resistance, stable resistance and sensitivity have been determined which is of particular practical importance.

**Material and methods:** To determine antibiotic sensitivity against isolates, discs of ampicillin, ampicillin sulbactam, amoxicillin, amoxicillin clavulanate, cefotaxime, ceftriaxone, tetracycline, ciprofloxacin and norfloxacin were used. Antibiotic sensitivity was determined by using petri dishes agar surface on lawn-grown cultures and antibiotic discs were placed at a distance of 1.5-3.0cm.



After drying turned-over, dishes were incubated at 37°C. The results were recorded after 18-20 hour incubation.

**Results:** The study of salmonella the resistance under the effect of various environmental factors has revealed very interesting practical outcomes. In particular salmonella is comparatively stable under various environmental factors. For example, in most cases salmonella is still alive at -45-80°C and perfectly undergoes the drying process. Under room temperature, they revive 40-100 days on various items. They survive up to 65 days under especially low pH conditions in water. The most interesting fact is that in dairy and meat products the salmonella not only survives up to 4 months, but it multiplies and doesn't change the organoleptic properties and shape of the products. The research has shown that salmonella is stable against salting, smoking and acid, but the bacteria decomposes as a consequence of thermal actions. For example they it dies within 35-40 minutes at +55- 60°C, but at + 80°C and over the microbes it dies within 1-2 minutes. Complete elimination of salmonella and product safety is guaranteed if the flesh is boiled within 2 or 3 hours. The given process completely ensures the destruction of salmonella in the deep layers of the flesh. For practical purposes it's particularly significant to test salmonella sensitivity against therapeutic concentrates of antibacterial preparations. Antibacterial preparations which are widely-used in medicine and veterinary practice such as: ampicillin, ampicillin sulbactam, amoxicillin, amoxicillin clavulanate, cefotaxime, ceftriaxone, tetracycline, ciprofloxacin and norfloxacin were involved in the experiment. Research outcomes are:

Sensitivity of Salmonella Strains to Antibacterial Preparation: Antibiotic Sensitivity (%); Stable resistance (%); Resistance (%)

1. Ampicillin: 84,0; 11,0; 5,0
2. Ampicillin Sulbactam: 84,0; 11,0; 5,0
3. Amoxicillin: 90,0; 8,0; 2,0
4. Amoxicillin Clavulanate: 88,0; 0; 12,0
5. Cefotaxime: 98,0; 1,0; 1,0
6. Ceftriaxone: 87,0; 1,0; 12,0
7. Tetracycline: 92,0; 2,0; 6
8. Ciprofl oxacin: 100,0; 0; 0
9. Norfl oxacin: 100,0; 0; 0,0

According to the data given, 16% of salmonella are stable against ampicillin and ampicillin sulbactam, 11% of them are stable resistant and 5% - express resistance. 10% - against amoxicillin and 12% - against amoxicillin clavulanate. Highly therapeutic effects were revealed in the cases of cefotaxime, ceftriaxone and tetracycline sensitivity is 98%, 87% and 92% respectively.

**Conclusion:** The study if antibiotic sensitivity to of salmonella determined that, 16% of salmonella are resistant to ampicillin and ampicillin sulbactam. Resistance to amoxicillin and amoxicillin clavulanate ranges within 10-12%. Salmonella sensitivity to ciprofloxacin and norfloxacin is 100%, allowing their use with a high therapeutic effect.

#### P4

### Pathomorphological changes in piglet liver during acute salmonellosis

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**Introduction:** This study shows the micromorphological changes occurring in the liver in the case of acute salmonellosis. Staining sections taken from liver were performed with hematoxylineosyn. The description of the micromorphological changes developed in liver is accompanied with photo illustrative material proving the changes. The researches have shown that the primary reason the development of the changes is blood circulation disorder which results in is the development of dystrophy, atrophy and necrosis. The goal of our research was to study the pathomorphological changes in liver in acute salmonellosis.

**Material and methods:** For the pathomorphological researches material was taken from the liver of dead, slaughtered and healthy pigs. For the ordinary pathohistological study the fixation of the research pathological material was performed in 10-15% neutral formalin-water liquid (prepared with tap water) and in ethanol-formalin. Embedding was performed in homogenic paraffin. For the purpose of the ordinary pathomorphological study, paraffin sections were stained with hematoxylin-eosin and picrofuchsin using Van Gieson's method. Histochemical research of glycogen was performed using the method of Shabadash. Pathohistological studies were also performed on the animals of the control group.

During the experiment isolate of pathogenic salmonellosis was recovered from dead and slaughtered animals and studied. For cultivation of isolated strains the following was used: a) meat-peptone broth (pH 7.2-7.4) b) 2% meat-peptone agar (pH 7.2-7.4) c) 0.7% agar (pH 7.2-7.4), d) Endo agar (pH 7.0-7.2), e) Clark nutrient medium (pH 7.2-7.4), f) Strogov nutrient medium (pH 7.0-7.4), g) Hiss medium (pH 7.0-7.4), h) Milk medium (pH 7.0-7.5). For study of biochemical properties the following was used: Nessler's reagent, acid fuchsin, solutions of: 1% hydroperoxidase solution, 10% plumbum acetic acid, 12% oxalic acid, 1% methylene blue, 10% potassium hydroxide, 4% sodium hydroxide. The study of isolated cultures was performed by microscopy, determining peculiarities of motility and growth on nutrient media, establishing biological properties and a serological study.

**Results:** The researches demonstrated that after affection of piglets with salmonellosis it ran mainly severely during 10 days. Changes characteristic to blood circulation disorder, hypertrophic and inflammatory processes mainly of exudative nature were observed. Macromorphologically cyanosis is obviously expressed in the liver. The organ is of grey-pink color because of which both the surface and cut are colorful, which is caused by unequal distribution of vein blood. The liver

has been widened and the edges-round. The given form of the disease of the liver is characterized by development of submiliaric necrotic foci, which is a change of diagnostic importance. As a result of the micromorphological study it was determined that the construction of liver lobes remained. Hepatocytes increased their protoplasm and were turbulent and poorly stained. The nucleus is light which is characteristic for parenchymal dystrophy. In separate sites, especially around the central vein, destruction-necrosis of hepatocytes occupying a small space and being foci correspondent to submiliar necrosis has been observed. Liver lobes are of unequal size. They may be sharply widened (lobes hypertrophy) in size or narrowed (lobes atrophy). On the whole, lobe hypertrophy is a characteristic symptom of acute salmonellosis. In such lobes overfilling sinus capillaries with blood are obviously expressed, being intensive in the centre of the lobe and then comparatively weakening in peripheries. Some hemorrhages occur in the lobes. There are some kinds of lobes, where there is focal perivascular infiltration and capillary stasis of leucocytes (mainly neutrophils). The central vein is sharply widened and overfilled with blood, containing hemolyzed erythrocytes. Sharp circulation dysfunction has been observed in the intralobe binding tissue. All kinds of vessels are overfilled with blood, the walls and adjacent tissues are widened as a result of which perivascular infiltration, stasis, hemorrhages, swelling are considered to be a coexisting process of the above-mentioned form, while proliferation is poorly existing.

**Conclusion:** Based on the micromorphological research carried out, it was determined that pathomorphological changes developing in acute salmonellosis are characterized by sharp disorder of blood circulation resulting in liver cyanosis, stasis, perivascular swelling, dystrophy of hepatocytes and miliaric necrotic foci in liver.

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## P5

### Immobilization and anesthesia of grey wolves (*Canis lupus, L*) in the Skopje ZOO using xylazine/ketamine combination

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Safe access to most animals that are residing in Zoo's is only possible after their immobilization. Reducing the stress that occurs during manipulation, transport and various procedures including surgeries require the use of sedation and anaesthesia.

The aim of this report was to analyze the effects of the anaesthesia by monitoring the time of induction, general anaesthesia and recovery from anaesthesia, as well as the behaviour during the whole anesthetic period in captive grey wolves.

In our case, we used combination of dissociative anaesthetic (Ketamine) and  $\alpha$ 2-adrenoceptor agonists (Xylazine). Injectable anaesthetic agents were applied through a remote delivery system, consisting of a dart and projector (blowpipe). Three captive grey wolves, two females and one male, at the age of 7 years, 2.5 years and 9 months, and body weight 30 kg, 27 kg and 19 kg, respectively, resident at the Skopje Zoo, were immobilized with an intramuscular injection of a combination of Xylazine (1mg/kg) and Ketamine (3.5 mg/kg). Drug doses were calculated by visual weight estimation. After the application of the anaesthetics, the first effects, like decreased motion and stumbling, were noticed after 6-8 minutes. Due to lower estimated weight, the male needed an additional dose of anaesthetic. The dose was recalculated for additional 5 kg and applied 27 minutes after the first injection. The average period of induction (from application of the anaesthetic to the safe manipulation with the animals), was 20.3 minutes. After immobilization, the weight was measured and the animals were placed in the transport box and sent to the Faculty of Veterinary Medicine in Skopje for further treatment (ovariohysterectomy and orchyectomy). Upon arrival at the University veterinary hospital, the animals received the same anaesthetic combination at a dose ranging from 0.25 to 1 mg/kg Xylazine and 2.5 to 3.5 mg/kg Ketamine, depending on the detected anaesthetic level. The average heart rate was 95 b/min. and respirations were 22/min. After recovery from anaesthesia, no adverse effects on their health or changes in their behaviour were noticed.

Based on the data obtained in this report, it can be concluded that the combination of Xylazine/Ketamine allowed a reasonably fast and smooth induction of anaesthesia, as well as secure immobilization and analgesia in order to perform safe manipulation and surgery of the captive wolves.

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## P6

### Study of endoparasites prevalence in foxes in the western part of Romania

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**Introduction:** Studies on the parasitism of foxes existing in the western hunting grounds of Romania are not numerous. Some species of endoparasites present a danger to human health. These endoparasites in foxes are also a source of infestation for domestic carnivores. The aim of the study was to identify those infested foxes (*Canis vulpes*) from the hunting grounds in western Romania.

**Material and methods:** During the period November 2013 - April 2014, 52 foxes (*Canis vulpes*) were

necropsied. Of these, 31 were males, 21 females and were aged between one and three years. We collected all the digestive tract to determine the endoparasites present in all bodies. We performed two examinations: macroscopic (gastrointestinal examination portions of the digestive tract) and microscopic (examination of faeces by flotation and sedimentation methods).

**Results:** In 43 corpses (83%) we identified nematode and cestode parasitism with the following prevalence: *Toxocara canis* 69%, *Trichocephalus vulpis* 23%, *Pterygodermatitis affinis* 9.6%, *Taenia pisiformis* 26%, *Mesocostoides lineatus* 36%.

**Conclusion:** We emphasize that this is the first study of foxes carried out in western Romania. This study reveals high prevalence of endoparasites and the risk of contamination of domestic carnivores and humans, equally.

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## P7

### The evaluation of atopic dermatitis impact on health-related quality of life of affected dogs and their owners in Romania

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**Introduction:** In the last few years atopic dermatitis (AD) has become widespread both in humans and dogs in Romania. This skin disease impairs the quality of life (QoL) of affected patients. It is known that AD is usually evaluated using a clinical approach and pruritus score. That's why several studies have been recently carried out to establish disease-specific questionnaires to assess both the QoL and health-related quality of life (HRQoL) in affected individuals (children with AD and their parents, owners with AD dogs).

**Material and methods:** In this respect, 100 questionnaires aiming to evaluate QoL and HRQoL both in owners and their AD dogs were prepared in Timisoara, Romania. Only 26 (26%) valid ones were returned from the owners. Answers were statistically analyzed to reveal the relationship between owner and his pet.

**Results:** In this study, this relationship was not influenced by the age of the owner, and the more severe the disease is, the more QoL is severely affected in both. Euthanasia is not an option for the pet owners from Timisoara, and the costs of establishing and applying the treatment appear to be not restrictive, showing a very close pet-owner relationship.

**Conclusion:** This is the first study of this kind conducted in Romania.

## P8

### Effect of optigen on some parameters of rumen fermentation in yearling rams

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A physiological experiment was conducted to evaluate the addition of Optigen® to the ration of yearling rams on rumen fermentation. Optigen® is a source of specifically protected non-protein nitrogen (NPN) with indirect controlled (slow) release in fore stomachs, specially designed for supplementation of ruminants' diets. Six yearling rams were used - Blackhead Plevan × Suffolk crosses, with initial live body weight of 45±2 kg. The animals were of uniform gender, body weight and origin. They were housed indoors, in individual boxes at the Experimental base of the Physiology Unit to the Faculty of Agriculture, Trakia University – Stara Zagora. The experiment consisted of two periods – control and experimental. The following parameters were investigated: hydrogen ion concentration (pH), ammonia concentration, total volatile fatty acid (VFA) concentration, total counts and generic composition of rumen ciliates, and in vivo cellulolytic activity in the rumen. It was established that the supplementation of yearling rams' ration with Optigen increased and stabilised rumen pH values, which varied within a narrow range – from 6.61 to 6.78. The differences between control period and post feeding hours 1, 2.5 and 5 were statistically significant ( $p < 0.001$ ,  $p < 0.05$ ;  $p < 0.01$ ). Optigen reduced the total volatile fatty acids and ammonia concentrations in the rumen content after feeding ( $0.05 < p < 0.001$ ). Optigen exhibited an inhibitory effect on ciliate fauna ( $p < 0.01$ ) as well as 1.3 times higher cellulolytic activity (9.02% vs 6.98% during the control period).

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## P9

### Laser surgery for the treatment of epiglottic retroversion in horse – One case

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Retroversion of epiglottis has been reported in horses as a relatively uncommon cause of the upper airway obstruction.

Two-year old crossbred colt was admitted to the Equine Clinic Brno because of the stridor audible during exercise. Resting airway endoscopy revealed pharyngeal lymphoid hyperplasia grade III/IV. Because the treadmill endoscopy

was not available at this time, the colt was discharged. Since poor performance and respiratory stridor during exercise were consistently observed, the horse was re-examined at the clinic one year ago. Physical examination and resting airway endoscopy was normal. The high-speed treadmill video-endoscopy of upper airways revealed retroversion of the epiglottis during inspiration resulting in obstruction of *rimaglottidis*, which appeared in trot (speed 4 m/s) and continued in gallop. Regarding the previous experience, the laser treatment was chosen. The intervention was performed in general anaesthesia. The tip of the epiglottis was grasped under visual control and the epiglottis was retroverted to make accessible its lingual surface. Using a 400 µm bare-fibre approximately 20 contact points were performed (15 W, 2-3 sec) until a visible mucosal reaction. After the recovery from the anesthesia, the first follow up endoscopy showed normal position and slight swelling of the epiglottis. The post-surgical treatment consisted of flunixin-meglumine for three days and rest for two weeks. The second follow up examination was performed 12 weeks after surgery. According to the owner the performance of the horse rapidly improved and no stridor was audible during exercise. The horse swallowed feed without problems. Shortening of the epiglottis was found out by resting endoscopy. During the high-speed treadmill examination rising up of the epiglottis was observed in trot and gallop during inspiration, but complete retroversion leading to obstruction of *rimaglottidis* was not visible anymore. Necrosis of the tip of the epiglottis developed as a complication and was caused most probably by grasping. But the role of the epiglottis in swallowing is controversial. The experimental evidence suggests that epiglottis may not be essential for the prevention of aspiration during swallowing in mammals. Therefore the laser surgical coagulation of the lingual epiglottic surface might be used as possible treatment of epiglottic retroversion in horses.

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## P10

### Fatal encephalitic aspergillosis in ZOO kept young swans

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**Introduction:** Aspergillosis is a common fungal disease affecting domestic, wild and captive birds. The most predominant causative agent is *Aspergillus fumigatus*, but other species like *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus nidulans* can be isolated from cases of avian aspergillosis. They are ubiquitous organisms and can be found anywhere (soil, feed, contaminated surfaces, decay material). In young birds aspergillosis

is characterized with acute onset resulting with high morbidity and mortality. Among the several forms of the aspergillosis, depending on the location of the lesions, there are numerous reports describing encephalitic aspergillosis in many avian species. This type of aspergillosis is usually present like focal granuloma in the cerebellum or cerebrum.

**Material and methods:** This study was performed on two dead swan one moth old from the Zoo garden Skopje, Macedonia. Clinical and anamnesis data were collected beforehand. Young swans demonstrated nervous signs with progressive nature lasting for one week. Necropsy was performed on the birds and tissues from the organs with lesions were collected for mycology and histopathology.

**Results:** Necropsy findings revealed airsacculitis with thickening and discoloration of the air sac membrane. Lesions in the brain were characterized as yellow to reddish focal granulomas. Mycology investigations revealed presence of *Aspergillus fumigatus* from both the brain and air sacs, while histopathology of the focal granuloma from the brain revealed presence of giant and mononuclear cells together with visible hyphae.

**Conclusion:** Aspergillosis can be a serious threat to captive birds, especially in young birds, often associated with fatal outcome. According to the age of the swans and time of the onset and severity of the clinical signs, birds were infected either *in ovo* or during the first days of life when the immune system is not fully developed and there is severe pressure from the environmental factors. Preventive measures for future hatchlings were recommended and successfully applied.

## P11

### Canine thelaziosis caused by *Thelasia callipaeda* in Bosnia and Herzegovina

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*Thelasia callipaeda* is a nematode belonging to the genus *Thelasia* which parasites in the conjunctival sac of domestic and wild carnivores. Clinical symptoms vary from conjunctivitis, lacrimation, eye congestion, photosensitivity, epiphora, blepharospasm and rarely keratitis, corneal ulcerations or blindness. Cases of thelaziosis have been reported in many parts of Europe, but not in Bosnia and Herzegovina. This report describes the first case of canine thelaziosis from Bosnia and Herzegovina, representing the first case in this region. Adult nematodes recovered from live animals were morphologically identified as *T. callipaeda*.

**P12****Fibrosarcoma of the mandible in Sharplanina mountain dog – Case report**

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**Introduction:** Tumors involving bones in dogs could occur both on the axial and the appendicular skeleton. Usually they manifest lameness (if the appendicular skeleton is affected) and regional mass. Among the canine bone tumors, the osteosarcoma (OS), is most common, and accounts for approximately 85% of skeleton malignances and approximately 5% of all canine neoplasms. Oral osteosarcoma manifests pleomorphic mesenchymal cells with marked anisocytosis and anisokaryosis. Multilobular osteochondrosarcoma (MLO) is a rare tumor that generally arises from the flat bones of the canine skull. Histologically, this tumor is composed of multiple nodules each containing a core of cartilaginous or bony matrix surrounded with thin layer of spindle cells. MLO primarily occurs in older and medium-to large-breed dogs and occasionally in small breeds, like Pekingese dog. Chondrosarcoma (CS) is the second most common canine primary bone tumor (5-10% of all primary bone tumors in dogs), and is characterized histologically by anaplastic cartilage cells with cartilaginous matrix. Hemangiosarcoma of bone is a rare tumor (less than 5% of all bone tumors), and affects middle-aged to older dogs of any size, although there is evidence of breed-related predispositions in German Shepherd dogs. Primary fibrosarcoma (FS) is also a rare tumor in dogs (less than 5% of all bone tumors) and it is difficult to distinguish it from fibroblastic OS histologically. It more often affects the axial skeleton than the appendicular skeleton, and is more common in young dogs.

**Material and methods:** A four-year old, male, Sharplanina Mountain Dog was presented at the University Veterinary Hospital within the Faculty of Veterinary Medicine in Skopje. It had a history of wounding in the apical region of the mandible. He had received common treatment (antibiotics and corticosteroids). Soon, at the site of the lesion, the gingiva proliferated and formed a mass. After the clinical examination, further diagnostic tests were performed: radiography, blood testing, cytology (FNA, Diff-Quick straining) and pathohistology analysis.

**Results:** The radiographic finding showed splitting of the apical region of the mandible, so surgical treatment was recommended. The FNA aspirate manifested small number of mesenchymal cells which showed signs of malignancy, with dark-violet colored cytoplasm. Nuclei

showed anisocariosis, light-violet color and many prominent nucleoli, which are a feature of the malign fibrosarcoma cells. This finding was confirmed with pathohistology analysis.

**Conclusion:** Surgical treatment included amputation of the apical region of mandible, including canine teeth and incisive teeth.

**P13****The usage of erythropoietin (EPO) in nonregenerative anemia as a result of chronic renal failure caused by chronic ehrlichiosis in dogs: Case report**

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**Introduction:** Anemia is characterized as insufficient concentration of hemoglobin due to decreased production, increased destruction or loss of red blood cells. It can be expressed as a packed cell volume (PCV), hematocrit, red blood cell (RBC) count, or hemoglobin level under the reference rates. Anemias can be caused by blood loss, hemolysis or inadequate production of red blood cells and are classified as: a) regenerative and b) nonregenerative. Ehrlichiosis, known as canine hemorrhagic fever is caused by rickettsia - *Ehrlichia canis*, usually transmitted by the infected ticks, *Rhipicephalus sanguineus* sp. that affects circulatory system, particularly the monocytes. Bleeding, anemia, lymphadenopathy, anorexia, depression, renal failure, are few of the clinical symptoms characteristic for Ehrlichiosis. Chronic renal failure occurs as irreversible and progressive nephrons damage which leads to reduced production of erythropoietin, a hormone that controls the production of red blood cells.

The aim of this report is to present a successful administration of recombinant human erythropoietin as a supplement of endogenous erythropoietin for stimulation of red blood cells production in patients due to renal failure.

**Material and methods:** The patient, 4 years old female, Maltese poodle was admitted in our clinic on 25.07.2013 due to signs of vomiting, lethargy, loss of appetite and weakness. Clinical examination revealed pale mucous membranes, normal body temperature 38.4°C, increased heart and respiratory rate and normal lymph nodes. Haematology, biochemistry, peripheral blood smear to exclude Babesiosis, antigen rapid tests of *Leishmania* and *E. canis* were performed.

**Results:** Laboratory examination revealed pancitopenia (RBC 3,35x10<sup>12</sup>; WBC 6.5x10<sup>9</sup>; Hgb 87 g/dl; Hct 26 %) highly increased levels of urea, phosphorus and creatinine (65,1 µmol/L; 2,7 mmol/L; 403 µmol/L) and positive rapid

test of Erythiosis. Treatment started by i/m administration of Tetracycline 5 mg/kg, i/v fluid therapy, along with supportive injection of 100 mg iron dextran + B<sub>12</sub> in order to manage anemia. Due to continuous loss of red blood cells, second week after the treatment (RBC 1,99x10<sup>12</sup>; WBC 6.0x10<sup>9</sup>; Hgb 56 g/dl; Hct 17 %) we prescribed 150 IU/kg s/c administration of Erythropoietin (Eprex 2000), initially three times per week. Slightly decreased values of creatinin and urea and improvement of blood counts were observed 6 weeks after the administration of human erythropoietin. Despite slight hypersensitivity, the treatment continues by s/c administration of 100 IU once a week along with 1 capsule of Ferroglobine daily.

**Conclusion:** Erythropoietin in animals is used for the treatment of anemia due to kidney disease. Recombinant human erythropoietin proved to be an excellent choice for treatment of nonregenerative anemia due to chronic renal failure.

#### P14

### Theileriosis case in a one-month old calf

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**Introduction:** Tropical theileriosis is a disease of cattle caused by *T. annulata* and causes considerable economic losses in herds.

**Material and methods:** A one month old, Holstein breed, female calf was the material of this case report. Blood smears were carefully examined under the oil immersion lens (100 x magnification) following Giemsa staining to detect *Theileria* piroplasm. Even the presence of a single piroplasms in erythrocyte was considered as a positive. The calf was treated with oxytetracycline (Primamycin/LA, 10 mg/kg im) or buparvaquone (Butalex 2.5 mg/kg im), Jactofer amp, % 5 Dekstrose, calcium (Kalsimin), vitamin C (Injacom-C) and vitamin B complex (Berovit B<sub>12</sub>).

**Results:** Fever, lack of appetite, lymphadenopathy, oedema at the eyelid, anaemia, jaundice, coughing and hard vesicular sounds in the lung were the prominent clinical symptoms. Petechial and ecchymotic hemorrhages were observed at the hairless areas of the skin, oral cavity and conjunctiva. While decreases were observed at the erythrocytes, total leukocyte, haemoglobin, hematocrite values, total protein and albumin level, while increases at aspartat amino transferase (AST), urea, total and direct bilirubin levels were seen.

**Conclusion:** As a result, exanthema on the animals' patient's back skin, increased liver enzyme level and the changes in blood parameters is related to liver degeneration, issued in erythrocytes to hemolysis and ticks toxins.

#### P15

### Anesthetic management and surgical repair of diaphragmatic hernia in cat: Case report

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Diaphragmatic hernia is a protrusion of viscera into the thoracic cavity due to radial or circumferential tear of the diaphragm. Nearly 85% of diaphragmatic hernias occur after blunt abdominal trauma such as motor vehicle accidents or falling from heights. Most frequently herniated organ is the liver followed by parts of the small intestines, spleen, pancreas, omentum, stomach and large intestines. The mortality rate is usually associated with the amount of herniated organs, location of the herniation and the time interval from the trauma to the surgery. Lung capacity loss, pulmonary contusion, hypovolemic shock, dyspnea, tachypnea, arrhythmias, pleural effusion, atelectasis, intestinal obstruction, are all major clinical findings in patients with diaphragmatic hernia.

The aim of this report is to present a successful anesthetic management, surgical repair and recovery of diaphragmatic hernia in a 7 month old, male cat. Two months before the admission at the University Veterinary Hospital the owner noticed breathing difficulties accompanied with coughing. Upon initial clinical examination dyspnea, abdominal breathing, absence of breathing sounds on the right side of the thorax, as well as normal body temperature and CRT were found. Plain and positive contrast abdominal and thoracic radiography revealed absence of the diaphragmatic line and lungs and cranial displacement of part of the small intestines. In order to restore normal ventilation surgical repair of the diaphragmatic hernia was scheduled. The patient was premedicated by i/m administration of 0,2 mg/kg Acepromazine maleate. Induction of surgical anesthesia was achieved by i/m administration of 10 mg/kg Ketamine and maintained using Isoflurane (1.5-3%) and manual ventilation. Midline celiotomy revealed a tear of the ventral right portion of the diaphragm, compression of the lungs, while parts of liver lobes, duodenum, pancreas and omentum were found into the thoracic cavity. Herniated organs were gently retracted into the abdomen and the rupture was closed in 2 layers with simple interrupted pattern using 2/0 PDS. Before final suture was closed, air from the pleural cavity was evacuated and negative pressure was achieved. The incision line was closed in a standard manner. Preoperative and postoperative analgesia was achieved with 2 mg/kg Ketoprofen and ventilation corrected with 6 mg/kg Aminofilin. Plain radiography revealed adequate closure of the diaphragmatic rupture and slight pneumothorax which was resolved on day 12 after the surgery.

**P16****Anesthesia and analgesia for caesarean section in dog**

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**Introduction:** The aim of this report is to present the use of balanced anesthesia and analgesia during a cesarean section case of gestating three-year old English bulldog that was brought to the Faculty of Veterinary Medicine, Belgrade with dystocia symptoms.

**Material and methods:** Pre-medication was carried out by intravenous application of Medetomidine hydrochloride (Cepetor, pharma - cp 1mg/ml) at a dose of 0,003 mg/kg. After two minutes, along with constant oxygenation, propofol was administered intravenously (Diprivan, Astrazeneca UK Limited, 10 mg/mL), slightly till the titration effect. After reaching effect, an endotracheal tube was applied and the general anesthesia was maintained using Sevoflurane (Sevoran 100 % Aesica Queenborough Ltd, UK). After reaching a surgical level of anesthesia, a reversal agent Atipamezole hydrochloride (Revertor, Cp-pharma, 5 mg/ml) at a dose of 0,003 mg/kg, was administered intravenously, in order to cease the action of Medetomidine hydrochloride. Before incision is was made, at the incision site, the infiltration of lidocaine chloride 2% (Lidocaine, Galenika 2%, 40mg/2ml was administered at a dose of 2mg/kg. During the fast surgical procedure and the removal of the last pup, a surgical plane of anesthesia was maintained with 3.5 % sevoflurane with concurrent analgesic treatment using Ketamine hydrochloride 10 % (1ml/100 mg Ketamidol, Wels, Austria), at a dose of 1 mg/kg i/v. Towards the end of the surgery the patient received NSAID, Carprofen (Rimadyl, Pfizer) at a dose of 4mg/kg, i/v.

**Results:** Premedication with medetomidine hydrochloride, in this case enabled a possible relaxation of the musculature. Induction of anesthesia with intravenous propofol application enabled rapid endotracheal intubation. The effect on the respiratory system of the fetus is reduced by using a low dose of sevoflurane. After intubation, intravenous application of atipamezole hydrochloride is given, as antagonists of medetomidine hydrochloride. After ten minutes of extubation, the patient was fully recovered and adopted the pups. All puppies began with spontaneous breathing and during the interventions were held in the incubator at the appropriate temperature, humidity and oxygen concentration, which was absolutely vital.

**Conclusion:** The presented case has described the use of a combination of short acting, rapidly metabolized anesthetics and analgesics that provides minimal negative impact on the patient and its newborn puppies. Reversible anesthetics are recommended since the duration of anesthesia for cesarean section should be as short as possible, with concurrent use of minimal doses of inhalation

anesthetics. Application of  $\alpha_2$ -agonists in premedication, propofol in the induction and maintenance of general anesthesia with sevoflurane inhalation under local anesthesia with lidocaine chloride 2% as a local anesthetic and ketamine hydrochloride 10%, as an analgesic, in the case of a caesarean proved an ideal combination.

**P17****A case of Monobrachial Peromelia in a 2 year old Holstein cow**

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**Introduction:** Peromelia is a severe congenital malformation of the limbs, including absence of the lower part of the extremity. It is one of the rarely observed malformations in animals.

**Material and methods:** *Monobrachial peromelia* was observed in a female, 2 year old Holstein cow in the right front limb. The cow was examined gross pathologically before and after slaughter.

**Results:** The cow was clinically healthy and in a good body condition. The proximal limb from the shoulder up to the radius and ulna was normally developed. Only normal scapulae and rudiments of the proximal segments of the radius and ulna were present. Normal skin covered the scapulae and the malformed radius and ulna. The cow has only one forelimb and marked angulations were observed at the left front limb. There was no other abnormality diagnosed.

**Conclusion:** *Monobrachial peromelia* was reported by using an anatomo-pathological method in a two year old cow. This is the first *Monobrachial peromelia* cow report in Turkey.

**P18****Insulinoma (pancreatic insulin-secreting tumor) in dog: Case report**Pandorce Trenkoska-Spasovska<sup>1\*</sup>, Emil Janev<sup>1</sup>, Ksenija Ilievska<sup>2</sup>, Tomco Lazov<sup>1</sup><sup>1</sup>*Veterinary clinic Dr. Naletoski-Skopje, Macedonia*<sup>2</sup>*Faculty of Veterinary Medicine, Ss Cyril and Methodius University in Skopje, Macedonia*

The pancreas is a glandular organ located close to the liver, stomach and small intestines with two main functions, exocrine and endocrine. The exocrine cells produce enzymes that digest food which are transported by pancreatic ducts to the intestines. The endocrine cells are located in small groups called islets of Langerhans which contain four cell types: alpha, beta, delta and F cells. The main function of the beta cell is producing

insulin, a hormone usually secreted in small amounts due to high blood sugar (glucose). Tumors of the pancreas may arise from exocrine or endocrine cells. Insulinoma is a rare type of malignant, fast growing tumor affecting beta cells of the pancreas. The beta cells primarily secrete small amounts of insulin in order to regulate high blood glucose levels which occur after meal. Due to insulinoma, regulation of serum glucose concentration is disrupted and excessive secretion of insulin by beta cells causes lowering of the blood glucose level below normal range (hypoglycemia). Insulinoma usually occurs in middle age to older dogs without sex predilection. Middle sized or large breeds of dogs are most commonly affected.

The aim of this report is to present a clinical case of a 10-year-old male West Highland White-Terrier. The patient was brought to our clinic due to progressive and episodic weakness and seizures, muscle tremors, loss of coordination, unusual behavior, lethargy and trembling. The owner noticed that the seizures became more frequent especially after fasting or before feeding. After application of a small amount of sugar or honey in the dog mouth, the seizures were reduced. Upon admission full clinical, neurological and laboratory examination was performed (complete blood count including serum biochemical panel-glucose, serum amylase, alanin aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine, albumin and insulin levels and urinalysis. Normal CBC and urinalysis, with mild hypoalbuminemia, low glucose concentration (1,6 mmol/l) and high insulin concentration (91,5 pmol/l) were noticed. Based on the results and clinical signs, pancreatic insulinoma was diagnosed. Despite the therapy and prescribed diet, 10 days after the treatment the owner decided to euthanize the dog.

Necropsy revealed dark-color and small amounts of tumor masses on the pancreatic surface. Beta pancreatic islet cell tumor was confirmed on pathohistological examination. Even if surgery is possible in conjunction with medical and dietary treatment the prognosis of insulinoma in dog is very poor with survival rates from 6-24 months.

#### P19

### Fatal snake bite in a brown bear (*Ursus arctos L.*): Case report

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Poisoning from snake venom in animals is an emergency which requires immediate attention or otherwise delayed and inadequate treatment may lead to untoward consequences and death. The present paper describes a case of venomous snakebite in a Brown bear cub (*Ursus arctos L.*) and its therapeutic management. The

brown bear cub which is found alone on the slopes of a mountain in southwest the part of the country was presented to the peripheral veterinary practice in Ohrid with a history of dullness, disorientation and excessive swelling around the left forepaw. It was diagnosed for snakebite based on the history and physical examination. The hematological parameters showed reduced values of hemoglobin, packed cell volume and increased total leukocyte count. The biochemical values showed elevated levels of alanine aminotransferase and creatinine. After immobilization of the animal, the treatment was done with fluids, corticosteroid, and broad spectrum antibiotic with careful monitoring. Despite the treatment which was initiated immediately, it was only partially effective, and the animal died one hour after the beginning of the course. Poisonous snakes are common in the mountainous part of Macedonia and, just like humans, wild bears especially their cubs are susceptible to the deadly venom of some species. The severity of the reaction to snake venom and prognosis in animals depends on a number of factors: on the type and species of snake, on how much venom was injected, on the location of the bite, on the age, health and body weight of the animal and crucially the time interval between the snakebite and the institution of treatment.

#### P20

### The use of ketamine, xylazine and midazolam combination for total intravenous anesthesia (TIVA) in surgical removal of abdominal testis of a stallion

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**Introduction:** Equine practitioner vets frequently anesthetize horses. In majority of cases performing short-term anesthesia (duration, 20 minutes). But there are substantial needs for long term anesthesia. The aim of this work is to present our experience with a long term and short term total intravenous anesthesia in horses.

**Material and methods:** In this paper we are presenting a result of anesthesia monitoring of a horse undergoing a surgical removal of the abdominal testis (complete abdominal cryptorchid). Sedation of the horse was conducted with xylazine, 1.0 mg/kg, iv, and midazolam 0,06 mg/kg, iv. The total anesthesia was induced using a combination of ketamine 2,2 mg/kg/iv, and midazolam 0,1mg/kg/iv. After induction, the horse was restrained and anesthesia was maintained with continuous intravenous drip of combination of drugs mixed in an infusion bottle containing midazolam (0.002 mg/kg/min), ketamine (0.03 mg/kg/min), and xylazine (0.016 mg/kg/min). Additional ketamine (0,03 mg/kg) and midazolam 0,03



mg/kg/iv was administered two times, if the horse moved the head or limbs during procedure.

**Results:** The duration of anesthesia was 80 minutes. During this time the cardiopulmonary parameters and reflexes were monitored continuously. The recovery from anesthesia was 30 minutes and the horse stood up in his first attempt.

**Conclusion:** midazolam, ketamine, and xylazine in combination produced TIVA in horses and can be used for short term and middle term, lasting surgical procedures in on field surgery.

## P21

### Maxillary osteosarcoma in a dog – Case report

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In this case maxillary osteosarcoma is pathologically described in a 13 year old, terrier female, dog. A mass showed in same characteristics, larger than others was detected in the same region about one month later. Its necropsy was carried out at the request of the owner. During necropsy, it was noticed to be firm, grayish-brown, 7 cm in diameter on the right maxillary region. At the center of the mass there appeared to be a crater, but it had irregularities at the edge. Histopathologically, it was anaplastic, ovoid-round shaped, osteositis and osteoblasts. In some areas, giant cells were detected. Calcification areas and cartilage tissue were come along with these areas.

## P22

### Phosphodiesterase type 5 (PDE5) inhibition attenuates cyclosporine A induced nephrotoxicity in mice

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**Introduction:** In the present study, renal protective effects of the phosphodiesterase type 5 (PDE5) inhibitors

in mice with calcineurin phosphatase inhibitor-related nephrotoxicity were investigated.

**Material and methods:** Fifty male mice were divided into five groups. Group 1 (G1) did not receive and medication; G2, received saline only; G3, received only CyA (30 mg/kg/d) by subcutaneous (SC) injection; G4, received only vardenafil (30 mg/kg/d) by the oral route; and G5, CyA (30 mg/kg/d/sc) + vardenafil (30 mg/kg/d) by the oral route. At the end of 28 days, platelet-derived growth factor A and C (PDGF-A, PDGF-C), transforming growth factor-beta 1 (TGF-β1), cyclooxygenase 1 and 2 (COX-1 and COX-2), and P glycoprotein (Pgp) expression levels were measured in the renal tissues. In addition, expressions of COX-1 and COX-2 genes were also determined by Real-Time PCR.

**Results:** Administration of PDE5 inhibitor restored renal dysfunction induced by CyA administration. PDE5 inhibitor administration significantly restored all the ligands with respect to controls. The relative expressions of COX-1 and COX-2 genes to GAPDH revealed that the maximum increase was obtained in the group combined CyA and vardenafil for the both COX-1 and COX-2 genes. **Conclusion:** The present results demonstrate that long-term oral treatment with PDE5 effectively prevents pathological kidney changes in the CyA induced nephrotoxicity.

## P23

### Two cases of mycotic rumenitis in a calf and a lamb

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**Introduction:** In this report, mycotic rumenitis was pathologically defined in a one week old male calf and a two-week-old lamb. At necropsies, common hemorrhage areas in rumen serosa and extensive necrosis in the mucosa of especially rumens and other forestomachs were seen. Besides, in the calf, there was a yellowish fibrinous liquid, which was coagulating when exposed to air, in the thorax, 1-2 cm in diameter pale areas in the heart, consolidated lobules in the left pulmonary lobes.

**Material and methods:** Tissue samples taken from organs were fixed in 10% formalin solutions and then routine processing procedure was performed. The sections, obtained from paraffin blocks in 5 micron thickness, were routinely stained with hematoxylin-eosin (HE) and some section also stained with periodic acid-Schiff (PAS) reaction.

**Results:** During histopathological examination, areas of wide coagulation necrosis were seen in the rumen walls along with hemorrhage, edema, fibrin clouds and some fibrosis. In addition, PAS stained fungal hyphae

were observed in these necrotic areas and vessels, some of which were thrombotic. In the lungs of the calf, catarrhal bronchopneumonia was determined, while interstitial pneumonia findings were observed in the lamb. Additionally, hemorrhages and myocarditis findings were seen in the heart of the calf. Fungal cultures were grown by inoculation from the rumen.

**Conclusion:** As a result, necrotic-hemorrhagic lesions, which are located from the mucosa to the serosa, should primarily be considered as mycotic rumenitis. It has been emphasized that this kind of cases in very young animals should take into account intrauterine infections.

## P24

### Thymoquinone: a new therapeutic challenge in canine leishmaniasis (Long term multiple therapy protocol)

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**Introduction:** Evaluation of Thymoquinone in Canine Leishmaniasis versus common long-term multiple therapy protocol.

**Material and methods:** Ten owned dogs (Mix Breed n:5, German Sheppard n:3, Doberman n:1, Jack Russel Terrier n: 1) were referred to the clinic with complaints of weakness, weight loss, ocular discharge and skin lesions. In the clinical examination, severe exfoliative dermatitis with ulceration on the pinnae, nasal depigmentation and ulceration, onychogryposis, bilateral periocular alopecia with mucousal pallor were found. Fine needle aspiration from popliteal lymph node was performed in all dogs and parasite amastigote was detected with Diff-Quik staining. The diagnosis was confirmed by PCR and IFAT performed on blood samples. Long term treatment protocol; Marbofloxacin (2.5 mg/kg, SID, PO, 1 month) with Allopurinol (30 mg/kg, SID, PO, 6 month) was applied in all dogs. In addition Domperidone (2 mg/kg, SID, PO) was used for one month and Thymoquinone (*Nigella sativa oil*) was used for 6 months.

**Results:** At the end of the 6 months of allopurinol therapy in dogs alongside the classic thymocinol domperidone therapy to be very successful in the clinical appearance of recurrence was not seen.

**Conclusion:** Canine Leishmaniasis classic domperidone and allopurinol treatment with the disease factor so much power improvement to be seen on account immune systems strong hold and for this purpose *nigella sativa oil* classical treatment of the next recurrence of disease to inhibit was concluded.

## P25

### Comparison of levels of certain heavy metals and minerals in cattle raised near and away from highways

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**Introduction:** Concentrations of certain heavy metals and minerals in cattle raised in Çankırı Province were investigated within the scope of this study. For this purpose, levels of heavy metals and minerals in blood samples obtained from 100 cattle raised in the province were assessed.

**Material and method:** The blood samples taken from jugular veins of the animals into anticoagulant free tubes were drawn after coagulation. Then, the serums were separated by centrifugation. Concentrations of certain heavy metals and minerals in the serums were analyzed on ICP-MS device (Agilent 7500a).

**Results:** Consequently, with the this study, levels of certain heavy metals and minerals in cattle raised near (300 m) and away from highways (2.5 km) in Cankiri Province were determined. Levels of aluminum (Al), iron (Fe), nickel (Ni) and zinc (Zn) in the cattle raised near highways were significantly different than the cattle raised away from highways ( $p < 0.05$ ). These results were Al  $105.2 \pm 35.04$  ppm, Fe  $122.6 \pm 34.76$  ppm, Ni  $2.37 \pm 1.21$  ppm, Zn  $0.22 \pm 0.15$  ppm in cattle raised near highways, and Al  $91.29 \pm 31.14$  ppm, Fe  $103.9 \pm 19.20$  ppm, Ni  $1.46 \pm 0.54$  ppm ve Zn  $0.14 \pm 0.08$  ppm in cattle raised away from the main road.

**Conclusion:** The risk of exposure to metals for humans and animals has increased today, due to the variety in agricultural production methods, unconscionable use of fertilizers and agricultural pesticides, contamination of human and animal food chains as a result of unsanitary storage of solid wastes and waste water, exhaust gases from vehicles and varying qualities of fuel-oil for vehicles, This may consequently lead to a decrease in the quality of food of animal origin.

## P26

### First report of the presence of ticks from the Hyalomma marginatum complex in three nomenclatures of territorial units for statistics (NUTS) level III regions in R. Macedonia

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**Introduction:** Diversity of tick species in the R. Macedonia still represents an unexplored field. Some

research studies showed that the Balkans in Europe are the natural habitat for persistence of the hard tick species such as *Hyalomma marginatum*. The importance of this tick lays in the fact that it is the main vector of a virus that causes Crimean Congo Hemorrhagic Fever (CCHF), a severe and potentially lethal disease to humans. Some other diseases such as Anaplasma, Ehrlichia, Rickettsia, Borre-lia, and Babesia/Theileria spp. have been found to be transmitted by this species of tick. In order to estimate the presence of all this diseases in R. Macedonia, especially the presence of CCHF, it is essential to prove the presence of *Hyalomma marginatum* tick species in this country and its regions respectively.

**Material and methods:** In the period of May-June ticks were collected from different breeds of cattle and sheep in three NUTS level III regions such as: Skopje region, North-eastern region, South-eastern region and Vardar region in R. Macedonia. Mostly open country habitats as well as arid open areas were chosen, since it is the most preferable habitat for this species of tick.

**Results:** A total of 136 ticks were collected and identified. The presence of the *Hyalomma m. marginatum* species was proven in three regions, except in the Vardar region, which might be due to a small number of collected ticks. Some other species such as *Rhipicephalus bursa* and *Rhipicephalus annulatus* were detected.

**Conclusion:** This is the first finding of *Hyalomma marginatum* complex tick species in the R. Macedonia. Since this tick species is the main vector of many pathogens, especially the CCHF, it is of great importance to estimate its habitats within all regions in the country, as well as the seasonal dynamics distribution of this tick species.

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## P27

### Genotyping of *Toxoplasma gondii* RH strain by multilocus PCR RFLP

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**Introduction:** *Toxoplasma gondii* is one of the most prevalent parasites around the globe, infecting all warm-blooded vertebrates including humans and birds. *T. gondii* possesses clonally population structures with 3 predominant lineages (types I, II and III) defined by different molecular techniques, such as multilocus nested polymerase chain reaction restriction fragment length polymorphism (Mn-PCR-RFLP). *T. gondii* RH strain has been continuously passaged in Swiss albino mice for use in Sabin-Feldman Dye Test in the Public Health Institutions of Turkey. In the present study, we have aimed to genotypically characterize *T. gondii* RH strain using Mn-PCR-RFLP.

**Material and method:** Genomic DNA was isolated from tachyzoites of *T. gondii* RH strain obtained from

mice peritoneum using a commercial tissue extraction kit (Qiagen, Germany) according to manufacturer. Genetic markers including SAG1, alt. SAG2, L358 and PK1 were analysed according to Su et al. (2010, Parasitology, 137, 1-11) and compared to digestion profiles of reference strains kindly provided by Dr. Chunlei Su, Department of Microbiology, The University of Tennessee, after resolving in 2.5% agarose gel by electrophoresis.

**Results:** The *T. gondii* RH strain had uniform typing band patterns of clonal type I in all markers evaluated. Analysis of further markers continues.

**Conclusion:** *T. gondii* type I strain has higher virulence compared to types II or III. Since a high prevalence of seropositivity both in animals and humans is evident in Turkey, further analysis to genotype new *T. gondii* isolates from varying hosts in Turkey will reveal significant data in determination of the epidemiology of toxoplasmosis.

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## P28

### Serum biochemistry of Barbary and Cameroon sheep

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**Introduction:** Barbary sheep (*Ammotragus lervia*) is an African ungulate retaining some primitive and unique characteristics which makes it particularly interesting for research. Cameroon sheep (*Ovis aries*) come from West Africa. They are similar to early breeds of sheep. The Cameroon sheep is smaller than other species of domesticated sheep. Factors such as nutrition, age, sex, breed and climate were known to affect biochemical and haematological parameters of clinically healthy animals. This study was carried out to investigate the effect of breed on biochemical parameters of Barbary and Cameroon sheep.

**Material and methods:** Healthy adult sheep (10 Barbary, 6 Cameroon) from both sexes, between 2 year and 6 years old constituted the material of this study. The animals were kept in a clean and hygienic environment in a zoo in Antalya. Blood samples were taken from fasted animals in the morning at the same time. Blood was withdrawn from vena jugularis into a vacutainer. Serum glucose (Glu), albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), calcium (Ca) and phosphorus (P) were measured by using IDEXX Vet-Test 8080 auto analyser. Statistical analyses were performed with SPSS. Wilcoxon rank sum test was used to compare the significant differences in male and female sheep in each group.

**Results:** The mean values of serum biochemical parameters of Barbary Sheep and Cameroon Sheep showed no differences for albumin, calcium and phosphorus. The

level of mean glucose values between Barbary sheep ( $6.52 \pm 0.33$  mmol/L) and Cameroon sheep ( $4.66 \pm 0.4$  mmol/L) were significantly different ( $P < 0.05$ ). There were significant differences for ALP, AST and ALT, between two the breeds. The ALP, AST and ALT values were  $521.80 \pm 17.00$  u/L,  $91.7 \pm 4.80$  u/L,  $45.70 \pm 1.40$  u/L in Barbary sheep and  $363.0 \pm 15.00$  u/L,  $56.50 \pm 3.00$  u/L,  $24.8 \pm 1.20$  u/L in Cameroon sheep respectively.

**Conclusion:** Biochemical values for Barbary and Cameroon sheep were similar to those reported for other wild and domestic sheep. No important difference occurred in these values except for ALP when compared with domestic sheep. Therefore it was concluded that biochemical parameters were similar in clinically healthy Barbary and Cameroon sheep.

## P29

### Alteration of oxidative stress parameters in bovine ephemeral fever disease

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**Introduction:** Bovine ephemeral fever (BEF) is an economically important viral disease of cattle and water buffalo. Outbreaks occur at intervals of two years in southeast Anatolia and it causes serious economic losses through deaths, loss of condition, decreased milk production, lowered fertility of bulls, mismothering of calves, delays in marketing and restrictions on the export of live cattle. The importance of the role of reactive oxygen species in the pathogenesis of viral diseases has increased in recent years. In this study, considering that elucidation of the disease can contribute new light into the pathogenesis of the disease, the oxidant- antioxidant balance in infected cattle in Sanliurfa region was investigated.

**Material and methods:** Total of 32 Holstein aged between 12 and 18 month, kept under the same care and feeding conditions, were used. The control group (Healthy group-HG) consisted of 15 cattle determined to be healthy as a result of clinical and serological examinations. The study group (Infected group-IG) consisted of 17 cattle diagnosed with ephemeral fever, after clinical

and serological examinations. Blood samples from the study group the were taken before and on the fifth day (Recovery group – RG) of treatment. Activities of GPx, Catalase and SOD and levels of TAC, TOS, CP, SH and MDA were measured.

**Results:** Levels of CP were significantly lower and TAC levels were significantly higher in IG compared to HG. Levels of SH and CP were significantly lower and CAT activity was significantly higher in RG compared with IG. Levels of SH and activity of GPx were significantly lower and CAT levels were significantly higher in RG compared with HG.

**Conclusion:** An oxidant/antioxidant imbalance in favour of antioxidants in ephemeral fever infected cattle has been determined. These results suggest that antioxidant therapy may not be considered among the therapies used in ephemeral fever. However, further studies are necessary in order to definitively delineate the pathogenesis of BEF. To the best of our knowledge, this is the first report showing an association between some oxidant / antioxidant parameters in BEF.

## P30

### Metastatic transitional cell carcinoma of the urinary bladder in a dog

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**Introduction:** Transitional cell carcinoma (TCC) is a malignant tumor originating from the transitional epithelium (the urinary tract system, kidney, ureters, urinary bladder, urethra and prostate) and is the most common urinary tract tumor in dogs. This is a cancer of older dogs (average age 9-11 years) and commonly localized in the trigone area of the urinary bladder. Microscopically, transitional cell carcinomas are divided into 2 patterns based on their growth. These are papillary project into the lumen or non papillary (sessile or flat) and infiltrating or non-infiltrating. Metastases in this tumor generally occur in the lungs and lymph nodes, less frequently present in the bones.

**Material and methods:** A 16-year-old female terrier dog with a history of hematuria was presented to the Lara Antalya Veterinary Hospital. After the clinical, radiological examinations and blood tests, the dog was euthanized and necropsy was performed. Following a routine necropsy, tissue samples were collected and processed. Afterwards, serial sections were cut for haematoxylin and eosin (HE) staining and immunohistochemical staining. Immunohistochemically, the routine avidin-biotin-peroxidase complex method was used with primary antibody against pancytokeratin and PCNA.

**Results:** Clinically, anorexia, hematuria, dysuria and urinary incontinence were seen in the dog. The chest

X-ray revealed a mass at least 4,9 cm in diameter and located on the left lung. CK-MB, AST and Glucose levels have shown marked increases in the serum biochemical analyses. While WBC value revealed increase, lymphocyte value revealed decrease at the blood analysis. Two days after admission the dog died and necropsy was performed. Macroscopically, a large portion of the bladder mucosa was infiltrated by the tumor and because of this; thickening was seen in the wall of the bladder. The tumor showed papillary projects into the lumen of the urinary bladder. In addition, a metastatic mass with a sharp margin was located in the left cranial lung lobe, measured 8,5x4,5x3,5 cm, weighed 140,4 g, grayish-white in color, firm to touch. The cut surface of the mass was homogenous and also grayish-white in color. In some parts of the cut surface, cavernous, necrotic and hemorrhagic areas were observed. Microscopically, papillary and infiltrating pattern was seen in the primary tumor area and metastatic mass. Immunohistochemically, positive pancytokeratin and PCNA immunoreactions were found in the tumor cells and diagnosis was confirmed by the immunohistochemistry.

**Conclusion:** In conclusion, the present case was investigated with clinical symptoms, radiological examination, blood tests, macroscopical, histopathological and immunohistochemical findings and was diagnosed as transitional cell carcinoma in the urinary bladder with pulmonary metastasis in a dog.

### P31

#### Clinicopathological findings of *Macrorhabdus ornithogaster* infections in budgerigars (*Melopsittacus undulatus*) Ozlem Ozmen<sup>1</sup>, Sima Sahinduran<sup>2\*</sup>

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**Introduction:** *Macrorhabdus ornithogaster* is a yeast organism containing a eukaryotic nucleus. *M. ornithogaster* colonizes the proventriculi and ventriculi of birds. This organism causes a chronic wasting disease, characterized by emaciation, weakness, high rates of morbidity and low rates of mortality, and has been described in canaries and budgerigars.

**Material and Methods:** One hundred ninety-two birds in 7 different flocks were presented to the Department of Pathology University of Mehmet Akif Ersoy, for diagnosis during the two years. All of them were infected with *Macrorhabdus ornithogaster*.

**Results:** Clinically vomits, inappetence, diarrhea and high mortality especially in young birds were characteristic findings. At necropsy, enteritis and atrophy of the pectoral muscle were the most common findings. Thickening of the proventricular and ventricular walls, covering of the proventricular mucosa by thick white mucus, ulceration and hemorrhage of the proventricular and ventricular mucosa, loosening of the koilin layer and hemorrhage

into the lumen of these organs were commonly observed. Native and Giemsa stained smears of the proventriculus revealed numerous *Macrorhabdus ornithogaster* agents. Amphotericin-B was the effective treatment. For prophylactic purposes, a 10% apple vinegar was advised and found effective.

**Conclusion:** The present results showed that *Macrorhabdus ornithogaster* infection can cause severe lesions and high mortality in budgerigars. Practitioners should consider this infection in budgerigars, especially in disease outbreaks characterized with diarrhea and high mortality in young birds.

### P32

#### Diabetes mellitus with multi-organ dysfunction in a cow Sima Sahinduran<sup>1</sup>, Ozlem Ozmen<sup>2</sup>, Necmettin Sarp Sevgisunar<sup>1\*</sup>

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**Introduction:** Diabetes mellitus (DM) is a state of chronic hyperglycemia usually accompanied by glycosuria. Primary DM is a disorder involving the beta cells that result in decreased insulin levels and hyperglycemia. DM in domestic animals has been most commonly reported in small animals but reported rarely in other species such as cattle, horse, goat and sheep. It is a quite rare condition in cattle. DM is generally accompanied with multiorgan disfunction in humans. Only fatty liver and DM combination reported in cattle.

**Material and methods:** A five year-old Holstein cow was brought to the Veterinary Medical Teaching Hospital from a farm in Burdur province with progressive emaciation, polyuria, polydipsia and decreased milk yield. According to the owner, she was very healthy, but in a period of 1 month she lost weight and suffered frequent urination. She avoided eating the last 3 days. On physical examination, the cow was depressed and her temperature, respiratory rate, heart rate were 38.4°C, 40 breaths/min, 90 beats/min respectively and she had shown moderately dehydration. Polyuria and polydipsia were observed.

**Results:** Blood and urine samples were taken twice in a 48 hour interval. Results of the urinalysis were within normal limits, except for glucose (1000, 1050 mg/dL), ketones (+++, +++), and pH (5.5, 5) values in the first and second samples. In the serum samples analysis, the first glucose sample was elevated at 38.11 mmol/L, and the second sample of glucose was 38.75 mmol/L.

**Conclusion:** In both (first and second) samples urine, glucose and ketones were very high, and in the serum biochemical analysis the glucose values were found very high, which is consistent with DM. The histopathological examinations showed decrease in the number and size of the pancreatic islets, as well as vacuolar degeneration of residual cells was observed. Immunohistochemical

study of all the pancreatic tissue revealed a marked reduction in the concentration and distribution of insulin, proinsulin, and amylin, but an increase in glucagon. At the microscopical examination, fatty liver, non-suppurative interstitial nephritis and edema pulmonum were diagnosed. This study reported that clinicopathological findings in a cow with multiorgan dysfunction.

### P33

#### Expression of CD3, CD79 and PCNA on tumor cells in Marek's disease

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**Introduction:** Marek's disease is a lymphoproliferative disease of chickens caused by the herpes virus and characterized by multiple visceral lymphomas or by inflammation and degeneration of peripheral nerves. After infection, the virus replicates in B-cells and during the next phase it starts cellular transformation and activation of T-cells followed by the development of lymphomas in several visceral organs, skin, muscles, nerves, and less frequently in the eyes. The aim of the paper is to determine the phenotype of neoplastic cells in Marek's disease by using immunohistochemical staining, as well as to determine the presence of the proliferating cell nuclear antigen (PCNA).

**Material and methods:** The following organs of 18 chickens naturally infected with Marek's disease were examined: liver, spleen, kidneys, proventriculus, lungs, heart, ovary and sciatic nerves. After the examination of sections stained by haematoxylin eosin, streptavidin-biotin (LSAB) commercial detection kit (ChemMate K 5003) was used for detection of CD3 T-cells, CD79 B-cells and PCNA. The commercial primary and secondary monoclonal antibodies were used in this immunohistochemical staining. The reaction was visualized by applying chromogen amino ethyl-carbazole (AEC) or diaminobenzidine (DAB).

**Results:** The most characteristic changes were observed by macroscopic examination on the liver and spleen. The liver was enlarged with multiple gray or yellow solid tumors. Spleen was also enlarged and dark red. Histological examination showed the tumor proliferates predominantly consisting of lymphoid cells of focal and diffuse character. Most of these cells were CD3 positive, while the intensity of CD79 expression was lower. PCNA expression was intense in proliferating neoplastic cells.

**Conclusion:** Immunohistochemical analysis revealed that lymphoma cells in Marek's disease are predominantly of T cell type, CD3+ phenotype with a very high proliferative potential and increased expression of PCNA.

### P34

#### Hypothyroidism in a miniature schnauzer dog: Case report

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Hypothyroidism is one of most frequently endocrinopathies in mid and large sized dogs. Diagnosis of canine hypothyroidism can be challenging for two main reasons: several diseases can manifest similar clinical signs and there is no ideal test for assessing canine thyroid function. The clinical signs are numerous, variable, often nonspecific and rarely pathognomonic. Change of quality and hair loss is one of the most common clinical signs; 25% of hypothyroid dogs have symmetrical bilateral alopecia. Overall, lack of one reliable test makes the assessment difficult. Currently, combined determination of serum concentrations of thyroxine (T4) and TSH is the easiest and most reliable way to assess canine thyroid function.

Nine year old male Miniature Schnauzer was brought to the University Veterinary Hospital in Skopje with the following history: lack of interest in physical activity, pain in the forelimbs. It had been treated several times with corticosteroids and antirheumatics, without improvements. Since October 2013 general conditions have worsened. Physical examination revealed various abnormalities: asymmetrical alopecia of the front of the neck down to the chest, excessively dry, brittle and easily felled out hair coat, dry skin, thick, puffy and darkly pigmented. Despite the weight gain and being somnolent, the patient didn't show symptoms of mental dullness.

Based on history and clinical findings, concentrations of the serum level of thyroxine (T4) and TSH were measured. The results showed low concentration of total T4 - 1.14 µg/dl (reference interval 1.50-4.50) and normal level of TSH 0.19 ng/ml (reference interval 0.03-0.40). The patient underwent 4 weeks of therapy with levothyroxine 20 µg/kg PO q24h. First symptoms of improvement (normal hair growth with lower quality) were observed 4 weeks after the treatment. During the therapy excitation, polydipsia, polyphagia, polyuria and gastritis were recorded. Slight improvement of laboratory findings of serum concentrations T4 and TSH were observed on the first control (1.41 µg/dl and 0.32 ng/ml respectively). Due to side effects, the prescribed dose of levothyroxine was reduced to 0.05 µg/kg PO every 72h.

The most common clinical signs associated with hypothyroidism are dermatological abnormalities as alopecia, seborrhea, and hyper pigmentation followed by metabolic disorders, lethargy, weight gain, exercise intolerance and heat seeking. Despite many available thyroid hormone supplements, levothyroxine (synthetic T4) is mostly preferred. In our study, due to the side effects of the treatment, the initial dose was reduced;

the patient was stabilized and continued with therapy. In order to establish definitive diagnosis of hypothyroidism, routine laboratory testing should be carried out.

### P35

#### Clinical signs and diagnostic procedures for detection of bovine viral diarrhoea

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Bovine viral diarrhoea (BVD) is a viral contagious disease characterized with changes in the digestive tract and is found in acute, congenital and persistent forms, resulting with high mortality in heifers and young cows. The aim of this study was to describe the clinical signs and possible diagnostic procedures for early detection of bovine viral diarrhoea, through cases of positive animals in two dairy farms. In two farms with 16 (5 heifers and 11 milking cows) and 2 (1 heifer and 1 milking cow) East Friesian cows, respectively, were identified as positive cases of BVD. The diagnosis was accomplished by recording the clinical signs of the animals, collecting blood samples for diagnosis with Ag-ELISA and hematology analysis, additionally on spot autopsy was performed for recording the morphological changes in targeted systems. In the observed farms, two milking cows and one heifer in both farms were showing intermittent fever (39.4°C – 40.8°C), inappetence, apathy, rumen atony (1 – 2 weak rumen contractions in 5 min.) and polydipsia; after 3-5 days of these symptoms, the nasal discharge, saliva with desquamous epithelium, erosions with clear boundaries in the gingival mucosa, diarrhoea and high level of dehydration were found in all affected animals. After 6 – 8 days from the first symptoms the pregnant milking cow in the first farm aborted in the seventh month of their pregnancy. The calf from the other milking cow, in the same farm, died 5 days post partum. In the second farm the affected heifer had bloody diarrhoea. The blood serum from all animals from the farms was tested with Ag-ELISA for diagnosis of BVD and the findings showed that the sero-positive samples were from the animals which manifested the previously described symptoms. The hematological analysis revealed non-significant differences in parameters between sero-positive and sero-negative animals. The autopsy was performed on the sero-positive heifer from the first farm, and the following changes were observed: rhinitis, erosions in the oral cavity, myocardial hypertrophy, intestinal submucosal bleeding and dark brown mucous liquid in the intestines. The analysis of animal movements in and out of the farms confirmed that there was no introduction of new animals at the farms and the origin of all sero-positive animals is only from one milking cow, confirming the trans-placental

transmission of the disease which could be used in the strategy for eradication of BVD present in the dairy farms.

### P36

#### Pathological characterization of myocarditis related to Foot and Mouth disease in two yearlings

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In this study, the necropsy findings belonging to two yearlings aged 10 and 15 days were evaluated in two different herds which have had peracute deaths. The herds of goats in which history it was specified that the kids died within 1-2 days of the illness, and with a ratio of mortality 25-30%, only a slight lameness, and no lesions have been observed in the mouth.

It was found out that both herds have not been vaccinated within the last year. Grayish foci, especially in the left ventricular muscles of yearlings were registered with variable sizes ranging from 1x3 to 3x5 cm. While this foci in the epicardium could be observed in the kid aged 15 days, it could be seen in the myocardium of the section surface in the other yearling. Hyalin degeneration and necrosis in the heart muscle, mononuclear cell infiltrations in the interstitial tissue and hyperemia and edema in the capillary vessels was detected during the microscopic examination of the section of the kids. However, this findings were intensive in the yearling aged 15 days. It was emphasized that vaccines should be administered regularly, even if the flocks in these areas seem free from Foot and Mouth disease.

### P37

#### Antibacterial and antifungal activity of ethanolic *Allium tuncelianum* extract

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**Introduction:** *Allium tuncelianum* is an endemic garlic species which is growing locally in Tunceli, Turkey. Garlic contains several biologically active compounds known as organosulfur compounds.

**Material and methods:** *Allium tuncelianum* was extracted using ethanol, and tested for its inhibitor activity against ten pathogenic bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*,

*Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella enteritidis* and six fungal (*Candida albicans*, *C.parapsilosis*, *C. krusei*, *Malassezia pachydermatis*, *Trichophyton mentagrophytes*, *Microsporum canis*) strains were determined by the macrobroth dilution method according to CLSI standards.

**Results:** The results showed that all the microorganisms used were sensitive to the extract. The ethanol *Allium tuncelianum* extract demonstrated the highest antibacterial activity against *Bacillus cereus* (12,5mg/ml). However, the ethanol extract at 0,78mg/ml had high antifungal activity against *Malassezia pachydermatis*.

**Conclusion:** According to the results of this study, *Allium tuncelianum* has significant potential as antibacterial and antifungal.

### P38

#### Existence of extended spectrum beta lactamase producing *E. coli* isolates in chicken

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The purpose of this study is to reveal the presence of Extended Spectrum Beta Lactamase (ESBL) producing *E. coli* isolates in the fecal samples of healthy chicken. 200 fecal samples were collected in total from 3 flocks in Burdur city in Turkey. One gram fecal sample was enriched in pepton buffered water for 24 hours and then 50 µl from enrichments were spread on Chromogenic *E. coli*/ Coliform selective agar supplemented with 2 µg/ml cefotaxime and 2 µg/ml ceftazidime. After incubation at 37°C for 24 hours, one colony from each positive sample was identified as *E. coli* by standard biochemical tests and Polymerase Chain Reaction (PCR) specific to *E. coli* 16S rRNA gene. The ESBL positive *E. coli* isolates were determined by CLSI Confirmatory Test using cefotaxime (30 µg) and ceftazidime (30µg) discs, together with cefotaxime + clavulanic acid (30 µg +10 µg) and ceftazidime + clavulanic acid (30 µg +10 µg) combination discs. The results were evaluated according to CLSI criteria.

Total 12 (6.0 %) fecal samples were found positive for ESBL producing *E. coli* isolates. Therefore, we state that ESBL producing *E. coli* isolates exist in the microbial flora of chicken in this part of Turkey. Since ESBL producing *E. coli* isolates in microbial flora of intestines can transfer resistance against extended spectrum cephalosporins to

pathogen and zoonotic microorganisms, the prevalence of ESBL producing *E. coli* isolates should be monitored in chickens by regularly conducted studies.

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### P39

#### The prevention of placenta retention and the increase of fertility through treatment with PGF2-α in postpartum dairy cows Luigi Turmalaj\*, Ilirian Bakiasi

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**Interdiction:** The objective of this study is to estimate the role of prostaglandins F2-α during the excretion of placenta, for the prevention of postpartum pathologies and fertility in dairy cows. The role of PGF2-α is well known for its stimulating abilities on the smooth musculatures and local immunity.

**Material and methods:** For the experiment, we chose two groups of animals belonging to the Holstein breed, each of them containing ten animals. The cows were 3-6 years old and had calved normally. The experimental group has been treated as follows: PGF-2α (Estrumate®), 2 ml via intramuscular 8 hours after parturition. The treatment was repeated 12 hours after the first one. The control group was not treated and was kept under survey so as to be compared with the indices of the experimental group.

**Results:** From the data of the study it resulted that: firstly, for the index of the placenta excretion the cows of the experimental group have realized it on average 13±2.4 hours after parturition, versus 19±4.1 hours after parturition to those of the control group. In the control group two cows have been recorded with placenta retention (>24 hours after parturition). For the index for endometritis and metritis, in the experimental group only one animal has displayed signs of endometritis; 10%, whereas in the control group only 3 cows, 30%. Thirdly, for the indices of fertility there are differences between the groups concerning both main parameters; that of Service Period (Calving to conception interval) and Index of insemination Services per conception. Thus the cows of the experimental group have their average service period 92±12.4 days versus 128±9.6 days. For the fertilizing insemination in the experimental group, 1.2 doses of sperm were used up versus 1.6 doses in the control group.

**Conclusion:** Considering the above mentioned results, we conclude that the use of PGF-2α after parturition affects positively the prevention of placenta retention, in the reveal of postpartum infections (endometritis and metritis) and in the improvement of fertility in cows.



**P40****An investigation of botulism outbreaks in dairy cattle in Bolu province**Sibel Yasa Duru<sup>1</sup>, Mehmet Şahal<sup>2</sup>, Atilla Beşkaya<sup>3</sup>, İdil Baştan<sup>2</sup>, Serkal Gazyağcı<sup>1\*</sup><sup>1</sup>*Kırıkkale University, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-71451 Kirikkale, Turkey*<sup>2</sup>*Ankara University, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-06110, Ankara, Turkey*<sup>3</sup>*Directorate of the National Food Reference Laboratory, TR-06170, Yenimahalle, Ankara, Turkey*

The objective of the present study was to describe an outbreak of botulism in a cattle herd. Of the 67 affected cattle included in the study, 10 were clinically examined and assessed for their haematological (erythrocyte and leukocyte counts, haemoglobin level, haematocrit value, thrombocyte count and differential blood count) and biochemical (trace elements: Mg, Ca, P, Mn, Cu, Se, Fe ve Zn; total cholesterol, direct and total bilirubin, albumin, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and urea) parameters. The animals, which fed on poultry manure due to inadequate grassland, displayed clinical symptoms of the disease as from 3 days after the ingestion of manure. Fifty seven Holstein and Brown Swiss dairy cattle of varying age died over a period of 9 days. The clinical symptoms observed in these animals were typical of botulism. For the effective control of botulism, the prevention of toxin formation and the elimination of toxin sources, it is required that measures including regular pasture management, mineral supplementation, appropriate disposal of carcasses and vaccination are taken. The present study provides not only a description of botulism, but also a general overview of associated clinical and laboratory findings.

**P41****Estrus synchronization in ewes out of the season**

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The ewes follow a seasonal reproductive pattern, what in the climate conditions of Albania means that sexual activity is manifested from May to August.

We assessed the efficiency of two hormonal protocols for oestrus synchronization in ewes outside the sexual season (during October 2011). For this reason two experimental groups, each comprised of 25 Lacon breed ewes, were formed. Both groups had the same breeding and physiological status (all are about 9 months), as well as management conditions. The treatment for the first group

was comprised of progestagen vaginal sponge insertion (Fluor Geston Acetate, 40 mg) for 14 days and the 500 IU of eCG (equine Chorionic Gonadotrophin) i/m was applied at the time of vaginal sponge removal.

The second group followed a similar protocol, but they were additionally treated with 0.1 mg/ewe gonadotrophin releasing hormone (GnRH; Fertagyl) 36h after the eCG application. The oestrus was detected by presentation of the ewes to ram 48h after the vaginal device was removed. The presented ewes were mated twice in the interval of 12 hours. The oestrus detection and lambing rate were determined for each group.

The oestrus was detected in 24 out of 25 ewes in each group (96 %). The lambing rate in the first group was  $2.1 \pm 0.12$  and  $2.3 \pm 0.2$  for the second group ( $P < 0.05$ ).

**P42****The variation of estrus in endocrinological levels and behaviour in Akkaraman ewes throughout the year**

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**Introduction:** In order to maximize lamb production and breeding strategies in sheep production (29,284 million, 2014) it is necessary to know the reproductive parameters of local breeds. The most popular breed in Turkey is Akkaraman breed (% 40 of all).

**Material and methods:** An investigation was carried out to determine the reproductive activity of endocrinological and behaviour in 11 Akkaraman sheep, (age=2-2.5 years, body weight =74±7.7) throughout the year in Middle Anatolian (39.57 N, 32.53 W). The onset of estrous was detected by using an apron ram for a 30-minute period, twice a day. Blood samples collected twice a week were analyzed by Enzyme immunoassay for progesterone ( $P_4$ , sensitivity in test 1.25 pg/ml), and by Radio immunoassay (125 I RIA, ICN, and sensitivity of test 10 pg/ml) for estradiol.

**Results:** Estrous cycle lengths and estrous lengths were (mean±SD) 18.33±1.03 day, 35.16±5.95 hours, respectively. The breeding season lengths were 283±63.36 day and 229.64±63.74 day according to hormonal and behaviour data, respectively ( $P \leq 0.0032$ ). Incidence of estrous was high between August and March, however the highest peak was in October. The lowest peaks were both in June and July. Plasma progesterone levels were changed between 0.01-9 ng/ml throughout the year and 0.2-0.16 ng/ml in anoestrus.  $P_4$  level on the 11<sup>th</sup> day of the luteal phase of estrous was 3.73±1.50 ng/ml (n=11). Plasma estradiol level was 8.42±2.51 pg/ml between 1<sup>st</sup> and 2<sup>nd</sup> day and 3.50±1.50 pg/ml on day 15. The second peak of estradiol determined on day 11 of the estrous cycle was 6.09±2.87 pg/ml (n=11).

**Conclusion:** Depending on the hormonal parameters, the breeding season is quite long and if the management conditions are appropriate, Akkaraman ewes can mate twice a year or three times in 2 years as natural.

**P43****Ultrasound measurements for monitoring of the first trimester of pregnancy in Bulgarian native goats**Manol Karadaev<sup>1\*</sup>, Ivan Fasulkov<sup>1</sup>, Nasko Vasilev<sup>1</sup>, Yoana Petrova<sup>2</sup><sup>1</sup>*Department of Obstetrics, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Trakia University, Student Campus, 6014 Stara Zagora, Bulgaria*<sup>2</sup>*Student of Veterinary Medicine, Faculty of Veterinary Medicine, Trakia University, Student Campus, 6014 Stara Zagora, Bulgaria***Introduction:** The purpose of this study was to establish the changes occurring in the first trimester of pregnancy in Bulgarian local goats by ultrasound measurements.**Material and methods:** In the study we used six goats of the Bulgarian native breed, aged between 2 and 4 years, weighing 48-56 kg, reared in the experimental base of the Faculty of Veterinary Medicine, Trakia University – Stara Zagora. All goats were subjected to a single ultrasound examination on days 17, 21, 28, 35, 42 and 49 of gestation. Ultrasound measurements included width of the uterine lumen, length of the embryo / crown-rump length (CRL), trunk diameter (TD), bi-parietal diameter (BPD), outer diameter of placentomes (ODP) and inner diameter of placentomes (IDP).**Results:** Our results indicate a statistically significant ( $P \leq 0.001$ ) increase in the size of the studied parameters in the first trimester of pregnancy. The establishment of the parameter width of the uterine lumen was possible to perform between days 17 and 49 of gestation. Ultrasound measurements of the crown-rump length was possible to be done after day 21 of gestation, trunk diameter – after day 28, while bi-parietal diameter, outer and inner diameter of placentomes – after day 42 of pregnancy.**Conclusion:** Ultrasonographic examination enables fast, accurate and non-invasive determination of the change in the parameters used for pregnancy monitoring in goats.**P44****Cryoprotective effects of four different extenders supplemented with Equex Past or different forms of egg yolk on sperm parameters**Omer Varisli<sup>1</sup>, Cigdem C. Sen<sup>1\*</sup>, Nurettin Aydilek<sup>2</sup>, Faruk Bozkaya<sup>3</sup><sup>1</sup>*Department of Reproduction and Artificial Insemination, Harran University, Faculty of Veterinary Medicine, 63100, Sanliurfa, Turkey*<sup>2</sup>*Department of Physiology, Dicle University, Faculty of Veterinary Medicine, 21100, Diyarbakir, Turkey*<sup>3</sup>*Department of Genetics, Harran University, Faculty of Veterinary Medicine, 63100, Sanliurfa, Turkey***Introduction:** Using frozen sperm from animals of high breeding values, substantially contributed to the development of animal production. When frozen sperm

is used for insemination of sheep, high fertility rates can only be achieved by laparoscopic methods. However, this method has limited use because of its difficulty. Low fertility rates in sheep inseminated with frozen sperm results mainly from the anatomical structure of female reproduction tract and sensitivity of ram sperm against cryopreservation due to its physico-chemical character. In order to obtain fertility rates near to those obtained by using fresh sperm, it is necessary to reduce damages during freezing or after thawing and to use more improved insemination techniques. The aim of this study was to investigate the cryoprotective effects of four different extenders supplemented with Equex Past or different forms of egg yolk on sperm parameters.

**Material and methods:** For this study, three healthy and fertile Awassi rams were used. Sperm samples of the rams were obtained by using an artificial vagina. Pooled sperm samples were frozen after adding TL-HEPES, modified Krebs's Ringer bicarbonate (mKRB), TES/Tris (TEST) and Salamon's tris-citrate containing 6% glycerol, different amounts of Equex Past (EP, 0.5, 1.0, 1.5%) and native (EY, 10%) or centrifuged egg yolk (CEY, 10, 20, or 30%). Sperm samples were filled into 0.25 mL straws, equilibrated at +4°C for 2 hours, frozen on liquid nitrogen vapour and stored in liquid nitrogen until they were thawed. The frozen sperm samples were thawed in a water bath at 37°C for 30 sec. Sperm samples were evaluated for motility, membrane integrity and acrosome integrity.**Results:** Motility and membrane integrity of sperm samples were significantly reduced, while acrosomal integrity was not affected by freezing in Awassi Rams. However, addition of 0.5-1.0% EP into TL-HEPES, mKRB and TEST significantly prevented motility loss due to freezing ( $P < 0.05$ ). The extenders with different egg yolk concentrations significantly affected the preserving ability of the solutions ( $P < 0.05$ ). Centrifuged egg yolk (CEY) added 20-30% to TL-HEPES and mKRB increased the preserving ability compared to 10% EY ( $P < 0.05$ ).**Conclusion:** The results indicated that use of CEY improved preserving ability of different extenders compared to native EY and would be useful for preparation of optimal extenders and development of a successful cryopreservation protocol for ram sperm.**P45****Improvement of the antioxidative status of pig ovaries by selenopyran treatment**Desislava Vasileva Abadjieva<sup>1\*</sup>, Elena Kistanova<sup>1</sup>, Yordan Marchev<sup>2</sup>, Radka Nedeva<sup>2</sup>, Chaika Vaisberg<sup>1</sup>, Rossen Stefanov<sup>1</sup>, Gennadiy Boryaev<sup>3</sup>, Mihail Nevitov<sup>3</sup><sup>1</sup>*Institute of Biology and Immunology of Reproduction- BAS, Sofia, Bulgaria*<sup>2</sup>*Agricultural Institute, BG – 9700 Shumen, Bulgaria*<sup>3</sup>*Penza State Agricultural Academy, RU - 2771 Penza, Russia***Introduction:** Selenium is an essential micro-element in animal diet due to its high antioxidative properties. As a part of selenocystein, it is an important constituent of

the glutathione peroxidase (GPx) enzyme, which has a big importance for cell protection from oxidative damage. The aim of the present work was the investigation of the selenopyran effect on the antioxidative state of pig ovaria.

**Material and methods:** The experiment was conducted with gilts of Danube white breed randomly divided into two groups with 9 gilts in each, between 120 - 228 days of age. The animals received equal basal diets without selenium additives. The experimental gilts (n=9) were injected intramuscularly with oil solution of preparation content 0,1 µg/kg live weight selenopyran (9-phenyl-symmetrical octahydroselenoxanthene) once per month. After slaughtering, the selenium content in the ovarian tissue was determined with the atomic absorption spectroscopy method. The GPx activity in ovary homogenates was measured using the colorimetric assay kit (BioVision). The expression of  $\gamma$ -glutamyl transpeptidase (GGT) in ovaries by immunohistochemical method. Data was estimated using one-way and regression analysis with STATISTICA (Ver.6.0 of the Stat Soft Inc.).

**Results:** The selenopyran treatment leads to increase in the selenium level in blood ( $P<0,05$ ). The high positive correlations between the selenium content in ovary and ovary weight ( $r=0,76$ ;  $P<0,05$ ) was established. The significant increase of the selenium level in blood ( $P<0,05$ ), as well as the higher activity of GPx (142,61±6,6 versus 122,28±3,4 U/gTP,  $P<0,05$ ) in the ovaries of the experimental group was observed. The GGT expression in the ovarian cortex cells and follicular fluid in treated gilts was an evidence of active transport of glutathione from blood to ovary tissue.

**Conclusion:** The selenium content as well as the GPx activity in ovaries show that the selenopyran treatment promotes the increase of the antioxidative defense in ovary of gilts.

#### P46

### Effect of different extenders and storage periods on motility and fertility of ram sperm

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**Introduction:** Artificial insemination with chilled-stored semen has become a technique in sheep breeding. Efforts to improve the preservation of ram semen are focused on the modification of the extender, as well as on the addition of various components to maintain motility and fertilizing capacity. The aim of this study was to test the effect of extenders with different sugar in their composition on the sperm motility in rams and pregnancy rate of ewe's following insemination with this semen.

**Material and methods:** Ten rams from the North-east Bulgarian fine-fleece breed were used in this study. Semen was diluted with different extenders: first with sucrose

(0.267), lactose (0.267) and rafinosa (0.267); second with sucrose (0.4) and rafinosa (0.4) and control extender with sucrose (0.4) and lactose (0.4). All series of experiments were repeated in triplicate. Total motility was determined with Sperm Analysis (SCA, Microptic, Spain). A total of 200 mature ewes of the same breed were used for cervical insemination with a sperm dose at the concentration of  $100 \times 10^6$  spermatozoa. Pregnancy was diagnosed 60 days after AI by - a real-time ultrasonic scan device (Alloka SSD 500).

**Results:** The sperm motility with extender 1 was significantly higher ( $P<0.001$ ) than other extenders after 240 min to 360 min. The survival of sperm cells of fresh ram semen in extender 1 and 3 were having similar values after 6 hours incubated at 39°C ( $P<0.001$ ). After 48 hours, only semen diluted with extender 1 indicated living and moving sperm cells (total motility =10.1%). The extender 1 showed higher values for pregnancy (80 %) in comparison with other extender (71.00 %).

**Conclusion:** Our experiments demonstrated that higher sperm motility after storage at 4°C for 24 hours and 48 hours was shown by the ram spermatozoa diluted with extender 1, which can be used successfully for the insemination of ewes.

#### P47

### Effect of cholesterol and 7-dehydrocholesterol loaded cyclodextrin on Simmental bulls sperm motility stored at 4°C

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**Introduction:** The aim of this study was to determine the effect of cholesterol-loaded cyclodextrin (CLC) and 7-dehydrocholesterol loaded cyclodextrin (7-DHCLC) in tris egg-yolk extender (T) for maintaining sperm motility at 4°C in bulls.

**Material and methods:** Forty ejaculates collected from three Simmental bulls were used, where the mixed ejaculates were divided into 7 groups, one as control (C); three at different CLC concentration and the other three at 7-DHCLC (1.5, 2.5, 3.5 mg/120x10<sup>6</sup>) concentrations in T. Diluted semen samples were transferred to the laboratory at +4°C in 45 minutes. Post-thawed sperm motility was determined for three days with computer aided sperm analyzer (SCA®, Barcelona, Spain).

**Results:** Initial total sperm motility rates assessed and C were 42,37±0,56, CLC (1.5, 2.5, 3.5) were 77,37±3,62, 67,62±3,16, 65,78±2,50; 7-DHCLC (1.5, 2.5, 3.5) were 74,93±1,94, 60,43±1,91, 59,82±2,72 respectively; and progressive motility rates were (C) were 15,35±0,49; CLC (1.5, 2.5, 3.5) were 30,38±2,91, 26,92±1,77, 28,42±2,26;

7-DHCLC (1.5, 2.5, 3.5) were 31,13±1,27, 26,95±1,23, 24,03±1,53 respectively. Sperm motility rates were 50 % higher than in each group apart from control at day 1. Sperm motility rates were reduced in each group; however CLC and 7-DHCLC 1.5 mg/120x10<sup>6</sup> motility rates decreased in the next 2 days gradually.

**Conclusion:** As a result, it was concluded that adding a different rate of CLC and 7-DHCLC to T which can then be used for artificial insemination practice for 2 days at +4°C. Moreover, CLC and 7-DHCLC 1.5 mg/120x10<sup>6</sup> groups were identified as the best groups for short time preservation.

#### P48

### Effect of different glycerol levels on cryopreservation of bull semen loaded with cholesterol or 7-dehydrocholesterol

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Forty ejaculates collected from three Simmental bulls were used in this study. Ejaculates were pooled and native semen samples were treated with 1,5 mg CLC, 7-DHCLC /120x10<sup>6</sup> cells. Pooled ejaculates were divided into 7 groups, one as control (C); at three different glycerol concentrations (3, 6 and 9%) with CLC and three at 7-DHCLC in Tris egg-yolk extender. Diluted semen was frozen in a 0,25 ml straw. Post-thawed CASA motility, abnormal spermatozoa and functional membrane integrity (hypo-osmotic swelling test) rates were assessed. Post-thawed CASA motility rates, C were 45,33±4,66, CLC (3, 6, 9%) were 9,07±2,10, 36,31±2,78, 36,17±3,70; 7-DHCLC (3, 6, 9%) were 13,13±3,17, 35,93±3,49, 56,80±1,64 respectively. Functional membrane integrity (hypo-osmotic swelling test) C were 44,29±3,96, CLC (3, 6, 9%) were 44,43 ± 3,24, 47,14±2,68, 53,00± 1,73; 7-DHCLC (3, 6, 9 %) were 36,14± 1,30, 41,86±1,77, 58,57±1,76 respectively. There was an interaction between CLC and 7-DHCLC with different glyserol concentration on motility and functional membrane integrity. Post thaw motility was highest in 9% CL 7-dehydrocholesterol (p< 0.001). Highest functional membrane integrity was found in 9% glycerol concentration which was treated with CLC and 7-DHCLC after thawing (p<0,001). There was no statistical differences (p>0,05) between groups according to abnormal spermatozoa rates.

This data indicates that, addition of cholesterol and 7 dehydrocholesterol (1,5 mg CLC and CL 7-dehydrocholesterol /120x10<sup>6</sup>) to spermatozoa membranes should be carried out with 9% glyserol concentration.

#### P49

### Kinetic parameters of cryopreserved boar semen

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**Introduction:** Despite the fact that cryopreserved semen is widely utilized for artificial insemination (AI), as one of the fundamental reproductive biotechnologies, in most of domestic animal species, its implementation in the swine industry is still at a very low level. It has been more than 50 years, since the first report concerning the boar semen cryopreservation was published (Polge,1956). Since then, number of scientist around the world have conducted many useful studies concerning the cryopreservation of boar semen. The method for gradual freezing of boar semen was published in 1975, and the same, with few modifications is still used for this purpose (Pursel and Johnson, 1975). The objective of this study was to determine the differences of kinetic parameters of boar spermatozoa, conserved by different procedures for cryopreservation.

**Material and methods:** In this study, 713 ejaculate were used, obtained from 18 sexually mature boars from breeds Yorkshire (n=10), Landrace (n=5) and Durock (n=4). The ejaculates were obtained using the gloved hand technique. The processing of the ejaculates according to the normal procedure was following the method described by Westendorf et al. (1975), modified by Thurston et al. (2001), while for the cryopreservation of sperm using the experimental procedure only the ejaculates with 90% motile spermatozoa after CASA evaluation were considered.

**Results:** The velocity of the VAP in liquid semen was 71,50±0,52µm/s, the TCP-semen 42,11±0,56µm/s, and the XCP-semen 51,23±1,05 µm/s, with high significant differences found between cryopreservation procedures (p<0.00005).The VSL in liquid semen was 47,03±0,40µm/s, the TCP-semen 22,02±0,53µm/s, and the XCP-semen 31,13 ± 0,93 µm/s, with high significant differences found between the cryopreservation procedures (p < 0,00005). VCL in liquid semen was 163,19±0,95µm/s, in TCP-semen 128,86±1,30µm/s, and in XCP-semen 132,66±2,23µm/s, where significance was found between cryopreservation procedures on the level of total processed ejaculate. ALH in liquid semen was 7,90±0,03µm, in the TCP-semen 7,53±0,05µm, and in the XCP-semen 7,37±0,08µm, there was no significance in the difference of this parameter between the cryopreservation procedures on level of the total number of processed ejaculates. BCF in liquid semen was 30,44±0,03Hz, in the TCP-semen 30,23±0,21Hz, and the XCP-semen 29,41±0,30, and there was no significance in the difference of the levels of this parameter between the cryopreservation procedures.

**Conclusion:** There were high significant differences of the values of the assessed parameters ( $p < 0.000005$ ) between different procedures for conservation of boar semen, as well as between the assessed breeds.

## P50

### Comparison of white blood cell counts in the perioperative period of ovariohysterectomy in free roaming dogs

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**Introduction:** In addition to the surgical procedure, which can be a potential stressor for dogs have undergone ovariohysterectomy, the period before and the period of recovery may also be sources of stress. The aim of this study was to compare white blood cell count and neutrophil/limphocyte ratio in free roaming dogs immediately after transport, 24 hour after housing in cages in stationary of veterinary practice, 30 minutes, 180 minutes and 24 hour after ovariohysterectomy.

**Material and methods:** The study was performed on a total of 30 female dogs 2 to 4 years old, under the programme for free roaming dog control, which have undergone ovariohysterectomy at the Faculty of Veterinary Medicine, University of Belgrade. Dogs were captured in public areas by the communal animal hygiene service and were transported to the clinic of a veterinary practice. All dogs were placed into cages size 1x1x1m. After 24 hour housing, the dogs were submitted to an identical preoperative and operative/anaesthesia protocol (ketamine, acepromazine, karprofen). After ovariohysterectomy, all dogs were returned into the cages. They were kept until they were returned to public locations. Blood was collected from the cephalic vein of each dog five times: immediately after transport, 24 hours after housing, 30 minutes, 180 minutes and 24 hours after ovariohysterectomy. Total white blood cell counts were measured using ADVIA 120 automatic cell counter. Statistical analysis was done using the Graph Pad Prism software. As data values were not normally distributed for all parameters, differences were tested using non-parametric Kruskal-Wallis test.

**Results:** Hematological analysis indicated that there was a significant leucocytosis ( $p < 0.05$ ) that is composed of significant granulocytosis, monocytosis and non-significant limphocytosis in dogs 24 hour after ovariohysterectomy. Also, significant higher leucocyte and neutrophil counts ( $p < 0.05$ ) were obtained immediately after transport and 180 minutes after ovariohysterectomy. There was a mild lymphopenia 30 minutes after ovariohysterectomy, but the difference was not significant.

**Conclusion:** The results obtained showed that the analyzed white blood cells were significantly influenced by stressors during perioperative period of ovariohysterectomy. Immune parameters may help to identify acute stress in the dogs, but they may be misinterpreted. Individual differences like the effects of an unknown breed or earlier

life experiences may further attribute to variability in stress responses. It is necessary to improve all procedures with free roaming dogs that could be potential sources of stress.

## P51

### Effects of lactational exposure to non-planar PCB-155 and planar PCB-169 on growth, biochemical and biomechanical parameters of maturing rat femur

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**Introduction:** Polychlorinated biphenyls (PCBs) are ubiquitous, highly persistent and bioaccumulative lipophilic environmental pollutants with a variety of potential health risks in wildlife, laboratory animals and humans. Exposure to PCBs induces a broad spectrum of toxic effects in various organs including bone. The aim of our study was to examine effects of a planar and a non-planar hexachlorobiphenyl (PCB-169 and PCB-155, respectively), individually and in combination, on selected biochemical parameters and femur growth, geometry, mineral density and biomechanical properties in rat offspring lactationally exposed to PCBs during the early postnatal period.

**Material and methods:** 21 lactating female Wistar rats, divided into four groups, were intraperitoneally administered PCB congeners dissolved in olive oil at different time points after delivery. In total, group 1 received 3 mg of PCB-169 per kg body weight, group 2 12 mg of PCB-155 and group 3 3 mg of PCB-169 and 12 mg of PCB-155. The control (group 4) was administered olive oil only. Offspring were sacrificed on postnatal day 22, body weights recorded, blood samples collected and femora dissected. Peripheral quantitative computed tomography (pQCT) and three-point bending test using purpose-made mechanical testing machine is currently underway to obtain data on femur geometry and biomechanical properties.

**Results:** Serum concentration levels of inorganic phosphate were significantly lowered in the PCB-155+169 and increased in the PCB-169 group compared to the control and the PCB-155 group. Total alkaline phosphatase activity was significantly decreased in

PCB-169 and PCB-155+169 groups. Changes in serum calcium and sex-by-treatment differences were not observed. Total femur length (measured from the top of the femur head to the most distal point of the medial condyle) was decreased in the PCB-169 and PCB-155+169 groups compared to the control. Femur length in the PCB-155 group was significantly increased compared to the control and to the PCB-169 and the PCB-155+169 groups. The diameter (measured at the narrowest part of the femur) was reduced in the PCB-169 and the PCB-155+169 groups compared to the PCB-155 group. Calcium, phosphorus and their ratio in inorganic substance of the femur were not significantly altered.

**Conclusion:** In conclusion, the preliminary results suggest that lactational exposure to PCB-155 and PCB-169 affected femur growth parameters. Effects of planar PCB-169 and the combination PCB-155+169 lead to shorter and thinner bones, while the non-planar PCB-155 seems to have the opposite effects on femur growth.

## P52

### Characteristics of the leg muscles of broiler chickens under different incubation factors

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**Introduction:** Modified incubation factors have a great influence on the development of broiler chickens, especially on the development of skeletal musculature. In this study, the influence of increased temperature and monochromatic green light on the characteristics of leg musculature of broiler chickens were examined.

**Material and methods:** During the experiment, eggs were divided into control and three treated groups. Treatments consisted of incubation of eggs under increased temperature (first treatment), monochromatic green light (second treatment) and simultaneously increased temperature and monochromatic green light (third treatment). Upon hatching, the chickens were transferred to brooders and grown until 42 days of age, when samples of skeletal muscle tissue (*m. biceps femoris*) were taken.

**Results:** Following the specific parameters that were observed (diameter and nucleocytoplasmic ratio of skeletal muscle cells and volume density of muscle connective tissue), it was determined that, in the late postnatal period of development, treated groups have the greater diameter and smaller nucleocytoplasmic ratio of

muscle cells, compared to those of the control group. On average, diameter of muscle cells was higher by 4.17%, while nucleocytoplasmic ratio was smaller by 37.5% in treated groups compared to the control group. In all groups, the volume density of the muscle connective tissue was approximately the same. These results can be explained by with influence of modified incubation factors on higher myoblast proliferation, which results in an increase of diameter and decrease of nucleocytoplasmic ratio of skeletal muscle cells during the postnatal stage of development.

**Conclusion:** It was concluded that temperature and light factors during incubation can influence the skeletal muscle development of broiler chickens, and hence their qualitative and quantitative characteristics.

## P53

### Visualization of structures in the mammary gland in Bulgarian native goats by B-mode, color doppler and 3-D ultrasonography

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**Introduction:** The purpose of this study was to determine the possibility for visualization of structures in the mammary gland of lactating goats by means of three ultrasound methods – two-dimensional (B-mode), color Doppler and three-dimensional (3-D).

**Material and methods:** In this research, we used 16 goats of the Bulgarian native breed, aged between 2 and 6 years, weighing 52-60 kg. An ultrasound examination of the mammary gland was performed by the technique “direct contact”. Teats were examined with linear transducer at a frequency of 12.0 MHz, while the glandular parenchyma of the udder – at frequency 5.0 MHz.

**Results:** The obtained results indicate that the use of a two-dimensional ultrasound can be used for visualization of all morphological structures in the mammary gland in a goat.

**Conclusion:** The color Doppler sonography is possible to use for visualization of changes in the mammary blood vessels that are located in the venous ring of Furstenberg and mammary parenchyma, and to differentiate them from the anechoic milk ducts. Three-dimensional ultrasound provides much more detailed information of the individual parts of the udder, which is a basis for comparison with pathological changes occurring in the mammary gland.

**P54****Morphometric differences of the right flippers of two dolphin species from the Adriatic Sea**

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**Introduction:** The aim of this study was to investigate and compare morphometric characteristics of the flippers in two species of dolphins which are morphologically very similar, but inhabit different environments. These are the bottlenose dolphin, resident species in the Adriatic Sea; and the striped dolphin, non-resident species in the Adriatic Sea.

**Material and methods:** The right flippers from 24 cetacean specimens were obtained from dead stranded animals found at the Croatian coast of the Adriatic Sea. External body measurements as total body length (TBL), cranial length of flipper (CrFL), caudal length of flipper (CaFL), and great flipper width (GFW) were taken. Morphometric data was mathematically analysed and compared between the two investigated species. For that purpose, authors used two mathematical indexes: the index of flipper measures ( $I_{FM}$ ), and the index of flipper measures across total body length ( $I_{FBL}$ ).

**Results:** Flipper length in most cetaceans has a constant relation to the body length. Index of flipper measures ( $I_{FM}$ ), as a calculation of all flipper morphometric parameters, shows much larger flippers in bottlenose dolphin than in the striped dolphin. Also, the index of flipper measures across the total body length ( $I_{FBL}$ ) shows that the bottlenose dolphin has a greater flipper in relation with body length.

**Conclusion:** Flexibility and precise maneuvering are found in cetaceans that inhabit more complex habitats, whereas high-speed maneuvers are used by cetaceans in the pelagic environment. The mean values of both indexes show the differences between the two investigated dolphin species, and bottlenose dolphin has wider and larger flipper than the striped dolphin. That result suggests more intensive use of flippers in the body movements of bottlenose dolphins than in the striped dolphins. The indexes that are proposed in this study, can be certain indicators of the flippers influence on maneuvering ability in various species of dolphins, and probably in other cetaceans.

**P55****Animal footprints on bricks from the Roman site of Cibalae, Croatia**

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**Introduction:** The determination of animal footprints gives valuable information about the animals and their coexistence and interaction with the humans of that time. According to the principles of Roman brick production, bricks were made by using removable mouldboards and allowed to dry in the open air before being fired. Bricks were dried on the roofed area which was open at the sides and during that time various animals moved around fresh bricks and left traces on them.

**Material and methods:** The animal footprints were investigated on four bricks which were collected in the Western necropolis of the Roman city of Cibalae (recently, city of Vinkovci) in the Eastern part of Croatia. The excavated site is identified as a grave, and it was dated in the range from the end of the third to the fifth century C.E. The largest brick is fully preserved and it has a regular shape. Other three bricks are fragments and have irregular shape.

**Results:** All bricks have footprints of front and hind animal feet which are predominantly of canide origin. The first and second brick fragment (B1, B2) have animal footprints originating from canids of medium body size. Also, B1 has two crossed lines deeply cut in the brick by the human, probably before the animal moved over the brick. The third brick fragment (B3) has eight footprints originating from hoofed animals of small body size, probably the roe deer. The largest brick (B4) has twelve animal footprints originating from canids and one print of a human hand. Also, that brick has one circular imprint which is probably some specific mark of the brick makers. Majority of footprints including the footprints of the roe deer, are shallow and they were probably imprinted while bricks were fairly stiff.

**Conclusion:** Generally, the range of canids body size is large and these results suggest the presence of at least three type of canids. Main characteristics of the canid traces are common for all canid family, and it is very difficult to distinguish it exactly. Canid traces found in the investigated bricks can be divided into canid groups corresponding to the dogs of small, medium and large breeds. Abundance of all described footprints reflects a presence of many animals around human habitations, and in combination with other zooarcheological data may complete the picture of fauna in the vicinity of humans and their settlements.

**P56****The rat adrenocortical *Zona glomerulosa* response to moderate heat – stereological and hormonal study**

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**Introduction:** Many studies have shown that high ambient temperature provokes activation of the adrenocortical *zona fasciculata* (ZF) within several hours. However, there is very few data concerning the effect of heat exposure on *zona glomerulosa* (ZG). An increase or decrease of serum aldosterone levels after one day exposure to heat stress was observed, while some data showed the absence of significant changes in the volume of ZG cells. Keeping in mind the ambiguous literature data regarding the effects of moderate heat exposure on ZG, our aim was to investigate the stereological and hormone secreting characteristics of ZG and its cells after 24-hour exposure of rats to  $35 \pm 1^\circ\text{C}$ .

**Material and methods:** The experiment was conducted on adult Wistar male rats. The experimental animals (n=7) were exposed 24 hours to moderately high ambient temperature ( $35 \pm 1^\circ\text{C}$ ), while the control group (n=7) was kept at room temperature ( $20 \pm 2^\circ\text{C}$ ). The left adrenal glands were fixed in Bouin's solution, serially cut ( $5 \mu\text{m}$  thick) and stained with hematoxylin-eosin. In order to evaluate the volumes of the ZG, every tenth section of the gland was analyzed at 100x magnification, while for the evaluation of nuclear and cytoplasmic volumes of ZG cells 1000x magnification was used; all with the M<sub>42</sub> multipurpose test system. Serum aldosterone levels were determined by aldosterone ELISA test. Duncan test was used for statistical analyses.

**Results:** The ZG in rats after 24-hour of heat exposure was very wide with presence of the adrenocortical proliferates consisting of ZG cells. The body weight and the absolute adrenal weight were decreased ( $p < 0.05$ ) by 15.3% and 11% respectively, in comparison with the controls. Absolute volumes of the adrenal gland and adrenal cortex were also decreased by 10.7% and 8.6% respectively, compared to the controls. The absolute and relative volumes of ZG as well as the total number of the cells in ZG were increased ( $p < 0.05$ ) by 14.7%, 12.2% and 17.1% respectively, while there were no significant changes in cellular and nuclear volumes of ZG cells. We've also found decreased ( $p < 0.05$ ) serum concentration of aldosterone by 79% in relation to the controls.

**Conclusion:** These findings indicate that 24-hour exposure of rats to moderate heat provokes increased activity of ZG, manifested through the increased relative volume and number of cells in this zone.

**P57****The Adriatic Sea bottlenose dolphin: histological structure of the ovaries**

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**Introduction:** The number of bottlenose dolphins (*Tursiops truncatus*) in the Adriatic is estimated at 220 – 250. Learning about the anatomy of the endangered animal species is a prerequisite for their successful protection.

**Material and methods:** The investigation was conducted on 16 females found dead in their natural habitats in the Croatian part of the Adriatic Sea from 1996 till 2010. The carcasses were thoroughly investigated and their age was estimated. At necropsy, both ovaries were taken for histology analysis. The samples were fixed in 4% neutral formalin, paraffin embedded, cut into  $6\text{-}\mu\text{m}$  thick sections and stained using routine haematoxylin-eosin method (HE), Mallory stain, Masson's trichrome staining method and Gomori's aldehyde fuchsin stain.

**Results:** The ovary was covered by a germinal epithelium, with the connective tissue tunica albuginea under it. The tunica albuginea thickened with age and was transformed into a dense fibrous layer. Primordial follicles, unilaminar primary follicles, multilaminar primary follicles and antral follicles were observed in the ovarian cortex. Mature follicles were found in none of the investigated animals. Primordial follicles characteristically surrounded the hilum and were grouped into clusters separated by dense bundles of collagen fibres. In older animals, the number of follicles decreased, the ovarian cortex was markedly thinned and the ovarian medulla thickened. The ovarian medulla was filled with a dense network of blood vessels. Clusters of small blood vessels reached nearly to the surface of the ovary and ramified around the fibrous corpus albicans. The arteries supplying the ovary appeared to be curved. Small arteries had outer and inner elastic membranes. The corpus luteum was composed of cells of irregular shape and uniform size. Some of the cells were vacuolated. In the ovarian medulla of all the investigated animals, rete ovarii was found.

**Conclusion:** The comparison of the histological structure of the ovary and the animal age estimate will yield valuable data on the reproductive cycles of the Adriatic bottlenose dolphin.



**P58****Scanning electron microscopic studies of the zonular apparatus in dogs**Dinçer Yıldız<sup>1\*</sup>, Murat Erdem Gultiken<sup>2</sup><sup>1</sup>Department of Anatomy, Faculty of Veterinary Medicine, University of Kırıkkale, Kırıkkale, Turkey<sup>2</sup>Department of Anatomy, Faculty of Veterinary Medicine, University of 19 Mayıs, Kurupelit, Samsun, Turkey

Dogs can hear and smell well as a consequence of their well-developed olfactory and hearing systems, but they cannot see well. In addition, cataract is one of the more frequent eye disorders in progressed age. Thus, we think that morphology of the lens and the suspensory apparatus of the lens can be investigated in detail. In this study, the eye balls obtained from dogs of different ages and gender which either died or were euthanatized due to a severe condition such as traffic accidents. The eye balls were investigated using sub-gross, histological and scanning electron microscopic (SEM) techniques.

The fibers connecting the ciliary process to the lens were observed in three different directions: anterior, equatorial and posterior. The fibers in the anterior-posterior directions exhibited many crossings at the lens equator. The length of the ciliary processes is approximately 17-20 µm; however, the ciliary processes giving origin to the zonular fibers varied between 138-145 µm in length. The connective tissue of the ciliary processes was rich in lymphatic vessels. The zonular fibers leave the ciliary processes as an extension of the fibrous sheath and give rise to thin branches near the lens. These branches eventually conjoin at the lens capsule.

hairs, was ejected with the placenta. The monster was examined and described, and analysed using a direct digital radiography. A macroscopic dissection was performed, during which tissue samples were harvested for histology analysis. The samples were fixed in 4% neutral formalin, paraffin-embedded, sliced in 6-µm thick sections and stained using haematoxylin-eosin method (HE), Masson's trichrome staining method and Gomori's aldehyde fuchsin staining method.

**Results:** The monster was of a regular oval shape, with a soft tissue excrescence similar to an umbilical cord, within which there were two blood vessels. In the radiogram, there was an oval mass of a soft tissue density embedding three opaque bone structures. The body cross section displayed large amount of connective and adipose tissues. Around centrally positioned bony parts, there were cartilaginous plates and muscle tissue bundles. Epidermal cells were vacuolated and the dermis formed of collagen fibre bundles and a dense network of elastic fibres with numerous dilated capillaries containing no blood cells. The subcutaneous white adipose tissue was of a lobar structure, and, between the lobes, there was a formation built of linearly threaded regular collagen fibre bundles. Individual striated muscle fibres were also observed. The bones could not be identified. They were of a trabecular structure, and formed of a woven bone tissue with numerous round-to-oval, randomly arrayed osteocytes. Due to significant underdevelopment of the monster and the presence of a healthy sibling, the malformation could be linked to the reversed arterial perfusion.

**Conclusion:** This is an obvious example of acardius amorphus and contributes to understanding of malformation with details on bone histology.

**P59****Morphology and radiology analysis of an *Acardius amorphus* monster in cattle**Snježana Vuković<sup>1\*</sup>, Katarina Špiranec<sup>1</sup>, Hrvoje Brzica<sup>1</sup>, Damir Stanin<sup>2</sup>, Koraljka Gracin<sup>3</sup>, Karmen Botka-Petrak<sup>1</sup>, Damir Mihelić<sup>1</sup>, Hrvoje Lucić<sup>1</sup><sup>1</sup>Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia<sup>2</sup>Department of Radiology, Ultrasound Diagnosis and Physical Therapy, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia<sup>3</sup>Postgraduate student, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

**Introduction:** *Acardius amorphus* is a rarely described malformation in domestic animals. It denotes an asymmetric monster twin with no functional heart linked to the placenta of the normally developed twin.

**Material and methods:** A case of acardius amorphus in cattle was morphologically and radiologically analysed. After a normal delivery of a healthy male calf, a round amorphous mass, entirely covered by skin with dense

**P60****Genetic polymorphism of the KAPPA - casein locus in the Holstein-Friesian dairy cattle in Republic of Macedonia**Nikola Adamov<sup>1\*</sup>, Dine Mitrov<sup>1</sup>, Lazo Pendovski<sup>1</sup>, Nikola Pacinovski<sup>2</sup>, Peter Dovc<sup>3</sup><sup>1</sup>Faculty of Veterinary Medicine, Ss. Cyril and Methodius University in Skopje, Macedonia<sup>2</sup>Institute of Animal Science, Ss. Cyril and Methodius University in Skopje, Macedonia<sup>3</sup>Biotechnical faculty, University of Ljubljana, Slovenia

**Introduction:** The bovine kappa-casein (κ-CN) is a phospho-protein with 169 amino acid residues encoded by the *CSN3* gene located on BTA6. This gene is around 13 kb long and is divided in a transcriptional unit (with 5 exons and 4 introns) and a promoter region. The fourth exon which is 517 bp long carries all the SNPs of the 14 *CSN3* genetic variants identified so far in the *Bos* genus. The two most common variants in the HF breed are A and B. The B variant differs from A at two amino acid positions: at 136<sup>th</sup> This substituted with Ile and at 148<sup>th</sup> Aspis changed with Ala. The C variant carries the amino acid His at the 97<sup>th</sup> position, while the amino acid Gly is

present at the position 155 in the E variant. The aim of this study was to optimize a laboratory method for genotyping of the most common *CSN3* variants as well as to determine the genotype and allele frequencies of this locus in the HF cattle population in the Republic of Macedonia.

**Material and methods:** Genomic DNA was extracted from full blood from 250 cows and from deeply frozen sperm doses from 20 different bulls. Blood samples were extracted with Phenol-Chloroform-Isoamil alcohol and the extraction from semen was done with sperm lysis buffer. The target DNA sequence was amplified with newly designed primers and the products were subjected to RFLP analysis for identification of  $\kappa$ -CN variants A, B, C and E. The correctness of the restriction analysis was checked by direct sequencing of the PCR products.

**Results:** The primers used for  $\kappa$ -CN genotyping amplify fragment of 458 bp. *Taq* I, *Hind* III and *Pst* I enabled the distinction of A and B alleles but none of them could identify E and C alleles. *Hae* III enabled differentiation of the E allele, while *Mae* II identified the C allele. The SNPs of the different alleles were identified by direct sequencing of the PCR products thus confirming the correctness of the RFLP genotyping. The chi-square test showed that the genotype frequencies of this locus were in the Hardy-Weinberg equilibrium.

**Conclusion:** The frequencies of the six genotypes AA (48,9%), AB (32,2%), BB (8,1%), AE (6,6%), BE (3,3%) and AC (0,7%) identified in this study are within the expected. In the HF breed most common are the variants A and B, allele E is found rarely, while allele C has not been detected in this breed. This study revealed two animals as carriers of the genotype AC, but they were crossbreds with the Brown breed. The genotypes of the  $\kappa$ -CN locus are in the Hardy-Weinberg equilibrium which suggests that this population is not under influence of any natural or artificial selection factor that favors some of the alleles over the others.

## P61

### Ultrasound determination of body condition of dairy cows

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**Introduction:** The aim of this research was to investigate a correlation between body condition score (BCS) and ultrasound determined skin and fat thickness over tuber ischiadicum (SFT), at dairy cows.

**Material and methods:** The investigation was carried out on 20 Holstein-Friesian dairy cows. The body condition score (BCS) and thickness of skin and underlying fat,

over tuner ischiadicum, was determined. The thickness of skin and underlying fat (SFT) was measured using an ultrasound device, with 8MHz linear probe. We employed a simple regression model (BCS vs. SFT), with BCS as a dependent value and SFT as independent value. The output shows the results of fitting a linear model to describe the relationship between BCS and SFT.

**Results:** The mean BCS was (3,66±0,34), while SFT was (1,3±0,39cm). The equation of the fitted model is  $BCS = 3,04 + 0,46 \times US$ . Since the P-value in the ANOVA table is less than 0,05, there is a statistically significant relationship between BCS and SFT at the 95,0% confidence level. The R-Squared statistic indicates that the model as fitted explains 27,12% of the variability in BCS. The correlation coefficient equals 0,52, indicating a moderately strong relationship between the variables. The standard error of the estimate shows the standard deviation of the residuals to be 0,3. The mean absolute error (MAE) of 0,232777 is the average value of the residuals. The Durbin-Watson (DW) statistic tests the residuals to determine if there is any significant correlation based on the order in which they occur in your data file. Since the P-value is greater than 0,05, there is no indication of serial autocorrelation in the residuals at the 95,0% confidence level.

**Conclusion:** The BCS is in correlation with SFT, and SFT can be used for condition scoring of Holstein-Friesian dairy cows.

## P62

### Welfare assessment and main concerns in dairy farms in Macedonia

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**Introduction:** The aim of this study was to determine the welfare state of dairy cows and to identify the main welfare concerns and priorities for improvement of the existing management and housing systems in Macedonia.

**Material and methods:** Twelve dairy farms (11 ties and 1 loose stall), with range of 15 – 74 cows per farm, in the north-western part of Macedonia, were selected as representing models for dairy farms in the country. The farms and the total number of 335 cows were assessed using the animal – based measures described in the Welfare Quality® Assessment protocol for dairy cattle. All measures were taken with the methods presented by Welfare Quality®, while the somatic cell counts from the milk samples were determined using Fossomatic 5000® (Foss Electric, Denmark), in accordance with the recommendations in Milk-Enumeration of somatic cells

(Guidance on the operation of fluoro-opto-electronic counters, ISO 13366-2:2006). The collected data were processed for calculating the criteria, principles scores and overall assessment for classifying farms, using the decision tree method, weighted sum and I-spline functions and alarm thresholds, by previously developed software.

**Results:** The percentage of very lean cows was  $18.21 \pm 2.87\%$ , in 11 farms the water points were bowls with two animals per drinker and water flow of 8 l/min. Regarding comfort around resting, the time needed to lie down ( $6.59 \pm 0.2s$ ), the animals lying outside of the lying area ( $18.13 \pm 6.27\%$ ) and the cleanliness of lower legs, udder and hindquarters ( $96.33 \pm 1.63\%$ ,  $79.38 \pm 4.86\%$  and  $94.91 \pm 2.68\%$ , respectively) are all considered as serious welfare problems. Only three farms were using pasture or outdoor run as part of their management system. The absence of injuries shows  $37.54 \pm 8.36\%$  of animals with mild and  $54.99 \pm 9.46\%$  with severe alterations; the lameness in the assessed farms was present with  $26.8 \pm 5.06\%$  and the percentage of mastitis ( $45.28 \pm 706\%$ ) is far above the set alarm threshold. Most of the animals, 82% are animals that allowed to be touched or approached at  $< 50$  cm, giving high score for human-animal relationship criterion. The total scores for the welfare principles were: good feeding  $40.73 \pm 4.28$ ; good housing  $23.10 \pm 4.56$ ; good health  $32.59 \pm 3.11$ ; and appropriate behavior  $21.59 \pm 2.84$ ; while the overall assessment classified 7 farms at acceptable level and 5 farms were not classified. **Conclusion:** Considering the findings, main concerns and immediate improvements that should be focused on are the housing conditions and adaptation of the management practices, in terms of allowing appropriate animal behavior which will significantly contribute to better welfare in the farms in Macedonia.

### P63

#### Sow productivity on commercial pig farms in the Republic of Macedonia

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**Introduction:** The objectives of the present study were to determine the production performances of sows in commercial pig farms in the Republic of Macedonia, to compare the differences in sow productivity data between small and large farms and to examine interrelationships of key production parameters among farms with different sizes.

**Material and methods:** The study was retrospectively based and included the annual (2012) analysis of the sow productivity data in small ( $< 200$  sows,  $n=4$ ) and large (200-1000 sows,  $n=5$ ) commercial pig farms. The data were collected from the farm record sheets,

statistically evaluated and compared with the known literature.

**Results:** Sow productivity was greater in the small farms compared to the large ones. The small farms had larger litter per sow (PBL), more pigs born alive (PBA), higher weaning weight (WW) and more pigs weaned per sow per litter (PWSL) than the large ones ( $p < 0.001$ ). Small farms also had greater farrowing rate (FR) ( $p < 0.01$ ). Higher replacement rate (RR), lower average parity (AP), greater number of litters per sow per year (LSY) and higher sow death rate (SDR) were observed in large farms ( $p < 0.001$ ). The large farms also had less non-productive days (NPD) than the small farms ( $p < 0.001$ ). Different intensity of correlations also were observed for several productive parameters among the farm groups.

**Conclusion:** The data obtained in this study shows that sow productivity in Macedonian pig farms is lower than in EU countries. Small herds are more efficient than the large herds. Despite all limitations, our study provides information for the veterinarians regarding reproductive parameters of sows and their interrelationships on Macedonian pig farms. Further investigation should be made in order to identify whether the specific management factors have effect on productivity of the breeding herd.

### P64

#### City of Ankara cattle fattening enterprises internal terms of trade, price, production and income fluctuations

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This study reflects the exchange of assets and the input and output differences with its results in the cattle fattening enterprises in Ankara, and also it does this with the relativist alteration between agriculture, livestock and industrial sectors.

The data for the research is collected from the city of Ankara and its 13 districts, and also the information is used from 123 which have been organizing beef cattle stock is used. The survey findings conducted on operations in the 13 districts and various villages affiliated with these districts and the data derived from the survey constitute the material of the research. Also, the research utilized the Turkish Statistical Institute data and various literature on this subject. Within the research, enterprises were chosen by random sampling through the pre-interviews conducted in 2010, 2011, 2012 with the enterprises. In choosing the enterprises, the intensity of the breeding activities in the districts and transportation were taken into consideration. One period is in May, the other one is in the time of the Feast of Sacrifice. In total, six surveys were done in three years. In the enterprises where the survey was applied in 2010, 2011 and 2012, the total number of animals which

was taken to stock, is 11.805 heads, 11.612 heads and 11.561 heads. In the calculations based on the analyzed data, in 2010, the General Terms Of Trade index value 100 changed into 85,7 and 75,3 respectively in 2011 and 2012; the General Income Terms Of Trade value which was 100 in 2010, changed to 84,1 and 73,6 respectively in 2011 and 2012. Secondary values on the other hand, the General Terms Of Trade index value which was 100 in 2006, had a little drop in 2008 and ends with 104,4; 134,8; 119,4; 121,3; in the years of 2007 and 2008. The General index of 2008, which was 100 in 2006, increased and reached 223,7 level in 2012.

## P65

### Pricing of veterinary services – Theory and practice

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**Introduction:** Prices are a key determinant of financial performance for virtually any business in either the public or private sector, and the veterinary practice offers no exception. To achieve consistent success, it is critical that a business understands the prevailing conditions in the marketplace when setting prices for its goods and services. Important issues to address include: 1. What are the prices of similar goods and services produced by other firms? 2. How important is price as a choice factor when customers select a source of these goods and services? 3. How do staff members view prices at the point-of-sale? 4. How are prices determined? These questions are especially crucial in small businesses such as those typical of the veterinary profession, where the decision-makers tend to be technical experts rather than trained business managers. Without this information, these decision-makers often have very little basis on which to develop a pricing policy.

**Material and methods:** The findings are based on desk-research, exploring of different articles and also interviews with veterinary practice managers regarding their experience and regular practice in pricing of veterinary services in R. Macedonia.

**Results:** The pricing strategy which a veterinary practice develops must reflect the try cost of delivering products and services to clients. An essential starting point for any pricing strategy therefore, to establish what these costs are. This article addresses the issues and challengers involved in costing and price settings of veterinary services in a small veterinary practice. First, various pricing strategies available to practice managers are explored. Finally, some general guidelines are given for keeping clients informed about prices in order to ensure transparency and clarity and thus maintain and even enhance the relationship practice – client.

**Conclusion:** When the social sector is with an intention of meeting a target level of profits instead of simply aiming for zero profit or maximizing the profit, a proper pricing policy needs to be set up. This study presents a model for optimal pricing for firms such as veterinary practices with a target profit motive instead of the normally expected maximum profit objective.

## P66

### The occurrence of teat hyperkeratosis in cows and its effect on milk somatic cell counts

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**Introduction:** The healthy condition of the teat end orifice is recognized as a significant factor in resistance to the mastitis pathogen, which is an important economic challenge in dairy herds. The occurrence of hyperkeratosis, which is defined as slightly swollen tissue consisting of either smooth or rough keratin plaque in a ring that surrounds the teat end orifice, can progress in a natural process from mild to severe and from erosion to scabs. The teat end, milk yield, lactation period, parity and the milking machine have an impact on the development of hyperkeratosis. In this study, the frequency of hyperkeratosis and its effect on milk somatic cell counts were researched.

**Material and methods:** A total of 2332 teats in 583 Holstein cows in different stages of lactation were evaluated on the base of the criteria of morphological structure of the teat end, milk yield and number of lactations. Macroscopic appearances of lesions on the teat orifice were classified and they were also photographed. Somatic cell counts (SCC) were evaluated using the fossomatic method.

**Results:** The SCC was determined to be less than 200.000 cells/mL in 72.8% of the quarters and greater than this in 27.2%. Forty-nine percent of the teat ends were normal, while hyperkeratosis was identified in 51%. Hyperkeratosis degrees of 1, 2, 3, 4 and 5 was determined in 17%, 13.8%, 9.2%, 5% and 6% respectively. The study findings demonstrated that there was a significant rise in SCC parallel to increasing severity of hyperkeratosis ( $P < 0.001$ ) and that even mild hyperkeratosis could result in an increase in SCC ( $P < 0.05$ ). A significant correlation was determined between severe hyperkeratosis and pointed teat ends ( $P < 0.001$ ). The relationship between hyperkeratosis and milk yield was determined to be significant ( $P < 0.001$ ). However, the connection between numbers of lactations with hyperkeratosis was not identified as statistically significant.

**Conclusion:** It was concluded that pointed teat ends have a greater tendency to develop hyperkeratosis. The development of hyperkeratosis increased as milk yield dropped and hyperkeratosis could result in a significant increase in SCC and consequently pose a risk to udder health.

**P67****Relationship between milk yield and somatic cell count**Halil Bocekli<sup>1</sup>, Omur Kocak<sup>2\*</sup><sup>1</sup>Mert Veterinary Clinic, Aydin, Turkey<sup>2</sup>Department of Animal Breeding and Husbandry, Istanbul University, Veterinary Faculty, Istanbul, Turkey

The study was carried out on 2591 test day data of 941 Holstein cows. The effects of somatic cell count (SCC) on milk yield were determined by the investigation of daily milk yield and SCC of cows.

Average daily milk yield was 27.67 lt. In terms of lactation parity, average daily milk yields were 21.26 litres for parity 1, 28.25 litres for parity 2 and 33.49 litres for parity 3 ( $P < 0.001$ ). Average daily milk yields with regard to SCC groups were 28.75 litres for group 1 (0-200 000), 27.48 lt for group 2 (200 001-500 000) and 26.78 litres for group 3 (500 001 and higher) ( $P < 0.001$ ). In SCC groups the differences between group 1 and the other groups were significant, while the differences between group 2 and group 3 were not significant. It was concluded in the present study that the increase in SCC resulted in significant decrease in milk yield.

**P68****The evaluation of the effect of somatic cell count on raw milk composition**Uğur Günşen<sup>1\*</sup>, Hüseyin Eseceli<sup>1</sup>, Can Günşen<sup>2</sup><sup>1</sup>University of Balıkesir, Bandırma Vocational High School, Department of Food Processing<sup>2</sup>University of Uludağ, Karacabey Vocational High School, Department of Food Processing

**Introduction:** Somatic cell count (SCC) is an indicator of the quality of milk. There is general agreement on the values of less than 100.000 cells/ml for uninfected cows. A threshold SCC of 200.000 would determine whether a cow is infected with mastitis. Cows with a result of greater than 200.000 are highly likely to be infected on at least one quarter and those greater than 300.000 are infected with significant pathogens.

Milk from mastitic cows may have off-flavors and may undergo deterioration of the milk fat and protein more quickly than milk from healthy cows. The aim of this study was to evaluate the relationships between SCC and some raw milk parameters (total dry matter, fat, protein, lactose and urea nitrogen).

**Material and methods:** Raw milk samples were collected from Brown Swiss cattle ( $n=30$ ) in two different dairy companies, in Bandırma District of Balıkesir Province of Turkey, in the period between November 2013 to March 2014. Totally 360 (180 in Farm-1 and 180 in Farm-2) raw milk samples divided into two groups, according to the mean levels of SCC in farms (Group-1: low

SCC  $\leq 107.000$  cells/ml and Group-2: high SCC  $> 107.000$  cells/ml in Farm-1, and, Group-1: low SCC  $\leq 172.512$  cells/ml and Group-2: high SCC  $> 172.512$  cells/ml in Farm-2. All the analysis were performed by Bentley FTS 400 Combi used FTIR technology. Statistical analysis was performed by Excel-2010.

**Results:** The mean levels of SCC were determined as  $41.944 \pm 28.184$  cells/ml for the first group and  $211.358 \pm 205.279$  cells/ml for the second group in the Farm-1, respectively. In the first group, mean levels of dry matter, fat, protein, lactose and urea nitrogen were determined as  $11.89 \pm 0.96\%$ ,  $3.28 \pm 0.83\%$ ,  $2.96 \pm 0.29\%$ ,  $4.81 \pm 0.23\%$  and  $14.05 \pm 5.31\%$ , respectively. In the second group, same parameters were calculated as  $11.99 \pm 0.97\%$ ,  $3.3 \pm 0.63\%$ ,  $3.01 \pm 0.43\%$ ,  $4.75 \pm 0.39\%$  and  $14.86 \pm 5.14\%$ , respectively. The mean levels of SCC were determined as  $55.117 \pm 38.229$  cells/ml for the first group and  $507.929 \pm 429.385$  cells/ml for the second group in the Farm-2, respectively. In the first group, mean levels of dry matter, fat, protein, lactose and urea nitrogen were determined as  $11.93 \pm 1.38\%$ ,  $3.39 \pm 1.21\%$ ,  $3.02 \pm 0.36\%$ ,  $4.67 \pm 0.33\%$  and  $10.11 \pm 4.05\%$ , respectively. In the second group, same parameters were calculated as  $11.97 \pm 1.45\%$ ,  $3.53 \pm 1.17\%$ ,  $3.1 \pm 0.49\%$ ,  $4.58 \pm 0.32\%$  and  $12.71 \pm 5.08\%$ , respectively.

**Conclusion:** It was concluded that, by the increase of SCC, milk dry matter, milk fat and urea nitrogen contents were determined to be affected, significantly ( $p < 0.05$ ) in Farm-1 (Group-1 : low SCC  $\leq 107.000$  cells/ml and Group-2 : high SCC  $> 107.000$  cells/ml), while milk lactose, and urea nitrogen contents were determined to be affected, significantly ( $p < 0.05$ ) in Farm-2 (Group-1: low SCC  $\leq 172.512$  cells/ml and Group-2 : high SCC  $> 172.512$  cells/ml).

**P69****A study on the method validation of LC-MS/MS reference method to determine the amphenicol residues in the samples of feedstuffs**Ali Özcan<sup>1</sup>, Uğur Günşen<sup>2\*</sup><sup>1</sup>Institute of Food and Feed Control Central Research, Department of Animal Products, 16036, Hurriyet, Bursa, Turkey<sup>2</sup>University of Balıkesir, Bandırma Vocational High School, Department of Food Processing, 10200, Bandırma, Balıkesir, Turkey

**Introduction:** Antibiotic residues in foods produced by animals may be the cause of numerous health hazards in humans. In the European Union and also Turkey, the usage of antibiotics in feedstuffs have been prohibited since January 1st, 2006.

Antibiotic residues in edible animal products are of great concern to regulatory agencies and consumers, so reliable methods for selective and sensitive detection of these residues are necessary to ensure food safety. The

aim of this study was to perform method validation of a LC-MS/MS reference method to determine the residues of amphenicol content in feed samples.

**Material and methods:** In the study, the samples of cattle fattening feed, not including amphenicol content, were used as research materials. The analysis was repeated with the samples of chicken fattening feed and cattle milk feed, not including amphenicol content.

**Parameters of LC-MS/MS:** For HPLC; flow rate: 0,2 ml/min, volume of injection: 50 µl, liquid phase: 80:20 methanol/water (v/v), for MS/MS; ionization mode: ESI -VE, API nebulizing gas pressure: 50 psi, drying gas temperature: 400°C, drying gas pressure: 35 psi, scan time: 0,4 sec, SIM width: 1,5 amu, needle: -4000V, shield: -400V, capillary: -55 V, detector: -1600V, CID gas pressure: 2 m Torr, Spray chamber temp.: 60°C, mass peak width in amu: 1,5, Quad 1: 1,5 and Quad 3: 1,5.

**Parameters of Method Validation:** Linearity, recovery (R) %, limit of detection (LoD) and limit of quantification (LoQ), repeatability and reproducibility were used as validation parameters.

Linearity was tested by using four different standard concentrations (0,5, 1, 1,5, and 2 µg/kg) in six repetitions. Recovery % was calculated by the spiked samples in three different standard concentrations (1, 1,5, and 2 µg/kg) in six repetitions in three days. LoD and LoQ were determined by using the calibration curve for linearity (LoD: 3 x SD/m, LoQ: 10 x SD/m, SD: smallest SD of calibration curve, m: slope of calibration curve). Repeatability were performed by using the spiked samples in three different concentrations (0,5, 1, and 2 µg/kg) in six repetitions. Reproducibility was studied by using the spiked samples of 1µg/kg by two persons in five repetitions and in four days.

**Results:** The results of the method validation were as follows: linearity for fluorphenicol, chloramphenicol, and tiamphenicol were 0.9975, 0.9972 and 0.9997, respectively. Recovery % for 0,5 MRL of fluorphenicol, chloramphenicol, and tiamphenicol were 103, 95.33 and 102, for 1 MRL were 97.85, 100.7 and 99.72, respectively. LoD and LoQ of fluorphenicol, chloramphenicol, and tiamphenicol were 28 and 35.01, 28.97 and 38.25, and 46.28 and 95.92, respectively. Repeatability for 0.5 MRL of fluorphenicol, chloramphenicol, and tiamphenicol were 3.7, 5.3 and 5.35, for 1 MRL were 4.3, 1.59 and 6.19, respectively. Reproducibility for 1 MRL of fluorphenicol, chloramphenicol, and tiamphenicol were 4.33, 3.74 and 5.35, respectively.

**Conclusion:** It was concluded that the present method validation study may be useful to formal or special laboratories, authorized by the Turkish Republic, Ministry of Agriculture for analysing the residues of amphenicol content. These laboratories, using this validated reference method, may assist exporters and importers, local producers, regulators and governments with detection of these compounds in feed and foodstuffs.

## P70

### Seasonal variations of Aflatoxin M<sub>1</sub> content in raw milk from Macedonia and estimation of consumers exposure

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**Introduction:** Milk has the greatest demonstrated potential for aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) introduction in the human diet. The frequency of occurrence of AFM<sub>1</sub> in commercially available milk and dairy products during the Western Balkan Countries outbreak in 2013, led to an increased concern about the establishment of measures to control AFM<sub>1</sub> contamination. In light of these concerns, a comprehensive surveillance program for AFM<sub>1</sub> in raw milk was established. The determined AFM<sub>1</sub> levels were used to calculate between-month and between-season variations of the toxin content, as well as the fluctuations of the consumer's exposure during the surveillance period.

**Material and methods:** A total of 3634 raw milk samples were collected from 48 dairies in the period February 2013-January 2014. The samples were tested applying the immunochemical screening method, and the positive samples exceeding the maximum residue level (MRL) were confirmed with high performance liquid chromatography (HPLC) with fluorescence detection (FD). Testing methods were validated and confirmed to be sensitive, selective, accurate and precise according to Commission Regulation 401/2006/EC and Commission Decision 657/2002/EC requirements. To evaluate statistical differences in the means between the data series, a t-test for independent samples has been applied (95 % confidence level).

**Results:** Regarding the between season variations of AFM<sub>1</sub> concentration in milk, unlike the common AFM<sub>1</sub> fluctuation pattern, the highest level has been detected in Autumn (32.4 ng/kg) and accordingly the calculated EDI was 0.108 ng/kg BW/day. The highest AFM<sub>1</sub> average concentration was observed in October 2013 (34.8 ng/kg). Accordingly, the estimated intake revealed to be 0.116 ng/kg BW/day. This could be explained to be due to the long drought in the second half of 2013 and lack of fresh feed. Overall, the average AFM<sub>1</sub> concentration for the survey period was 24.2 ng/kg with the respective intake of 0.081 ng/kg BW/day. A gradual decline of the AFM<sub>1</sub> concentrations in milk was observed for the period studied, confirming the effectiveness of the measures taken for control of milk production facilities.

**Conclusion:** To ensure the safety of milk for human health, it is extremely important to avoid providing feed contaminated with AFB<sub>1</sub> to cows. Hence, regular

monitoring of not only the AFM<sub>1</sub> level in milk, but also the AFB<sub>1</sub> level in feed, will be required to protect the public, especially infants and young children, against AFM<sub>1</sub> toxicity.

## P71

### Incidence of ochratoxin A: Current situation in some food products

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**Introduction:** Ochratoxin A, a nephrotoxic mycotoxin mainly produced by *Asperillus ochraceus* and *Penicillium verrucosum*, has been shown to contaminate a wide variety of commodities (cereals and their products, grapes, wine, dried wine fruits-figs, coffee, nuts). There is growing evidence that this mycotoxin has poor effects not only on body weight, feed intake and feed conversion in animals after consumption of contaminated feed, but it is also involved in the etiology of Balkan endemic nephropathy. OTA exerts nephrotoxic, immunotoxic, teratogenic, genotoxic, mutagenic and carcinogenic effects. For this reason the International Agency for Research on Cancer evaluated OTA as a possible carcinogen in humans (group 2B). The MRL for OTA content in food has been regulated by legislation worldwide and it is in the range from 0,5 to 10 µg/kg for different commodities.

**Material and methods:** Total of 40 corn flour, 11 polenta, 38 wheat flour, 63 grits, 63 bread, 15 breakfast cereals, 13 green coffee, 18 frozen corn, 3 pasta (dry) and 33 strudel samples were brought to our laboratory by inspectors or from the food operators themselves during 2013-2014. The HPLC-FLD and fluorometry with immunoaffinity column clean-up were the methods used for determination of OTA. The extraction and purification of samples was done according to AOAC Official method 2000.03 (for HPLC-FLD) and according to Instruction Manual (for fluorometry).

**Results:** Total of 273 samples were analyzed for OTA content. Most of them (218 samples) were with OTA concentration level below LOD (79,8%). Eighteen (18) samples were positive in accordance with legislation. Among them, 10 strudel samples (30,3%) show OTA content over the MRL in the range of 3,3-9,1 µg/kg, 6 corn flour samples (15%) were with OTA concentration level in the range of 3,2-5,0 µg/kg and 2 grits samples (5,1%) were with OTA content over the MRL in the concentration range of 3,9-5,7 µg/kg. None of the following samples: polenta, wheat flour, bread, breakfast cereals, pasta, frozen corn and green coffee, surpassed the legislation limits suggested by the official agencies.

**Conclusions:** OTA was found in 55 samples (20,1%) tested with levels ranging from 0,14-9,1 µg/kg for different commodities. Although 79,8 % of samples were

with an OTA concentration level below LOD, the number of positive samples (6,6%) should not be neglected. The strategies for ensuring food safety should be directed to the current human exposure to OTA in relation to the safety guidelines for OTA, taking into account what can be reasonably achieved following good practices at all stages of production.

## P72

### Occurrence of ochratoxin A in Macedonian wines

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**Introduction:** Ochratoxin A (OTA) is a mycotoxin produced by the fungi *Penicillium verrucosum*, *Aspergillus ochraceus* and *Aspergillus carbonarius*. It possesses carcinogenic, nephrotoxic, teratogenic, immunotoxic and possibly neurotoxic properties. The international Agency for Cancer Research (IACR) has placed OTA into the B2 group i.e. among substances potentially carcinogenic for humans. The total intake of OTA due to wine has been provisionally estimated by the Codex Alimentarius Commission to 15%. In accordance to EU Regulations Commission (EC 123/2005) wine and other wine and/or grape must based beverages should comprise maximum concentration of 2.0 ng/ml of OTA.

**Material and methods:** Quantitative determination of OTA in wines and grape musts after their clean-up on immunoaffinity columns was investigated using HPLC method with fluorescence detection according to AOAC method (2001.01). In duration of 4 consecutive years (2011-2014), 189 samples of variety bottled wines and grape musts (86 red wines, 90 white wines, 11 rose wines and 2 grape musts), which originated from different parts of Macedonia, were analysed.

**Results:** OTA was detected in 30% of samples, in a concentration level up to: 0.349 ng/ml, 0.716 ng/ml and 0.163 ng/ml in red wines in 2012, 2013 and 2014 respectively; 0.079 ng/ml, 0.238 ng/ml in white wines in 2012 and 2013 respectively; 0.315 ng/ml in rose wines in 2013; 0.137 ng/ml in grape must in 2013. Overall OTA concentration detected in samples in 2011 and 2012 (in both red and white wines) was below LOD (0.043 ng/ml). In 2013 the mean concentration level was 0.076 ng/ml in rose wines and 0.137 ng/ml in grape must. In 2014, only in red wines the overall OTA concentration was over the LOD (0.059 ng/ml). None of the samples exceeded the maximum limit of OTA concentration.

**Conclusion:** In general, levels of OTA were higher in red wines than in white ones, corresponding to the comprehensive published findings. It is interpreted as a consequence of the differences in the winemaking procedures for both types. The overall OTA concentrations

didn't show big fluctuation during the years and are far below the proposed limit. All samples fulfil the regulations of the European Commission regarding maximal concentration of OTA in wine and other wine and/or grape must based beverages.

### P73

#### Validation of ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry method for determination of thyreostats in bovine urine

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**Introduction:** Thyreostatics are veterinary drugs that may be used in livestock production as growth promoter. They cause retention of water in the subcutaneous and muscular tissues as well as in the gastro-intestinal tract by inhibiting thyroid hormone production; thus administration of thyreostats results in increasing of body weight. On the other hand, residues of thyreostats in meat pose a risk for human health due to the teratogenic and carcinogenic effect. For this reason, in 1981 the European Union banned their use in animal production. The aim of this study was validation of the UHPLC MS/MS method for determination of thyreostats in bovine urine.

**Material and methods:** Our study included four thyreostats: thyouracil (TU), methylthyouracil (MTU), propylthyouracil (PTU) and tapazole (TA). Besides these thyreostats, dimethylthyouracil was used as an internal standard. Negative bovine urine was used for validation. Before extraction, the pH of the urine was adjusted to 1. Then derivatisation of the samples was performed with 3 iodobenzylbromide, 1h at 40°C and liquid/liquid extraction was performed with ethyl acetate. The method was validated according to 2002/657/EC. The decision limit (CC $\alpha$ ), detection capability (CC $\beta$ ), accuracy, precision and reproducibility were validation parameters which were obtained with validation procedures. The calibration curve for all standards was from 1.0 to 30  $\mu$ g kg.

**Results:** The linearity of the method showed good correlation for all standards with  $r^2=0.9913$  for TA,  $r^2=0.9977$  for TU,  $r^2=0.9969$  for MTU and for PTU  $r^2=0.9946$ . The accuracy was evaluated by determining the recovery of spiked urine samples on three concentration level at 5 $\mu$ g/l, 10 $\mu$ g/l and 15 $\mu$ g/l. The recovery for TA was from 98.22 to 123%, for TU was from 81.11 to 102.33%, for

MTU was from 92.89 to 93.67% and for PTU the recovery was from 92.33 to 106.33%. The precision of the method ranged from 4.58 to 17.45% and the reproducibility of the method was from 1.61 to 20.70%. The value from CC $\alpha$  were 2.26 $\mu$ g/l for TA, 10.71  $\mu$ g/l for TU, 2.79 $\mu$ g/l for MTU and 2.22 $\mu$ g/l for PTU. CC $\beta$  were found to be 2.93 $\mu$ g/l for TA, 16.75 $\mu$ g/l for TU, 3.60 $\mu$ g/l for MTU and 3.04 $\mu$ g/l for PTU.

**Conclusion:** The UHPLC-MS/MS method for detection and identification of four thyreostatics compounds in urine was developed. Good linearity, good precision, recovery and reproducibility, make this method applicable for determination of thyreostats in bovine urine.

### P74

#### RP-HPLC determination of 5-hydroxymethylfurfural in honey from the Republic of Macedonia as a control parameter of its quality with some analytical aspects: a random collection study

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The decomposition of sugars produces the organic compound 5-hydroxymethylfurfural, also known as *HMF*. *HMF* affects the color and taste, thus manufacturers monitor its levels in some foods, including honey. *HMF* production increases with extended shelf life and/or heat exposure, and is used commonly as an indicator of heat and storage changes in honey. But, *HMF* is not a harmful substance in the levels present in food. According to the Codex Alimentarius Standard for Honey (1981), "*HMF* content of honey after processing and/or blending shall not be more than 40 mg/kg", and "in the case of honey of declared origin from countries or regions with tropical ambient temperatures, and blends of these honeys, the *HMF* content shall not be more than 80 mg/kg."

The *aim* of this work was to develop, test and implement simple, fast, accurate, rugged and robust chromatographic method for determination and quantification of 5-hydroxymethylfurfural in honey.

In this research we used HPLC system automated Varian ProStar with ternary high pressure mixing pump, Autosampler 410 with column oven and Diode Array Detector 330, controlled by software Varian-Star Version 6.31; the chemicals were Ph.Eur. grade, products of Merck Darmstadt, Germany. Several columns were tested: LiChrosphere C18 125 x 4 mm, 5mm; LiChroCart C18e 125 x 4 mm, 5mm, protected with C18e pre-columns 4 x 4 mm; Eurosphere EC Knauer C18 250 x 4,6 mm, 5 mm and Perkin Elmer Brownlee C18 Pecosphere 33 x 4,6 mm with 3 mm particles.

The extraction procedure was simple, using demineralized water; the monitoring signal at 282 nm, column oven set



at 30°C, flow rate 1ml/min and injection volume 10 or 20 ml.

In order to test the separation technique reproducibility and ruggedness, we tested usage of pure water and acidified with organic and inorganic acids, with combination of methanol or acetonitrile on 3 different HPLC columns. External calibration curve of *HMF* concentrations was constructed and applied, ranging in 0.1, 0.5, 1, 2 and 5mg/ml in water, which were further multiplied with 50 to calculate real concentrations.

In our random collected samples of honeys from different origin, date and place of manufacturing, we found very different concentrations of *HMF*, from 4.2mg/kg up to 670 mg/kg. According to our random study, systematic approach to collection, archiving and evidence of Macedonian honeys is recommended, prompting implementation of regulative for honey adulteration quality control.

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#### P75

### Implementation of drug quality control methods in Macedonian veterinary medicine; Preliminary investigations with metronidazole and ciprofloxacin

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Quality control of two different antibiotics frequently used in veterinary and human medicine, Metronidazole and Ciprofloxacin hydrochloride, was considered and performed. Metronidazole is nitroimidazole antibiotic used for prevention and treatment of bacterial and parasitic infections in animals and Ciprofloxacin is a second-generation fluoroquinolone antibiotic used for treatment of bacterial infections in animals. They are available in various pharmaceutical dosage forms for local or systemic action. Metronidazole is included in the British Pharmacopoeia (Veterinary) 2012 with full monograph, whereas Ciprofloxacin hydrochloride is not covered by the British Pharmacopoeia (Veterinary) 2012, but has a monograph in the main part of the British Pharmacopoeia 2012.

In our research three different HPLC system were used: Varian ProStar, Perkin Elmer Series and UPLC Shimadzu Prominence XR. The chromatographic columns used were LiChropher RP Select B 75 x 4 mm with 5 µm particles and Discovery C18 100 x 4,6 mm with 5 µm particles. All used chemicals were of Ph.Eur. quality and all purchased from Merck Darmstadt, Germany and demineralized water was an in-house product with conductivity less than 2 µS. Working standards for active substances, Metronidazole gel 0,75 % and Ciprofloxacin film coated tablets 500 mg were purchased from Replek Farm Ltd. Skopje, Macedonia and Ciprofloxacin hydrochloride eye

drops 3 mg/ml were purchased from a local pharmacy. Chromatographic methods used for both analytes were compendial, with minor modifications made for experimental purposes.

Minor modifications of the pharmacopoeia prescribed chromatographic conditions, in both cases, led to better chromatographic parameters, good resolution and shorter analysis times. Optimized methods can be used for: determination of Metronidazole in gel formulation, for its simultaneous quantification with preservatives present in the formulation and even for identification and quantification of its specified impurity, 2-methyl-5-nitroimidazole; determination of Ciprofloxacin hydrochloride in film coated tablets and eye drops and identification and quantification of its specified impurities. These slightly modified and optimized pharmacopoeial methods for quality control of Metronidazole and Ciprofloxacin dosage forms used in veterinary medicine can be successfully applied in laboratories for quality control of veterinary medicines. This way, we aim to prompt the establishment of laboratories for quality control of veterinary medicines sold in veterinary pharmacies and ambulances in our country and the implementation of the existing regulations for testing veterinary medicines.

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#### P76

### Production of primary biomolecules in different varieties of oriental tobacco (*Nicotiana tabacum L.*)

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**Introduction:** In the last decades most of the researchers have taken tobacco (*Nicotiana tabacum L.*) as a central concern, because of its agro-economic meaning, its significance in the phytochemical industry, as a model for the effect of plant viruses, physiology of mineral nutrition and genetic researches. When it comes to the parameters which describe the quality of the tobacco plants, they are not strictly defined, but they mostly observe the concentration of primary and secondary biomolecules. For these reasons, approaches to research of oxidative status and parameters accurately define the quality of the raw material.

**Material and methods:** The object of analysis are the varieties of the most exploited types of tobacco in the Republic of Macedonia: type Prilep variety P-66, type Jaka variety JK-125/3 and type Basma. The leaves of different vintages and belts in tobacco mature simultaneously, but picking starts gradually from the lowest leaves when they have reached their so-called "technological maturity". As

material for analysis, fermented raw material was taken from two harvests of tobacco crops in 2011 and 2012 including three insertions: lower, middle and upper. The experiment included following analyses: quantitative determination of the content of total and soluble carbohydrates (spectrophotometric method), quantitative analysis of total nitrogen and proteins (Kjeldahl) and testing of the oxidoreductase activity (catalase enzyme activity).

**Results:** Examined parameters directly affect the quality characteristics of fermented tobacco and they alter the physical and features flavors of tobacco products. The carbohydrates are one of the most important biomolecules and in mature leaves of tobacco, they can be represented up to 40-45% of dry matter. The harvest of 2012 in all varieties of tobacco, shows larger concentrations of total and soluble carbohydrates, unlike 2011 where the concentrations are lower.

**Conclusion:** Carbohydrates as primary metabolites are inversely proportional according to the concentration of proteins and total nitrogen. The results also showed larger concentration of proteins and nitrogen in the harvest of 2011 than that of 2012, which confirms that, prolonged fermentation of the raw material decreases the content of carbohydrates and increases the concentration of proteins and nitrogen, analogous to the reduction of the quality of tobacco. The enzyme catalase has a defensive role in the fermentation process from different microorganisms and it shows largest activity at the variety JK-125/3 which is one of the most resistant species to Tobacco mosaic virus (TMV).

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#### P77

### Natural radioactivity in uncultivated soil in the surrounding of Skopje

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**Introduction:** Natural radioactivity of the environment and the associated external exposure that is due to gamma radiation depend primarily on the geological and the geographical conditions and exist at different levels in the soil of every region of the world. The measurement and the understanding of the behavior of natural radionuclides in the environment is very important, because natural radiation is the biggest contributor for the external dose in the world population. The objective of this study was focused on determination of the concentrations of activity of  $^{226}\text{Ra}$ ,  $^{232}\text{Th}$  and  $^{40}\text{K}$  in samples of uncultivated soil, collected from different locations in the surroundings of Skopje. The concentrations of activity of  $^{226}\text{Ra}$ ,  $^{232}\text{Th}$  and  $^{40}\text{K}$  in the collected soil samples were assessed with gamma spectrometry and the technique for registration and monitoring.

**Material and methods:** In order to measure natural radioactivity in soil, samples of uncultivated soil were collected from 14 locations in the surroundings of Skopje in May 2012, including 3-4 samples of uncultivated soil according to the recommendations of IAEA. The measurements of the concentrations of activity of  $^{226}\text{Ra}$ ,  $^{232}\text{Th}$  and  $^{40}\text{K}$  in the collected soil samples were determined by using HPGe gamma spectrometer and the technique for registration of the monitoring of fission.

**Results:** In the current study it was found that the specific activity of these radionuclides in the soil is not uniform, however it is different in different soils, depending on the geological or the typographical character of the area. Also, the measured values of concentration of activity of the radionuclides  $^{40}\text{K}$ ,  $^{226}\text{Ra}$  and  $^{232}\text{Th}$  in all soils that are being examined are within the world range indicated by international organizations. Due to the long half-life of  $^{232}\text{Th}$ , the values of  $^{232}\text{Th}$  are higher than the values of  $^{226}\text{Ra}$  in all uncultivated soils in all the places that were being examined. In terms of the activity in regard to depth, no mutual connection is found between the activity and the depth for  $^{226}\text{Ra}$  and  $^{232}\text{Th}$ . The measured values of radioactivity show that it is randomly distributed in different depths of the soil that was being examined.

**Conclusion:** One can determine that the activity of all natural radionuclides in uncultivated soil is significantly lower than the measurements that are performed at the cultivated soil. This is due to the application of different fertilizers that are applied on agricultural fields in recommendable quantities, that may increase the level of radioactivity in soils. The results and data from this study are useful as a foundation, for the preparation of a radiological map of the studied area, as well as for enrichment of the world data base.

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#### P78

### Investigation of methicillin and vancomycin resistance in *Staphylococcus aureus* isolated from goat milk with mastitis

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In this study, the phenotypical and genotypical resistance of *S. Aureus* isolates, from goat milk with mastitis, to methicillin and vancomycin were investigated. For this purpose, a total of 466 milk samples were collected from 233 goats in the Burdur province. Out of 43 *S. Aureus* isolates from goat milk samples, 7 isolates according to disk diffusion test and 5 isolates according to minimal inhibitory concentration (MIC) values were found phenotypically resistant to methicillin, but none of these isolates carried the *mecA* gene encoding methicillin resistance. All *S. Aureus* isolates did not harbor the *vanA* gene (encoding vancomycin resistance) and these isolates were also susceptible to vancomycin according to the disc diffusion test and MIC. As a conclusion, it is stated that

the phenotypic methicillin resistant *S. Aureus* isolates can be responsible from mastitis in goats and be transmitted to humans through goat milk and milk products.

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## P79

### Most common toxic plants of Turkey to animals: classification by target species/ systems and management of poisoning cases

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This paper is a review of the most common toxic plants and provides classification by their toxic effects on the systems and/or organs of animals. It also aims to provide an ways to best manage poisoning and will be of value to occupations within the scope of animal science. There are over 30 plants commonly reported to be toxic to animals where 11% of poisoning cases of small animals are due to plants. History taking and identification of the plant has a vital importance, as well as the practice of procedures for saving lives and support procedures in cases of suspected animal poisoning. Decontamination (emesis, gastric lavage, prevention of absorption for oral exposure, enhancement of elimination (diuresis, peritoneal dialysis and hemodialysis, exchange transfusion or blood transfusion, gastrotomy), and symptomatic and supportive care (keeping the airway open, prevention of arrhythmia, hypotension and collapse, convulsion, acute renal failure and cerebral edema, managing shock, promoting acid-base balance, treatment of hypoglycemia, hypothermia, hyperthermia) are the basic principles of treatment in toxicological cases including plants. We will provide information on the botanical characteristics of plants for the purpose of identification, applications for decontamination, toxic principles and target systems or organs for diagnosis and procedures of treatment for ideal manage the best management of this issue.

Some of the plants are toxic. Their use in homes, gardens or landscapes has become widespread as a result of developments in plant production methods. The cases of poisoning are increasing every year and the success of the diagnosis and management of the cases is not well known due to limited publications. One of the most significant parts of the management is the correct identification of

the plant. This has a vital importance because it will provide information such as which toxic principles the plants possesses, what kind of effects it has which systems or organs and whether there is any antidote or special treatment The toxicity characteristics of plants are used in the places where animals lives such as houses, parks, gardens and roadsides must be considered. These properties also must be specified in the label at the point of sale and use. In addition, due to regional differences which play a role in the toxicity of plants, poison control centers and professionals must have data on this issue, too.

## P80

### The effect of mild heat with/without lactic acid on bean sprouts contaminated with Shiga toxin producing *Escherichia coli* O157:H7, O103, O111, O145, O26 and comparison of variations between strains

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**Introduction:** Fresh produce-associated foodborne illnesses outbreaks have highlighted the need for more effective decontamination methods to ensure the safety of fresh produce.

**Material and methods:** In this study, *Escherichia coli* O157:H7 and non-O157 Shiga toxin producing *Escherichia coli* (STEC) O103, O111, O145, O26 strains used to contaminate bean sprouts and decontamination with/without lactic acid (v/v) 1.5%, 2%, 2.5% at 20, 40, 50 °C for 3 min was performed. Sterile distilled water was used for the control groups. Before the process, all food matrices were evaluated for non-O157 STEC and *E. coli* O157 contamination using ISO 16654:2001 with immunomagnetic separation.

**Results:** The results showed no significant decrease ( $p>0.05$ ) in the group where decontamination was conducted solely using sterile distilled water at 20, 40, 50 °C. On the contrary, significant decrease ( $p<0.05$ ) in pathogen numbers was observed in the groups with lactic acid addition. The most effective combination was 2.5 % lactic acid with 50 °C with a decrease of 4.00 log<sub>10</sub> cfu/g in pathogen numbers There was no significant difference between the numbers of each different *Escherichia coli* O157:H7 and non-O157 STEC O103, O111, O145, O26 strain ( $p>0.05$ ).

**Conclusion:** The results of this study indicates that mild heat combined with lactic acid is a promising decontamination technique for reducing the numbers of *E. coli* O157:H7 and non-O157 STECs.

**P81****Effect of decontamination with lactic acid solutions at different temperatures on cucumbers contaminated with *Escherichia coli* O157:H7 and non-O157 STEC**Abdullah Dikici<sup>1\*</sup>, Ahmet Koluman<sup>2</sup>, Mehmet Çalıcıoğlu<sup>3</sup><sup>1</sup>Department of Food Engineering, Faculty of Engineering, Tunceli University, Tunceli, Turkey<sup>2</sup>National Food Reference Laboratory, Ministry of Food, Agriculture and Livestock Ankara, Turkey<sup>3</sup>Food Hygiene and Technology Dept., Veterinary Faculty, Firat University, Elazığ, Turkey

**Introduction:** Recent outbreaks showed an increase in Shiga toxicogenic *Escherichia coli* contaminated fresh produces. This study aims to determine the bactericidal effect of different lactic acid concentrations combined with mild heat on pathogens of concern and to assess variations on serogroups.

**Material and methods:** In this study, *E. coli* O157:H7 and non-O157 Shiga toxin producing *E. coli* (STEC) O103, O111, O145, O26 strains used to contaminate cucumbers and decontamination with/without lactic acid (v/v) 1.5%, 2%, 2.5% at 20, 40, 50 °C for 3 min. was performed. Sterile distilled water was used for the control groups. Before the process, all food matrices were evaluated for non-O157 STEC and *E. coli* O157 contamination using ISO 16654:2001 with immunomagnetic separation.

**Results:** The results showed no significant decrease ( $p>0.05$ ) in the group where decontamination was held conducted solely using sterile distilled water at 20, 40, 50°C. On the contrary, significant decrease ( $p<0.05$ ) in pathogen numbers was observed in the groups with lactic acid addition. The effective combination was 2.5 % lactic acid with 50 °C with a decrease of 4.00  $\log_{10}$  cfu/g in pathogen numbers. There was no significant difference between the numbers of each different *E. coli* O157:H7 and non-O157 STEC O103, O111, O145, O26 strain ( $p>0.05$ ).

**Conclusion:** The results of this study suggest that mild heat combined with lactic acid is an applicable parameter which would provide additional value in public health to reduce the numbers of *E. coli* O157:H7 and non-O157 STECs.

**P82****Effects of liquid humate to supplemented drinking water on the performance and eggshell quality of hens in different laying periods**

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**Introduction:** The purpose of this study was to determine the effects of liquid humate (Farmagulator Liquid®)

supplemented to drinking water on the performance (feed intake, feed conversion ratio, egg weight, egg production) and the eggshell quality (shell strength and thickness, shell ash, cracked egg ratio) of hens in different laying periods.

**Material and methods:** A total of 420 Lohmann Brown 26 weeks old hens were divided into two groups (control and liquid humate) and fed with standard layer diets. Liquid humate (salts of humic acids) product (Farmagulator Liquid®) was supplemented to the drinking water of the treatment group at 0.25ml/l. The treatment group received liquid humate from the 26<sup>th</sup> to the 90<sup>th</sup> week of age. Data was collected at early (26<sup>th</sup> – 42<sup>nd</sup> weeks), middle (42<sup>nd</sup> – 74<sup>th</sup> weeks) and late (74<sup>th</sup> – 90<sup>th</sup> weeks) periods of laying.

**Results:** Egg production improved ( $P<0.05$ ) in the liquid humate group in the middle and late laying period, but did not improve in the early and total laying periods. Feed intake decreased ( $P<0.05$ ) in the early laying period while feed conversion ratio (FCR) improved ( $P<0.05$ ) in the middle laying period by supplementation of liquid humate. In the liquid humate group, the cracked egg ratio decreased ( $P<0.01$ ) in all laying periods. Also, shell ash increased in the middle ( $P<0.001$ ), late ( $P<0.01$ ) and total ( $P<0.001$ ) laying periods, while shell thickness increased only in the late laying period ( $P<0.05$ ) by liquid humate supplementation. Shell strength, mortality and egg weight was not affected by liquid humate supplementation.

**Conclusion:** The results of this study indicate that liquid humate salts in the drinking water of layers has improving capacity on the cracked (damaged) egg ratio in all laying periods and FCR in the middle laying period.

**P83****Fatty acid compositions in the abdominal fat of broilers**

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**Introduction:** This research was carried out to find out the fatty acid composition of oils added to the chicken rations as an energy source, and to examine the effects of these oils on broiler performances, and reflection of the fatty acid compositions in abdominal fat.

**Material and methods:** In this study a total of 230 Peterson x Avian commercial hybrid broiler chicks of 1-day-old were used. These animals were divided into 5 groups including 46 chicks in each. Diets of trial groups were prepared by adding 5 % of each of the following: sunflower oil (as control), cotton oil, corn oil, flaxseed oil and tallow by means of mixer. In the present study, the fatty acid composition of oils was determined first. Chickens were selected based on their body weights in a close range as much as possible. Feed and water were supplied *ad libitum* throughout the 49-days experimental period. After 14, 28, 42 and 49 days, all chicks were weighted with an electronic scale and feed intake values were determined by weighing the rest of feed at the same days and feed conversion rates were determined. Broilers

were sent to slaughter on day 49 of the trial. Abdominal fats and carcass of all animals were weighted separately. In this way, carcass and abdominal fat ratio of each broiler was determined. Crude nutrient in feeds were analysed using the Weende analysis method.

On the last day of the trial, abdominal fats from chickens chosen randomly from each group were collected. They were mixed to obtain a homogenous mixture. Their oil fractions were determined by using the ether extraction method. These oil samples were esterized and the concentration of the long-chained fatty acids in the abdominal fat were determined. Data obtained was subjected to analysis of variance using one way ANOVA procedures.

**Results:** As part of this research, results of the analysis used in this trial, as well as rations, daily feed intake, feed conversion ratio, live weights, fatty acid compositions of abdominal fat were obtained and compared with the literature data.

**Conclusion:** It can be said that compositions of fatty acids from animal products that are produced for human consumption could be changed depending on the nutritional conditions; to decrease the risk of heart-vessel disease, the composition of fatty acids added to poultry rations are of importance and using flaxseed and fish oil in poultry rations would have a subsequent effect human health in a positive manner by increasing omega-3 fatty acid quantities in animal products.

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#### P84

##### **Factors that affect microbial feed spoilage**

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Feed spoilage is a metabolic process that causes feed to be undesirable or unacceptable for animal consumption due to changes in the sensory and safety characteristics. Spoiled feed may be unsafe to eat, i.e. they may cause illness because there can be pathogens or a toxin present, as well as changes in texture, smell, taste, or appearance. Spoilage of feed also involves any change which renders feed unacceptable for animal consumption and may result from a variety of causes, which include: insect damage; physical injury due to freezing, drying, burning, pressure, radiation; activity of indigenous enzymes in plant and animal tissues; chemical changes not induced by microbial or naturally occurring enzymes.

The numerous sources of microbial spoilage come from undesired yet ubiquitous micro-organisms which can originate from the natural habitat, e.g. soil, water, air, spoiled raw materials, biofilms on the surface of equipment, personal hygiene of food workers. Considering the variety of feeds and the methods used for processing, it is apparent that practically all kinds of

microorganisms are potential contaminants and can cause changes in appearance, flavor, odor and other qualities of feed. These degradation processes include putrefaction, undesired fermentation and rancidity.

Feed unfit for consumption may not necessarily be spoiled and may contain a high number of food poisoning bacteria without presenting any 'negative' characteristics. Microbial deterioration of feed is evidenced by alteration in the appearance e.g. color changes, pockets of gas, soft texture, color, odor and flavor or slime formation.

Feed spoilage is wasteful and costly, it can adversely affect the economy and can erode consumer confidence. A good knowledge of the feed characteristics and its microbial ecology is therefore a priority for feed business operators. Common groups of foods spoilers are lactic acid bacteria, acetic acid bacteria, filamentous fungi and yeasts, Enterobacteriaceae, Pseudomonadaceae and spore forming bacteria.

In order to control or at least minimize contamination, a feed safety system is mandatorily focused on: good manufacturing practices, good hygienic practices, sanitary practices, rapid movement of food through the processing plant, well-tested preservation procedures, microbial specifications of raw materials and end products.

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#### P85

##### **A high incidence of adulteration and mislabelling in meat products**

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**Introduction:** As the world population rises, the demand for meat products continues to escalate in almost all regions of the globe, especially in developing countries.

Aside from price, other aspects for selection of meat products include quality and nutritional attributes. Consumers are increasingly aware of their health and are demanding more information on the origin, composition and safety of the foods they consume. Due to their high market value, meat products are often targets for species substitution and adulteration. Preventing adulteration of meat foods with less desirable or objectionable meat species is important for economic, religious and health reasons. In addition, determination of the species of origin of the meat components in meat products is an important task in food hygiene, food control, food codex and veterinary forensic medicine.

**Material and methods:** Analytical methods for detection of meat species adulteration are imperative for quality assurance and regulatory purposes. Identification of species origin in heat-processed meat products requires a different approach from methods used for raw meats, which detect heat labile serum proteins. DNA – based methods are recognized as the most appropriate means to detect such fraudulent practices, however, these have

not been extensively employed for the authentication of meat products available in Macedonia. Enzyme-linked immunosorbent assay (ELISA), which is highly sensitive, specific and practical, is the most effective and widely used method for detecting meat species in meat and meat products. In this study sandwich ELISA test kits (ELISA-TEK, Gainesville, FL, USA) were used to determine meat species in retail meat and meat products in Macedonia that do not comply with the standards of the Macedonian food legislation (Official Gazette of the Republic of Macedonia, No.52/2011). A total of 200 samples were analysed (45 chicken ham, 45 chicken and pork ham, 30 chicken pariser, 15 chicken frankfurters, 15 pork frankfurters, 10 sujuk, 30 chicken salami, 5 pork sausages, 5 beef sausages).

**Results:** The results emerging from the studies indicated that meat species substitution occurs more regularly in processed meat commodities, for instance in ground, comminuted, cured and value added products. One possible reason for this may lie in the fact that deliberate substitution with cheaper species is more difficult to detect in such products by visual observation than it is in fresh, intact meat. Processing techniques often lead to changes in the appearance, colour, texture and even flavour of meat products, meaning that the origins of constituents can be easily disguised in the meat mixture. The results showed that 46.7% of the chicken ham samples, 43.3% of the chicken pariser samples, 66.7% of the chicken frankfurter samples, 53.3% of the pork frankfurter samples, 20.0% of the sujuk samples, 6.7% of the chicken salami samples, 40.0% of the pork sausages and 60.0% of the beef sausages were found to contain undeclared species.

**Conclusion:** Based on the results of this study, the adulteration of high-quality meat and meat products with their cheaper counterparts is a problem of the meat industry in Macedonia. To protect consumers and to avoid unfair competition, governmental food-control institutions must continuously regulate meat and meat products using effective methods.

## P86

### Comparison of high oxygen (High-Ox) and low dose carbon monoxide (CO) packaging on the textural parameters of beef meat

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**Introduction:** The effect of high oxygen modified atmosphere packaging (High-Ox) against low dose carbon monoxide (CO) packaging was investigated on the texture

profile analysis parameters of beef meat during shelf life in retail display storage.

**Material and methods:** For this purpose, the sirloin meats prepared from the *M. longissimus dorsi thoracis* muscles of male animals slaughtered at 1,5-2 years of age have been separated into 2 groups; each group has been packaged with predetermined gas mixtures (High-Ox; 80:20:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> and CO; 0,4:30:69,60/CO:CO<sub>2</sub>:N<sub>2</sub>). The packaged meats are kept at 4°C for 14 days and evaluated for the texture (Texture Profile Analysis - TPA) values and sensorial (tenderness, chewiness and gumminess) parameters.

**Results:** As a result, the meat quality of beef meat packaged under low dose CO could be preserved during retail display storage like in the first days. The texture values of beef meat gave better results during the whole shelf-life compared to High-Ox packages. The sensorial perception of beef meat was also parallel to the instrumental evaluation.

**Conclusion:** It was concluded that low dose CO packaging can be considered as an alternative application to preserve the textural quality of red meat in retail display.

## P87

### Effect of packaging techniques on the colour and oxidative stability of beef meat during refrigerated storage

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**Introduction:** The effect of different packaging techniques containing O<sub>2</sub>/CO<sub>2</sub> gas mixtures at various concentrations on the colour and oxidative stability of raw beef meat was evaluated during refrigerated storage at 4°C.

**Material and methods:** For this purpose, the sirloin meats prepared from the *M. longissimus dorsi thoracis* muscles of male animals slaughtered at 1,5-2 years of age have been separated into 4 groups; each group has been packaged with predetermined gas mixtures (ambient air, vacuum, 80:20:0/O<sub>2</sub>:CO<sub>2</sub>:N<sub>2</sub> and 0,4:30:69,60/CO:CO<sub>2</sub>:N<sub>2</sub>). The packaged meats were kept at 4°C for 21 days and determined for the instrumental colour (CIE L\*, a\*, b\*), metmyoglobin and thiobarbituric acid values and sensorial (red colour and off-odour) parameters.

**Results:** As a result, the colour and oxidative stability of raw beef meat packaged under modified atmosphere with low dose CO performed better than in other packages. The redness of beef meat could be preserved like in the first day and the high concentration of CO<sub>2</sub> in the package prevented the increase in the lipid oxidation values of raw meat.

**Conclusion:** Even though, usage of O<sub>2</sub> as package gas in red meat allows the formation of the desired red colour, the high O<sub>2</sub> level within the package risks the shelf-life of the product by increasing the lipid oxidation level. To overcome this problem, usage of low dose CO as packaging gas can be considered as an alternative application; but the formation of the bright red colour during long-term storage may mask the deterioration signs.

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**P88****Antibiotic resistance of nonpathogenic *Listeria* spp. strains isolated from foods**

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**Introduction:** *Listeria* spp. are gram-positive, motile, non-sporulated bacteria that can be recovered from soil, water and vegetables. They have been isolated from various surfaces, food processing plants and contaminated foods. *Listeria* spp. have been reported as susceptible to antibiotics active against gram-positive bacteria, but many authors reported antibiotic resistance of strains isolated from foods. Many *Listeria* are developing resistance to most currently used antibiotics and there are frequent reports of bacteria which are resistant to almost all available antibiotics. Many antibiotic resistant bacteria in foods are saprophytic or commercial in habit, their resistance genes can be transferred to other food-

borne bacteria, including pathogenic species within the gastrointestinal tract.

**Material and methods:** The antibiotic resistance to antibiotics currently used in veterinary and human therapy of 24 nonpathogenic *Listeria* spp. strains isolated from different foodstuffs was determined by disk diffusion method CLSI M02-A10 (2009) and CLSI M31-A3(2008). The *Listeria* spp. isolates were tested for resistance to penicillin, amoxycylav, streptomycin, gentamicin, chloramphenicol, tetracycline, ciprofloxacin, erythromycin, sulfamethoxazole/trimethoprim, cefalothin, cefotaxime and vancomycin, produced by OXOID and BioMerieux.

**Results:** *Listeria* spp. 24 strains were identified as *Listeria innocua* - 12, *L.welshimeri* -7 and *L.seeligeri* - 5. One *Listeria innocua* strain was resistant to tetracycline isolated from duck liver and 2 isolates were resistant to penicillin, amoxycylav, chloramphenicol, tetracycline and vancomycin isolated from hot smoked trout. All tested strains of *Listeria welshimeri* and *Listeria seeligeri* were susceptible to all examined antibiotics. All tested *Listeria* spp. strains were susceptible to gentamicin, ciprofloxacin and cefotaxime. Data for microbial resistance of none-pathogenic *Listeria* spp. were discussed through the point of view of genes transfer to representatives from genus *Listeria* playing role in food safety

**Conclusion:** Low level of microbial resistance was detected against tested antimicrobials. Only 3 *L.innocua* strains showed resistance to tetracycline, penicillin, amoxycylav, chloramphenicol, tetracycline and vancomycin. They were isolated from food matrixes (fish and fattened duck) which could be treated with antibiotics in the period of growing and fattening.