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# **ABSTRACTS OF DAYS OF VETERINARY MEDICINE 2010**

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## EMERGING ISSUES IN FOOD-BORNE DISEASES

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Food-borne diseases include infections caused by bacteria, viruses and parasites. These pathogens cause millions of cases of sporadic illness and chronic complications as well as large and challenging outbreaks over many states and nations. Improved surveillance that combines rapid subtyping methods, cluster identification, and collaborative epidemiologic investigation can identify and halt large, dispersed outbreaks. Outbreak investigations and case-control studies of sporadic cases can identify sources of infection and guide the development of specific prevention strategies. Better understanding of how pathogens persist in animal reservoirs is also critical to successful long-term prevention. In the past, the central challenge of foodborne disease lay in preventing the contamination of human food with sewage or animal manure. In the future, prevention of foodborne disease will increasingly depend on controlling contamination of feed and water consumed by the animals themselves.

**Keywords:** food borne diseases, *Salmonellae* spp., *VTEC*, *Campylobacter* spp., *Listeria monocytogenes*, gastroenteric viruses

Veterinarians are in constant contacts with animals and their owners, which gives them the opportunity to influence the circumstances in which the animals are being kept. Depending on where they work and whether they get in direct contact with an animal, veterinarians either have a direct or indirect impact on animal welfare.

Expert associations, such as veterinary chambers, play an important role in the veterinary service. Providing documents, such as codes of veterinary ethics and codes of good practice is indispensable in dealing with questions on humanness of veterinary procedures. Thus, the role of veterinarians in securing animal welfare is of great importance. In the process of balancing the interest of the owner and the welfare of the animal, veterinarians are frequently faced with the decision making responsibility.

These decisions need to be science-based so as to assure that animals are treated in a way that does not harm them.

**Keywords:** animals, veterinarians, animal welfare

## CLASSICAL SWINE FEVER- YESTERDAY, TODAY AND TOMORROW- THE CROATIAN EXPERIENCE

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Classical swine fever (CSF) is a highly contagious viral disease of the Suide family. Even though attempts to control and eradicate CSF such as the use of vaccines in some countries or a strict stamping out policy as in the European Union are implemented, outbreaks of the disease keep occurring and causing problems almost worldwide.

The causative agent of CSF is a member of the genus Pestivirus within the family Flaviviridae. CSF virus is antigenically and genetically closely related to other members of the Pestivirus genus such as bovine viral diarrhea virus and border disease virus. The disease can develop as an acute, chronic or prenatal infection form, mostly depending on the virulence of the isolate, immune status of the animal, breed and age. Therefore, recognizing the disease is often more difficult than expected and requires thorough investigation and the use of modern laboratory techniques.

Until end of 2004, Croatia has been implementing a vaccination policy to control the disease. From then on, the legislative for the control of CSF has been harmonized with the EU legislative and based on two documents: the Directive considering measures for the

## VETERINARY SERVICE AND ANIMAL WELFARE: THE ROLE OF VETERINARY PRACTITIONERS IN PROTECTION OF ANIMALS

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A historical overview of development of human perception of animal suffering tells us that some eastern religions have fostered a particularly protective attitude towards animals. Jainism for example is regularly presented as an example of an animal-friendly religion and culture. Contrary to jainistic approach, the European philosophy needed to evolve from seeing animals as »automata« to "discussing animal rights". Today's understanding of animal welfare is based on the principle of five freedoms and is generally assessed applying three aspects: physical health, evaluation of affective states and ability of an animal to perform natural behaviors.

recognition, control and eradication of classical swine fever (OG 187/04) and the Diagnostic manual (OG 16/2005). Apart from the mentioned documents, to ensure preparedness of the country for a possible re-introduction of the virus, two National control programs have been developed and implemented jointly, by the Ministry of Agriculture, Fisheries and Rural Development of Croatia and the Croatian veterinary institute in Zagreb. One of the programs includes surveillance of domestic pigs, whereas the other is carried out for the surveillance of wild boars.

From 1995, lots of effort and education has been invested into prompt and accurate diagnosis of CSF in Croatia. The Croatian veterinary institute, Laboratory for CSF, molecular virology and genetics in Zagreb is the National Referral Laboratory for the diagnosis of CSF and carries out all the testing due to the National Programs. It has been accredited in 2007 and participates in ILCT from 1999. The laboratory provides all modern methods for CSF diagnosis as well as education and training programs. According to the laboratory results from 1997 until 2004, two major genotypes of CSF virus have been identified in domestic pigs and wild boars from Croatia (genotype 2.1. and 2.3.). Genotype 2.1. was isolated during 1997 when an outbreak of CSF has appeared throughout Europe, mostly in Germany and the Netherlands. The isolate is not common for Europe and it is hypothesised that it was introduced from Far East into Europe. Isolates 2.3. have been recognized in wild boars and domestic pigs and are common in Central European countries and West European wild boars. After banding vaccination in Croatia, an outbreak of CSF appeared in 2006 and 2007. The isolates were clusters of CSF virus type 2.3., but genetically differed from the ones that have been isolated in previous years. The isolates were genetically related to some "old isolates" from Western Europe and some recently identified isolates from Serbia and Bosnia and Herzegovina.

The last confirmed cases of CSF were in 2008. However, implementations of strict control measures and surveillance programs have resulted in the absence of the virus since March 2008, even though a limited number of antibody positive wild boars are still present in two districts in Croatia. The data collected show that most of the positive animals originate from animals older than two years of age.

Today, Croatia is CSF free in domestic pigs. However, surveillance programs and constant education of all involved in CSF control will be provided in the future as well. Therefore, even though CSF is a challenge for the veterinary service, it is also a reminder to be alert and prepared for all scenarios that contagious diseases may develop.

**Keywords:** CSF, pigs, Croatia, European Union

## MASTITIS IN EWES

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*Staphylococci* are the main etiological agents of intramammary infections in ewes. *Staphylococcus aureus* is the predominant organism isolated in ovine clinical mastitis, while coagulase-negative *staphylococci*, traditionally considered as non-pathogenic or of low pathogenicity for the mammary gland of domestic ruminants, are the most prevalent isolates in subclinical intramammary infections. The annual incidence of clinical mastitis in ewes is usually lower than 5%, however severe mastitis outbreaks caused by *Staphylococcus aureus* are not uncommon. Antibiotic therapy regimes generally formulated for the bovine mammary gland are often unsuccessful in eliminating existing *Staphylococcus aureus* udder infections. At the time of weaning and start of milking (6-8 weeks after parturition) in a flock of 180 dairy ewes, 29 cases of peracute gangrenous clinical mastitis appeared, during a 10 days period. In 26 cases (90%), *Staphylococcus aureus* was determinate as the causative agent. In the described case, diseased animals were treated intramammary and parenteral with a cephalosporin preparation designed for cows, immediately after detection of inflammatory changes in the mammary gland. Despite of immediate treatment, which was based on results of laboratory findings, 9 of the animals (31%) died, 14 (48%) lost the affected udder half and only in 6 cases (21%) functional recovery was achieved.

**Keywords:** clinical mastitis, ewes, *Staphylococcus aureus*, treatment

## TACKLING THE PROBLEM OF NON-AMBULATORY COWS

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In view of providing safe cattle production for consumers, needs one of the greatest concerns of veterinary service is to ensure the well-being, treatment care and transport of the animals. Extra attention on the part of the practitioners should therefore be focused on the cows that are down for any reason. It is reported that the majority of inhumane treatment (handling) is being exerted on the animals that are unable to stand up by themselves. A clinician should give a prompt diagnosis and prognosis. The term "downer cow syndrome" has been changed to "non-ambulatory cow". The term is applied to cows that are down (regardless of the time parameter) and are unable to stand up or move without being dragged or carried. According to the literature, the annual incidence of non-ambulatory cows is between 0.4% - 2.1%. However, in Slovenia there is no exact data available. In the present study the data sent by 6 vet practitioners from different parts of Slovenia were analyzed. It has been assessed that in 2009 in herds with 10,573 cows, 721 (6.82 %) were non-ambulatory. The data suggested that large proportion of non-ambulatory cows after therapy of metabolic derangements, infectious or toxic diseases and injuries recovered (85.4%) and died or euthanized (14.6%). Typical clinical and atypical hypocalcaemia (86.6%), toxic metritis and mastitis (8.8%), and injuries or dystocia (4.6%) were main cause factors for cow recumbency. Non-ambulatory cattle should be treated as medical emergencies. Especially cows that are non-ambulatory for 6 hours or more are considered to have a poor prognosis. High-quality nursing care should be provided to non-ambulatory cows. Downer cows should be maintained in sternal position and repositioned every few hours, altering between left- and right-sided sternal recumbency. Lifting devices (well-padded hip camps, slings) and hobbles should be readily available to assist with non-ambulatory cattle. Personnel should be trained and competent in the use of lifting devices or hobbles. Annual incidence of non-ambulatory cows is important as a lost source of income and of the good information to make prevention strategy for production diseases, injured, and possible for appearance for bovine spongiform encephalopathy (BSE).

**Keywords:** cattle production, non-ambulatory cow, Slovenia, production diseases, BSE

## COCCIDIAN INFECTIONS IN HOUSED LAMBS IN KOSOVO

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In this study are investigated 10 lamb farms in Kosovo with 22 up to 100 lambs per farm, to investigate coccidian infection spread on lambs with age 3 to 15 weeks. Also we monitored zoo hygienic conditions, temperature and humidity of floor in the farms, and floor was treated with the disinfection material (chlor chresol 2%) to study if we will reduce or eliminate cause of coccidiosis in the farm. After collecting samples of feces from floor and directly from rectum, they were analyzed with floating method. These analyses were repeated every 2 weeks, parallel with measuring temperature and humidity in the farm and in the floor. In every farm we found coccidian infection in lambs with *Eimeria* spp.. The highest intensity of infection it was in the end of month February and in the beginning of month April, respectively in the period when lamb were in ages between 4 and 9 weeks. During this infection period temperature in the farm were between 20 to 30°C, humidity in the floor was between 80 up to 90%. In all farms where a massive elimination of *Eimeria* spp., oocysts, in lambs present were clinical signs and death, respectively in the farm 1 6 deaths (6/100), farm 2 with 3 deaths (3/40), farm 3 with 4 deaths (4/70), farm 4 with 1 death (1/22), farm 5 with 3 deaths (3/45), farm 6 with 4 deaths (4/51), farm 7 with 5 deaths (5/55), farm 8 with 6 deaths (6/90), farm 9 with 2 deaths (2/90) farm 10 with 8 deaths (8/60). After treating the floor with disinfection material (chlor chresol 2%) after some hours we had reduction and elimination of *Eimeria* spp., oocysts, with this the infection strength decreased rapidly on young lambs.

**Keywords:** Coccidiosis, lamb, oocistes, temperature, humidity

## CRIPTOSPORIDIOSIS OF SMALL RUMINANTS

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Cryptosporidiosis is a zoonotic parasitic illness that causes diarrhea caused by *Cryptosporidium* spp. Cryptosporidium parasites can be found anywhere in the environment that is contaminated by animal droppings or human waste. Cryptosporidium oocysts can contaminate soil and the food grown in it. Sources of disease include parasite-containing stool, food, and water. Cryptosporidiosis are important parasitic disease of small ruminant – sheep and goats, with clinical signs at animals 4-10 days old. Presences of great number



of cryptosporidial oocyst are usually at adult animals without clinical signs of disease. At sheep and goats were established *Cryptosporidium parvum*. At young animals cryptosporidiosis had high morbidity and mortality rate. Symptoms of acute cryptosporidiosis include lack of appetite, and weight loss. Clinical signs are yellow diarrhea with odor smell, and some time are present a blood. Animals had abdominal pain, anemia, lost of appetite, dehydration, tenisms, weakens and lost of weight. Some animals do not develop into chronic cases and become carriers. After infection, animals resist the organism, develop a mild infection that is self limiting, or soon sicken and die. Some animals may exhibit fevers or signs of respiratory distress, but these may be secondary conditions from opportunistic microorganisms that have infected the animal in its weakened condition. Younger animals are much more susceptible to infection than adults. In studies done with lambs, five-day-old lambs had diarrhea for 9-10 days and suffered from a high rate of mortality. Sixty-day-old lambs showed no symptoms when they were infected and adult sheep completely resisted infection. There is an indication that adults develop immunity to *Cryptosporidium*, yet this immunity does not seem to be passed to their offspring. Pathological changes are present in small intestine. In acute cases were thickens gut wall, edema, hyperemia at cecal and colon mucus. There are present numerous hemorrhage, and present of mucofibrinal seam dark brown colored. Those pathological changes drawl resorption and induced clinical signs of disease. Consequence are significant increase of kid accrescence, its weakens and less develop. A significant number of drugs have been tested and found ineffective for treating cryptosporidiosis, including most drugs normally used to treat coccidian. So far, no treatment has been found. The best control of cryptosporidiosis in goats comes from lambs and kids getting adequate immunity through colostrum soon after birth.

**Keywords:** cryptosporidiosis, goat, kid, lamb, sheep  
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## NATIONAL SURVEILLANCE AND ERADICATION PROGRAMS FOR VIRUS DISEASES IN BULGARIA (AFTER THE ACCESSION TO EU)

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Animal disease outbreaks can have devastating consequences to the national livestock population and

agriculture. They can result in heavy economic costs (the 2001 Foot-and-Mouth crisis cost over 13 billion euros in the UK alone) and can lead to problems in animal welfare and the environment to destroy public confidence in farming and animal products, and food safety more generally (e.g. consumer reaction to poultry products in certain Member States during the 2006 avian flu outbreaks).

In the EU, a great deal of progress has been made in recent years in the field of animal health. CVO Bulgaria established harmonised surveillance control and eradication program measures to prevent disease outbreaks and to deal with them effectively when they do occur. For that purpose now Bulgaria has 7 national surveillance and eradication programs for virus diseases in place. Most of them are without vaccination strategy for control and we use vaccination only where it can bring about real improvements, such as in the fight against rabies. So we adopted a key principle of the new EU animal health strategy - "prevention is better than cure". Now we can already seen some examples, such as avian flu, FMD, bluetongue of how a pre-emptive or preventative approach to animal health threats can really work. Biosecurity measures at farm level, at borders and in the movement of animals can play a key role with disease surveillance and emergency preparedness and are the great achievements of these programs. The objective is to invest more in preventive measures and our controls system, thereby reducing disease outbreaks.

An important task of each Member State country for better success and profit with disease surveillance and control programs will be to evaluate it priorities on the basement of geographic, socio-economic and epidemiologic level. Then to develop a modern laboratory network system working under the ISO-17025 standard and keeping an high level of diagnostic capacity and expert knowledge for certain diseases.

**Keywords:** EU, member state, avian flu, FMD, bluetongue, laboratory network system

## PUBLIC HEALTH AND FOOD SAFETY IN VETERINARY MEDICINE EDUCATION

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Globalized world, developing production and consumption conditions of food industry made to revitalize the branches of Veterinary Medicine, especially food science, compulsory. Veterinary Medicine Food Technology and Public Health is accepted as one of the most well rounded program due to its cause and

result effects. Socially and economically developed countries re-interpreted boundaries of food sciences and related branches, and adopted themselves according to increasing technological opportunities as a requirement of increased production and demand. Veterinarian doctors should re-introduce the meanings of to be food science veterinarian and public health issues as a requirement of national and scientific ethic. Food Science Veterinary Education is too broad to be limited as serving only production and technology of food animals, and its present practice area, education should be increased to the level of world standards, the level it deserves, for the sake of animal and public health.

**Keywords:** public health, food safety, animal health, food industry

## STRENGTHENING FOOD SAFETY IN ALBANIA

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Food safety and healthy nutrition are both essential to the health of the people and are therefore important public health areas that the health systems has to deal with. Today, Albanian consumers have improved access to information on food hazard and are making increasing demand to minimize risks.

Albania during these last years have invested significant efforts in the pursuit of wide-ranging reforms of its health sectors, addressing issues of financing, organization and management of health services.

**Keywords:** food safety, public health, food hazard, health services

## CHARACTERIZATION OF LISTERIA MONOCYTOGENES ISOLATES ANTIMICROBIAL RESISTANCE FROM SMALL RUMINANTS AND POULTRY SLAUGHTERHOUSE ENVIRONMENT AND CARCASSES

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Antimicrobial resistance profile was tested in 64 isolates of *Listeria monocytogenes* from small ruminants and poultry slaughterhouses in Republic of Macedonia. Isolates came from premises environment swabs (such as tools, equipment and containers), carcasses of slaughtered animals, lambs small intestine content and poultry caecal content (31 from small ruminants and 35 from poultry). Antimicrobial profile was performed based on standard diffusion agar test using antimicrobial discs with 13 antimicrobial substances. Investigation of phenotypic similarity between isolates was examined using the automated VITEK 2 Compact system bioMérieux with AST cards having 19 antimicrobials. Efficient treatment of listeriosis is based on antimicrobial resistance profile. Phenotypic similarity among isolates can locate sources of contamination in the food chain, and thus to successfully implement measures for their elimination. All *Listeria monocytogenes* isolates had resistance toward lincomycin, neomycin and nalidixic acid using diffusion agar test and benzylpenicilin, ampicilin/sublactam, Imipenem and fosfomycine using cards for automated VITEK 2 bioMérieux. Research does not establish a significant difference in resistance between isolates obtained from different type of slaughterhouses. Also isolates from intestinal content showed similar resistance and they are not species dependant (poultry and lambs). Most of the isolates from intestine and surfaces had identical antimicrobial susceptibility, providing evidence for fecal origin of contamination of carcasses and equipment in the slaughterhouse.

**Keywords:** *Listeria monocytogenes*, antimicrobial profile, phenotypic similarity

## PREVALENCE AND MAIN ASPECTS OF DIAGNOSIS, TREATMENT AND PREVENTION OF CANINE VISCERAL LEISHMANIASIS IN R. MACEDONIA

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Canine leishmaniasis is an important zoonotic disease that is endemic in more than 70 countries, as well as in the Mediterranean region. It presents a veterinary and public health problem, because usually it is fatal if not treated in people and dogs. Little was known for the presence of this disease in R. Macedonia. The first published data of its presence dated from 2004 and still it is neglected disease, because of the lack of experience

in recognition of the variety of clinical signs, haematological and biochemical profiles, interest in diagnostic tools and the very low level of public awareness. Estimated AP (apparent prevalence) of 32, 3% and TP (true prevalence of 28, 2%) of canine visceral leishmaniasis among all kind of dogs in R. Macedonia is undeniable proof for determination of our country as an endemic region. Having most of the symptomatic, as well as oligosymptomatic and asymptomatic dogs undetected and untreated, not using repellent impregnated collars on dogs in the period of risk (March –October), having Alopurinol as the only available drug, present a strong evidence that the role of veterinarians in diagnosis, treatment and prevention of canine visceral leishmaniasis must be improved.

**Keywords:** *Leishmaniasis, Dog, Prevalence, Diagnosis, Treatment, Prevention, R. Macedonia*

## ANATOMY OF VETERINARY BUSINESS

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Anatomy is scientific discipline which studies the structure and the construction of the living beings. But not only living beings, have a natural destined of their construction and structure. The orders of things in society are reflecting in the natural order, very often. Possession of construction and structure is one of the fundamental requirements for successful functioning of the social segments starting from the smallest, to the greatest segments. The business is no exception from this rule, but in opposite, it should have its own construction and structure. If living creature has not spine or brain, for example, it would not be possible its existence. The business should also have its own “spine” and its “brain”. The maintaining of a private veterinary practice represents a business like any other. If we exclude the specifics of the profession, we will receive basic, essential structure, like structure of any other business. It is therefore chosen this title - Anatomy of the business, to the veterinary practitioners to bring the need for basic knowledge in business. Just as anatomy has its constituents with specific detail and studied organic systems that make up this structure, such as bones and muscles or locomotion apparatus, brain and nervous system as a sensuous instrument, internal organs such as the system that regulates the entry and output of goods and their movement through the body, heart and blood vessels as a system that supplies the body with necessary nutrients. If some cell of the body is excluded from this system it will die.

**Keywords:** *veterinary practice, business, clients, anatomy*

## SEASONAL VARIATIONS OF SERUM BIOCHEMICAL PARAMETERS IN DAIRY COWS

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Milk production in high productive dairy cows importantly depends on normal functioning of many metabolic processes, on which the feeding regime and zoohygienic conditions have the main impact. This is particularly related with s.c. “transition period” (3 weeks prior to 3 weeks after calving). The aim of this study was to find eventual influence of a season (summer and autumn, respectively) on the serum concentrations of some biochemical parameters, which are indicators of the energetic (glucose, total proteins) and the mineral status (calcium, phosphorus, sodium, potassium). The samples of the first group were taken in June (summer group), and from the second group in October (autumn group). Among the parameters which were investigated, glucose, total proteins and phosphorus have higher values in autumn group, and the other parameters have higher values in summer group, with exception of potassium, which had values almost identical in both groups. No significant differences of means were found for any of these parameters between two groups. The cows from the summer group had significant high positive relation between glucose and phosphorus ( $r=0,77$ ), and mid negative relation between glucose and potassium ( $r=-0,43$ ). The same situation was also in the autumn group ( $r = 0,76$  between glucose and phosphorus, and  $r = -0,47$  between glucose and potassium, respectively).

**Keywords:** *dairy cows, metabolic profile, transition period, lactation*

## MYCOPLASMA DISEASES IN SHEEP AND GOATS AND EPIDEMIOLOGY OF CONTAGIOUS AGALACTIA IN REPUBLIC OF MACEDONIA

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Numerous Mycoplasma organisms are isolated from sheep and goats but very few are directly connected to certain disease. The two most important are *Mycoplasma agalactiae* as main cause of contagious agalactia (CA) and *M. capricolum subsp. capripneumoniae* as causative agent of contagious caprine pleuropneu-



monia (CCPP). Several countries close to Macedonia, including Croatia, Bulgaria, Greece and Albania, are continuously reporting clinical CA. This fact, as well as some indicative clinical cases initiated the investigation, particularly having in mind the fact that CA in Macedonia has been absent for more than 20 years. Two investigations were carried out. The first was screening investigation carried out on 381 serum samples and 171 milk samples, randomly taken from 19 sheep and 3 goat flocks from different parts of Macedonia, covering the most of its territory. The second was targeted investigation, investigating sheep flock with clinical CA. Twelve serum and 20 milk samples were collected for the laboratory confirmation. Besides the finding of typical clinical signs for CA in the flock, the 12 serum samples were all highly positive on indirect ELISA. The 20 milk samples, cultured on Eaton media gave 10 isolates, confirmed by GIT and PCR as *M. agalactiae*. The epidemiological investigation showed that several clinical cases were present previous few years where CA like symptoms were noticed for the first time in goats from 3-4 different holdings. They were treated twice with *Geomycin R* evidently improving the clinical picture. Next year the disease occurred again and also spreaded in surrounding flocks. One infected flock was treated with recommended doses of enrofloxacin with significant improvement of overall clinical picture and only individual relapses of the disease. Different strategies can be applied for the control of contagious agalactia, such as *stamping out* at the beginning of the appearance of the disease, through antimicrobial treatments with large doses of antibiotics and vaccination with inactivated vaccines with various successes. General sanitary measures in the flock such as milking hygiene can help significantly in the control of the disease.

**Keywords:** *Mycoplasma*, contagious agalactia, epidemiology, ELISA, PCR

## THE EFFECT OF TESTOSTERONE ON THE HISTOMORPHOLOGY OF THE ADRENAL CORTEX IN TESTECTOMIZED RATS ACCLIMATED ON DIFFERENT AMBIENT TEMPERATURES

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In this study the effect of testosterone on the histomorphology of the adrenal cortex in bilaterally testectomized, rats acclimated to different ambient temperatures was examined. The animals were acclimated on room (20±2°C) and hyperthermic temperature (35±1°C).

The acclimation to the hyperthermic temperature was performed in special heated chamber, with controlled temperature and relative humidity of 30-40%, where animals were constantly kept for 30 days. After acclimation, the rats from both ambient temperatures were bilaterally testectomized and the analyses were made 14 days after the operation. Testosterone application was performed during the last 4 days of the experiment, when the hormone was given i.m. in doses of 1mg/100g body weight/day. The analyses were made 24 hours after the last application. The obtained results have shown that testosterone provokes cytomorphological changes in the adrenal cortex, regardless to the previous thermal acclimation. The results showed increased lipid amount into the spongiosocytes; destruction in the cytoarchitecture of the cortex; appearance of groups of cells with hyperchromated nuclei as well as cells without clearly distinct, visible borders in the two inner zones; increased occurrence of "dark" cells in zona fasciculata and discontinuity of the zona glomerulosa. These changes were more pronounced in heat acclimated rats, where a large hyperemia was evidenced as well as significant increase of the nuclear volume and area in all corticocytes. A significant increase in the relative weight of the adrenal gland of heat acclimated rats was also registered. From the obtained results we can conclude that testosterone had a similar effect on the cytoarchitecture of the adrenal cortex in animals from different ambient temperatures, which was more pronounced in heat acclimated rats.

**Keywords:** testectomy, testosterone application, adrenal cortex, morphometry temperature, rat

## APPLICATION OF COMPUTER SOFTWARE IN REPRODUCTION MANAGEMENT OF DAIRY HERDS (ALTAMATE, VALUE BULDER)

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The widespread application of AI and realization of its full potential depends largely on the use of frozen semen obtained from genetically superior sires. Beside the implementation of quality control standards in the production of the genetic material, it is imperative implementation of controlling the obtained results af-

ter its utilization on field. For that purpose, the market leader for deep frozen semen production and its global distribution Alta Genetics, designed specific software in order to obtain adequate selection of the sires, which genetics will be used in particular dairy herds, based on its production, conformity and health traits, on the level of the herd, as well as the same traits on an individual level.

**Keywords:** AI, deep frozen semen, Alta Genetics

## USING OF e-MARKETING IN VETERINARY PRACTICE PROMOTION

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We are living in the world of information. Digital eras in which we are living are changing way of interaction among people, and interaction between men with the wider publicity. Nowadays, even most distance village has internet access. Internet become part of almost every home and this trend is visible in Macedonia, also. The researches show that e-mail became most used way of communication. Internet connection offers many possibilities to the user – from visiting web pages, e-mail communication, interactive profiles to the social networks like Facebook, My Space, Hi 5, etc., till the buying products and services only with one mice clicking. From the other side, Internet provides business possibilities for cheap and simple presentation of products and services to the potential clients. It is very rare to find companies which has not their own web page and which didn't use internet for self promotion and selling. Veterinary companies like hospital, practices etc are not exception. They are coming in the tertiary business sector which is offering services, and in this moment it is most increasing economical sector in the world. The services we can define like acting, efforts or performance, which are economical activities which are producing profit and values for the customers. This is the reason why veterinary practice should have own web page in which it will promote its corporative image.

**Keywords:** internet, business, clients, veterinary practice, marketing

## DEGENERATIVE MYELOPATHY IN DOGS

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One of the chronic progressive disorders of the spinal cord in dogs is the degenerative myelopathy (DM). The most predisposed age in dog is 5 to 14 years, while rarely noted in younger, there is no gender predisposition. This disorder most commonly appears in dogs of the German shepherd breed, but it can appear in other breeds too. The main changes about this disease are degeneration of the myelin, especially in the thoracolumbar segments of the spinal cord and the dorsal nerve roots. The progression of the disease is slow and can last months to years. Undoubtedly, diagnosis is made by examinations of the CSF and establishing elevated level of protein segments, especially concentration of myelin basic protein (MBP) for evaluate of demyelinating lesion.

**Keywords:** dog, degenerative myelopathy, CSF, MBP.

## THE IMPACT OF THE CURRENT EMOTIONAL STATE OF STUDENTS ON ACHIEVEMENT TEST TASKS FROM CLINICAL SUBJECT

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The survey was conducted on a group of 24 students from third year of the Faculty of Veterinary Medicine, Skopje, while taking the exam on the subject "Clinical and laboratory diagnosis". Tests were used to determine the current emotional status by providing answers to key question "How do you feel right now". A questionnaire of 47 subjects with the possibility of response from 1 to 5 was given. Answer 1 shows the weakest and 5 the strongest intensity, of the presence of positive or negative emotions. Students were divided into two groups of 12 students. Unlike the second group, the first group before the exam and the notice of completing the questionnaire is induce positive (encouraging word and small gift). The questionnaire was completed before and after by both groups. It is established that more positive emotions are present, happiness and relaxation, more reduced presence of the negative emotions of fear, anxiety, unhappiness and anger. Both groups showed a sense of relaxation and pleasure before the start of the exam, which means the situation of the famous "academic" stress appear posi-

tive emotions, which is a very good sign for those who prepare and take the exam. It is common practice in the teaching process; students are asked how they feel at a given moment. The changes in this policy should go in the direction from the possible achievement of a positive mood among the students, to improve the future relationship veterinary doctor - animal. A positive approach to student clinical subject and the absence of anxiety, should result in a positive approach to clinical practice and working with animals.

**Keywords:** *positive and negative emotions, lack of anxiety, positive educational environment, clinical subject.*

## EFFECTS OF AGE AND BREED ON TRACE ELEMENTS (Fe, Zn, Cu, Pb, Cd) CONCENTRATIONS IN OFFAL AND MUSCLES OF CATTLE

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In this study, the effects of age and breed on the trace elements of different offal and muscles of cattle were investigated. The used samples in this study were taken from offal (liver, kidney, heart) and muscles (*M. longissimus dorsi*, *M. longissimus thoracis*, *M. psoas major*, *M. intercostalis internus*, *M. semispinalis capitis*, *M. biceps femoris*, *M. biceps brachii*) of 7 East Anatolian Red (EAR) and 5 Montofon breed cattles from Erzurum slaughterhouse. Concentrations of iron, zinc, copper, lead and cadmium of taken samples were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The results obtained from this study showed that there aren't significant differences ( $P > 0.05$ ) in the content of trace elements in the samples with the age and breed. The order of trace elements in the offal samples of cattles and their concentration ranges in 1 mg/kg (ppm) was Cu (56.88)>Fe (54.64)>Zn (34.29)>Pb (0.17)>Cd (0.08). On the other hand, the order of trace elements in the muscle samples of cattles and their concentration ranges in 1 mg/kg (ppm) was Zn (53.25)>Cu (49.95)>Fe (26.97)>Pb (0.18)>Cd (0.03). The highest trace element concentrations were obtained from offal cattles in the 4-year-old and in the EAR breed. According to findings, trace element concentrations (especially copper) in all samples were higher than the prospective values.

**Keywords:** *Trace element, Offal, ICP-AES, Cattle*

## SOME BIOCHEMICAL PARAMETERS IN SERUM OF DIARY COWS IN TWO FARMS

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The maintenance of energetic and mineral balance is essential for optimal milk production in high productive dairy breed cows. This is most critical during the so-called "transition period" (3 weeks prior to 3 weeks after the calving). Metabolic processes related with milk production are maintained by many regulation mechanisms, which are highly depend on feed intake. The level of feed intake is genetically conditioned, and there are variations between different breeds, and also individual variations. Because of this, we made investigation of some biochemical parameters in serum of dairy cows in two farms with same conditions in management and feeding. The investigated parameters were glucose and total proteins (indicators of energetic status), and also calcium, phosphorus, sodium, potassium, magnesium and chlorides (indicators of mineral status), respectively. The values of these parameters on the two farms (mean, with standard deviation and standard error of mean) are given in the text. The cows in farm II had significantly higher values of calcium ( $p < 0.05$ ) and phosphorus ( $p < 0.001$ ), and significantly lower values of chlorides ( $p < 0.01$ ), then cows in farm I. Also, the cows in farm I had significant mid positive relation between total proteins and sodium ( $r = 0.49$ ), and significant high positive relation between calcium and phosphorus ( $r = 0.65$ ). The cows in farm II had significant high negative relation between potassium and chlorides ( $r = -0.77$ ).

**Keywords:** *dairy cows, metabolic profile, transition period, lactation*

## ERYTHROCYTE SEDIMENTATION OF DOGS AND CATS CLINICAL APPLICATION IN SMALL PRACTICE

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Erythrocyte sedimentation (ES) is simple and real laboratory test. ES presents sedimentation velocity of

red cells in standard time and temperature under the Earth gravity. Whole blood can be taken from vein or capillary and mixed with anticoagulant. The ratio of whole blood and anticoagulant should be 4:1. Erythrocytes membrane is negatively charged, which maintains the stability of erythrocytes in plasma. During the inflammatory process as a response of the immune system, acute phase proteins are produced (fibrinogen, immunoglobulins, glycoproteins). That is the reason for rapid accumulation and formation of Rouleaux aggregates. The most of the animals have relatively slow ES (except horses), but in different pathological conditions it could be changed. Different methods can be used for laboratory testing, depending of blood taken amount. They can be divided into micro and macro methods. Macro methods for ES are: ES Westergreen, ES Traubner, ES Seditainer. Although the sedimentation is insensitive and nonspecific method, it is still held in a routine application. Nonspecific is because the increased value does not indicate the type of pathological process, because it can be common in many diseases. From the other side, this method is insensitive. Many other diseases, which could cause animal's death, could be accompanied by normal values of SE. Sedimentation rate is not pathognomonic for specific disease, but it could be used for accessing the health status of the animal. Errors of erythrocyte sedimentation rate may occur with irregular manipulation of the sample. ES rate is a laboratory method which could give us in a short time, useful diagnostic information, to indicate the possible pathological process. Performing this method does not require expensive equipment, so, it is recommended to become a routine analysis. Supported by other laboratory tests this method could help us in making a precise diagnosis, and thus success in the treatment of small animals in practice.

**Keywords:** sedimentation rate, inflammation, macrohematocrit method, microhematocrit method, routine method

## CLINICAL AND LABORATORY DIAGNOSIS OF SERTOLI CELL TUMOR IN DOG - CLINICAL CASE

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Sertoli cells are positioned in palisade layers, surrounding the functional testicular tissue. They provide nutritional ingredients for spermatogenesis and secrete certain hormones - estrogen, inhibin and Müllerian in-

hibiting hormone. Sertoli cell tumors (SCT) often occur unilaterally in cryptorchid dogs. Right unilateral SCT is more common than left, with concomitant atrophy of the contralateral testis. A 9 year old, mixed breed dog was admitted to the clinic, with scrotal swelling. Palpation of the swelling revealed a hard palpable mass, surrounded by fluid. Bilateral symmetrical alopecia in the abdomen area, swelling in the posterior mammary complex and gynecomastia were noticed. The left testis was palpable, but atrophic. Fluid from the scrotal swelling was taken and fine needle aspiration was performed on testicular parenchyma as well as regional lymph nodes, for further cytology examination. Cytology smear from the FNA have shown neoplastic criteria in the testicular samples while negative metastatic finding in the lymph node samples. A lot of neoplastic cells were evident on the cytology smear from testicular parenchyma. The nucleus had metachromatic characteristic and cytoplasm show increase metabolic activity. According the cytology finding, it was a SCT. The fluid aspirate examination has shown low cellular content and protein concentration of 20 g/l. According hematological parameters, moderate non-regenerative anemia was determined, as well as thrombocytopenia and mild leukopenia. Orchiectomy was indicated as radical surgical treatment. Two months later, clinical control and blood analysis, have shown correction of hematological status and normalization of parameters within the reference values. The SCT was diagnosed upon characteristic clinical appearance of a hormonal active tumor. This endocrinopathy have caused paraneoplastic syndrome. The myelosuppressive effect of the estrogen hormones on bone marrow is evident from the blood count changes such as generalized pancytopenia or nonregenerative anemia, as well as leukopenia and thrombocytopenia. Postoperative blood analysis is useful to monitor possible metastatic activity of the tumor.

**Keywords:** testicular parenchyma, Sertoli cell tumor, hormonal activity, cytological analysis, hematological analysis

## CHARACTERISTICS OF WHITE SHEEP CHEESE FROM MALESH REGION IN EASTERN MACEDONIA (MALESH'S CHEESE)

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Malesh's cheese "Maleshevsko sirenje" is prepared from the natural unpasteurized milk of sheep in the Malesh Region in Eastern Macedonia. Similarly, white cheese from unpasteurized milk is produced in South and Eastern Europe countries including Bulgaria, Serbia, Croatia, Greece, Albania, and Turkey. White cheese produced in different area has own different specific characteristics due to the geographic origin, specific flora and water (environment) and unique traditional processing technology. In Malesh region there are around 40,000 sheep mainly domestic breed "Ovchepolka" and "Sharplaninka" and some are cross breeds with virtenberg and awassi. Sheep in Malesh region during entire lactating period graze freely on the pasture and drink water from mountain creeks. Malesh's cheese is divided in three categories based on the fat content, ranging from 45 % to 55%. In dry matter (50%-55%) content of protein is 20-25% and minerals are 3-5%. Ingredients of the cheese are in the natural form and there are not free radicals because the milk is not heat treated. For more then 40 years the region is free of brucellosis. The content of radionuclide Cs-137 in Malesh's cheese is 0, 6 Bq/kg (max allowed is 370 Bq/kg). Malesh's cheese has mostly all dominant good characteristics. In the EUREKA 2001 in Bruxells, Malesh's cheese was recognized with "Golden medal for quality". This quality of Malesh's cheese need to be maintained, unified and certificate like "brand". Traditional technology of Malesh's cheese producing and environmental characteristics of the region will be described.

**Keywords:** Malesh's cheese, unpasteurized milk, traditional, technology, brucellosis, quality, Cs-137 Eureka, golden, medal.

## HAEMATO-BIOCHEMICAL VARIATIONS IN BROILER CHICKENS FED AFLATOXIN CONTAMINATED FEED

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This study was undertaken to examine the toxic effects of different levels of aflatoxin B1 in broilers. For that purpose, 220 15-day-old Cobb 500 broiler chickens

were fed 2.5 and 4 mg/kg of aflatoxin in their diet for 10 and 20 days and 100 chickens were maintained as control group (0 mg/kg). After the intoxication period, a clearance period was established of 1, 2, 4 and 8 days and blood was taken for analyses. No morbidity or mortality due to the aflatoxicosis were observed during the trial period. Hematological data showed significant reduction in haemoglobin, haematocrit, packed cell volume, total erythrocyte count, trombocyte and lymphocyte, whereas significant increase was observed in heterophil counts. Serum biochemical analyses revealed a significant decrease in serum total protein, albumin, inorganic phosphorus, calcium and creatinine levels, contrary to increased serum glucose level and significantly higher serum ALT, AST and GGT. No changes in the relative weights of liver and kidneys were observed in both experimental groups, while body weight gain and feed consumption were decreased. In conclusion, it can be stated that biochemical alteration produced by aflatoxin administration, together with haematological changes observed, may aid in disease diagnoses.

**Keywords:** broilers, aflatoxin, serum parameters, haematology

## BONVITAL® (Enterococcus faecium DSM 7134) EFFECTS UPON BROILER PERFORMANCE

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A feeding trial was conducted to investigate the effects of dietary supplementation of a single-strain probiotic (Enterococcus faecium DSM 7134) on broiler performance. During the trial period of 36 days, 4000 Cobb 500 broiler chicks were randomly divided into three experimental groups equally A, B and C, receiving commercial probiotic Bonvital (Schaumann, Austria) starting from day 1, 20 and 27 after hatch. One group of 1000 chicks was considered as a control, fed standard diets without probiotic administration. Body weight gain, average weight gain, feed consumption and feed conversion rate were measured on a daily basis. Results were discussed compared to control and between experimental groups. Daily body weight gain, average daily weight gain and feed conversion rate were significantly ( $p < 0.05$ ) increased by the dietary administration of the probiotic Bonvital in group A, compared with other experimental groups and with the



control. Daily body weight gain and feed conversion rate were increased in group receiving probiotic from day 20 compared to the group receiving the probiotic from day 27. The study suggests that administration of the probiotic Bonvital from day one after hatch (starter diet) had more positive effect on broiler performance compared to probiotic administration in grower and finisher diets. As a conclusion, it can be stated that the use of Bonvital shows better economic efficiency in broiler chicks when used starting from day one after hatch and reducing farmer's expenditures in the same time.

**Keywords:** probiotic, Bonvital, broiler, growth performance

## MODIFICATION TECHNIQUE OF TIBIAL TUBEROSTY AVULSION FRACTURE REPAIR IN DOG: CLINICAL CASE

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Fractures of tibia and fibula are relatively common in dogs and cats, and comprise 21% of long-bone fractures. In dogs, proximal tibia represents a union of tibial apophysis, proximal tibial epiphysis and proximal tibial metaphysis. Proximal tibia has two separate ossification centers, proximal tibial epiphysis and tibial tuberosity apophysis. Avulsion fractures occur due to muscle contractions observed during hyperflexion of the stifle joint mostly in immature animals at age of 4-8 months. The aim of this report is to present successful result of a surgical treatment of an avulsion fracture of tibial tuberosity in male, 6-months-old, Jack Russell terrier. Upon admission, the taken history of the patient revealed falling accident from nearly 1.8 meter. The clinical examination, have shown high degree of lameness of the left hind leg, obvious pain during palpation and distinct soft tissue swelling present in the stifle and patella region. The patella was moved towards the femoral trochlear groove. Lateral radiograph revealed an avulsion fracture of tibial tuberosity. The patient was premedicated with 0,1 mg/kg Acepromazine maleate. Surgical anesthesia (TIVA) was achieved by bolus infusion of 5 mg/kg Ketalar and 1 mg/kg Xylazine and maintained with intermittent boluses of the same combination in total of 1/2 of the induction dose. In order to provide sufficient intraoperative analgesia, 4 mg/kg Carprofen was applied during the premedication. Fixation of the tibial tuberosity was achieved by application of one Kirchner wire through the avulsive

fragment into the cortical bone of the proximal epiphysis and cortical screw was placed at the beginning of crista tibiae, 3 cm distally of the avulsion. The inserted pin was secured to the screw by cerclage wire. The leg was additionally fixed by Ehmer sling during the following 10 days, until suture removal. The implants were removed 90 days after the surgery, based on confirmation of bone healing by radiography and normal gait of the patient.

**Keywords:** tuberositas tibiae, avulsion fracture, dog

## PREVALENCE OF THERMOPHILIC CAMPYLOBACTER SPP. AT A POULTRY PROCESSING PLANT

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Campylobacteriosis is the most frequently reported zoonotic disease in humans in the European Union (EU). In 2004, an incidence of 51.6 cases per 100,000 inhabitants was reported by 24 EU member states. The incidence varied widely between countries, ranging from <0.1 to 303 cases per 100,000 inhabitants. Poultry meat is assumed to be one of the main sources of foodborne Campylobacter infections in accordance with the greatest proportion of Campylobacter-positive food samples. There is lack of information regarding to prevalence of campylobacteriosis in human population and prevalence of campylobacter in poultry chicken as main source for food contamination in Republic of Macedonia. Therefore the aim of this study was to assess the possibility of carcasses cross-contamination at a poultry processing plant. The prevalence of thermophilic Campylobacter species in broiler chicken caecal samples and carcass surfaces was investigated. The samples (caeca and carcass swabs) were analyzed according to ISO 10272-1:2006 Microbiology of food and animal feeding stuffs - Horizontal method for detection and enumeration of Campylobacter spp. - Part 1: Detection method. Thermophilic Campylobacter spp. was isolated from 34 (51.5%) of total 66 caecal samples examined and from 16 (72.7%) of total 22 carcass samples taken. Overall, 88.0% and 12.0% of the isolates were identified as *Campylobacter jejuni* and *Campylobacter coli*.

**Keywords:** thermophilic Campylobacter spp, broiler chickens, caecal samples, carcass swabs

## ANALYSIS OF GOAT MILK BY MID-INFRARED SPECTROSCOPY

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Goat's milk quality depends on many factors that are related to both the animals (breed, number and stage of lactation) and the conditions of production (region, diet, rearing system), which have a predominant influence on the quality of subsequent goat's milk products. The objective of this study was to evaluate the possibility for determination of fat, lactose, protein, non-fat solids and total solids in raw goat milk using the Milkoscan 4000 instrument. Traditional methods for this purpose are time-consuming and costly.

Foss Electric (Hillerød, Denmark) has developed and manufactures the large scale system Milkoscan 4000 for testing raw milk samples for individual analytical needs and for the management of herd feed, health and management using the single-beam mid-infrared optical system. It has the possibility of measuring 8 constituents in the following measuring ranges: fat 2-15%, protein 2-10%, lactose 2-10%, solids 2-20%, citric acid 0,1-0,5%, urea 0,01-0,10%, freezing point depression 450-550 m°C.

In this procedure the sample is illuminated with light of wavelengths in the mid-infrared region of the electromagnetic spectrum and filters placed between the infrared source and the sample cuvette, isolate the wavelength specific for the constituents to be measured, such as fat, protein, solids or lactose of a sample. From this information the instrument measures the constituents that the user wants to know.

Calibration procedure was made with 10 samples with results obtained through routine methods. Sixty samples of goat milk were used to evaluate the possibility of using this instrument in the every-day work in goat milk analysis.

This study showed that the mid-infrared spectroscopy is a potentially useful technique for evaluating the composition of raw goat milk. Despite the relatively small number of samples included in the calibration model, mid-infrared spectroscopy was found to be a reliable method for determination of milk constituents.

**Keywords:** goat milk, milk quality, Milkoscan 4000

## SIGNIFICANCE OF THE PROFICIENCY TESTING IN THE DAIRY LABORATORY

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Quality assurance of the analytical results is an obligation of all analytical laboratories which tend to be credible. In order to obtain accuracy and precision of results each laboratory implements suitable programmes according to EN ISO/IEC 17025:2006. Since 2008, the Laboratory for milk quality at the Faculty of veterinary medicine is accredited according to the above mentioned norm. Validation of analytical methods and verification of validation parameters are part of good laboratory practice. Quality of analytical results is assured by use of reference methods and certified calibration standards and by participation in international proficiency trials which are organized by dairy laboratories from Germany, Italy and Slovenia. The Laboratory for milk quality at the Faculty of veterinary medicine has its own protocol of quality control which is based on analytical procedures and methods which are traceable, accurate, reliable and applicable for certain purpose. ISO 17025 stands for International Standards Organization, and 17025 is the code for the standard for competency of testing laboratories. In general, ISO accreditation stands for "You say what you do and you do what you say." Laboratories write down all their procedures in Standard Operating Procedures (SOPs). Then, the laboratory needs to show that it actually performs the procedures according to these SOPs. An important component of ISO 17025 is a competency evaluation of the laboratory. This means that the laboratory must show that it not only follows procedures, but also comes up with the correct result. External proficiency programs and statistical process control is used to evaluate the quality of the laboratory results.

**Keywords:** proficiency testing, ISO 17025, dairy laboratory

## SOME QUALITY PROPERTIES OF KURUT, A TRADITIONAL DAIRY PRODUCT IN TURKEY

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The aim of this study was to investigate some chemical and microbiological properties and the mineral content of kurut, a traditional dairy product produced by drying the buttermilk. A total of 43 kurut samples produced from buttermilk by churning of yoghurt (YG; n

= 16) or by churning of cream (TG; n = 27) were collected from Erzurum and Bingöl provinces of Turkey. The samples of TG and YG groups contained aerobic mesophilic bacteria ( $3.1 \pm 2.20$  log cfu/g,  $0.25 \pm 0.89$  log cfu/g), coliform bacteria ( $1.04 \pm 1.61$  log cfu/g, < 10), *lactobacillus* ( $2.71 \pm 2.49$  log cfu/g,  $0.29 \pm 1.05$  log cfu/g), *staphylococcus-micrococcus* ( $0.25 \pm 0.99$  log cfu/g,  $0.45 \pm 1.32$  log cfu/g), *lactococcus* ( $2.87 \pm 2.02$  log cfu/g,  $0.20 \pm 0.71$  log cfu/g), yeast and mould ( $2.14 \pm 2.27$  log cfu/g,  $0.85 \pm 1.63$  log cfu/g), respectively. Microbial content of TG group was significantly higher than that of YG group contents. Average levels of moisture, total ash, salinity, acidity, fat, pH, protein of TG and YG groups were ( $15.48 \pm 4.48\%$ ,  $12.4 \pm 2.33\%$ ); ( $10.76 \pm 4.90\%$ ,  $14.31 \pm 3.23\%$ ); ( $8.62 \pm 3.92\%$ ,  $9.73 \pm 1.30\%$ ); ( $1.34 \pm 0.51\%$ ,  $2.13 \pm 0.38\%$ ); ( $22.56 \pm 9.08\%$ ,  $11.28 \pm 2.66\%$ ); ( $4.22 \pm 0.58$ ,  $4.01 \pm 0.13$ ); ( $51.15 \pm 10.73\%$ ,  $48.79 \pm 12.84\%$ ), respectively. Minerals in samples were determined by WDXRF. There is need in scientific studies towards determining the quality of kurut, modernizing its production and keeping conditions and making consumption widespread.

**Keywords:** Kurut, Turkey, Microbiological composition, Chemical composition, Mineral composition, WDXRF

## OCCURRENCE OF AFLATOXIN M1 IN UHT MILK IN ERZURUM, TURKEY

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In this study, 150 UHT milk samples were analyzed for aflatoxin M1. They were obtained from supermarkets in Erzurum city. The occurrence and concentration range of AFM1 in the samples were investigated by ELISA method. Fifty -nine percent of the UHT milk samples contained AFM1. AFM1 levels in 16 (10.7%) UHT milk samples exceeded the maximum tolerable limit of the European Community and the Turkish Food Codex. It was concluded that high AFM1 level is an important problem threatening the public health in Turkey.

**Keywords:** Aflatoxin M1, UHT milk, ELISA

## DETERMINATION OF AFLATOXIN M1 LEVELS IN SOME CHEESE TYPES CONSUMED IN ERZURUM, TURKEY

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In this study the total 304 cheese samples (85 white cheese, 75 kashar cheese, 62 civil cheese, 82 cream cheese) were examined in terms of AFM1. The AFM1 content and concentrations of the samples were researched by competitive ELISA method. Determinable limit was 50 ng/kg and it was determined that white cheese samples included 82.4% AFM1, kashar cheese samples 80%, civil cheese samples 19.4% and cream cheese samples 84.2%. According to European Commission limit (250 ng/kg), the sample incidence exceeding the acceptable limits were 27.1%, 34.7%, 17.1% in white cheese, kashar cheese and cream cheese samples, respectively. The sample ratio exceeding the limits regulated by Turkish Food Codex (500ng/kg) was determined in white cheese, kashar cheese and cream cheese samples as 16.5% (14/85), 14.7% (11/75) and 6.1% (5/82) respectively, any sample exceeding these limits was not met in civil cheese samples. As understood from these results, high AFM1 level determined in some cheese types is an important problem threatening the public health in Turkey.

**Keywords:** Aflatoxin M1, Cheese, ELISA

## DETERMINATION OF AFLATOXIN M1 LEVEL IN BUTTER SAMPLES CONSUMED IN ERZURUM, TURKEY

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In this study; the levels of aflatoxin M1 (AFM1) in 80 butter samples were determined. They were obtained from supermarkets in city center of Erzurum. The presence and concentration range of AFM1 in the samples were investigated by competitive enzyme-linked immunoabsorbent assay (ELISA) method. AFM1 was found in 66 (82.5%) samples at levels ranging from 10 to 121 ng/kg with mean concentration of  $30.4 \pm 23.9$  ng/kg. The levels of AFM1 in 13 (16.3%) samples were higher than the maximum legal limit accepted by Codex Alimentarius Commission (CAC). None of the contaminated butter sample exceeded the legal limit regulated by Turkish Food Codex (TFC) for AFM1. The results indicated that contamination of the butter samples with AFM1 in high level could be a potential hazard for public health.

**Keywords:** Aflatoxin M1, Butter, ELISA

## AFLATOXIN M1 LEVELS IN RETAIL YOGHURT AND AYRAN IN ERZURUM IN TURKEY

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This study was carried out to determine the presence and levels of aflatoxin M1 (AFM1) in yoghurt and ayran consumed in the province of Erzurum, Turkey. For this purpose, a total of 80 yoghurt samples and 80 ayran samples were randomly obtained from markets. The samples' AFM1 content and concentrations were examined by competitive ELISA method. AFM1 at detectable level (5 ng/kg) was found in 87.5% of the yoghurt samples and in 90.0% of the ayran samples. AFM1 levels in 16 (20%) yoghurt and in 11 (13.6%) ayran samples exceeded the maximum tolerable limit of the Turkish Food Codex. The positive incidence of AFM1 in the yoghurt and ayran samples ranged from 10 to 475 ng/kg and from 6 to 264 ng/kg, respectively. It was concluded that high AFM1 level is an important problem threatening the public health in Turkey. Therefore dairy products have to be controlled routinely for presence of AFM1 contamination by the public health authorities.

**Keywords:** Aflatoxin M1, Yoghurt, Ayran, ELISA

## MICROBIOLOGICAL PARAMETERS OF WATER FROM WELLS AND NATURAL SOURCES IN THE VICINITY OF SKOPJE

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This study was made to determine the microbiological quality of water from wells and natural springs in the vicinity of Skopje, used for drinking. We've examined 27 samples of water. The samples were tested according to Book of rules on water safety (sl.vesnik 46/2008), on the following parameters: total number of bacteria at 37°C and 22°C, intestinal enterococci, coliform bacteria, *E.coli*, *Pseudomonas aeruginosa* and sulfide-reductive anaerobic bacteria. None of the tested samples did not give satisfactory results. Analyzing each parameter separately, 24 samples had increased numbers of bacteria at 37°C and 22°C, intestinal enterococci were detected in 6 samples. In 18 samples the presence of coliforms was detected, in 6 sample *E.coli* was found. In all samples the presence of *P.aeruginosa* was determined, and in none sulfide-reductive anaerobic bacteria. Although none of the samples give a satisfactory result, in 18 samples, all from wells, the presence of coliform bacteria and intestinal enterococci was detected, which indicates fecal contamination of the source and a possible presence of pathogen bacteria.

**Keywords:** well water, spring water, coliform, *E.coli*, intestinal enterococci, *Pseudomonas aeruginosa*

## NUTRITIONAL FEED SUPPLEMENTS – LEGISLATION IN RM

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Feed manufacturing today can be hardly imagined without the use of nutritional feed supplements. Due to technological reasons, feed supplements are intentionally added during production, processing, preparing, packaging, transport or storage, where they become a part of the final product.



Those differences are reflected in the necessity for change in professional approach, as well as in raising awareness of feed manufacturers, aimed at greater competitiveness and effectiveness, as well as at faster discovering of all possible risks of uncontrolled feed supplementation. Increasing level of responsibility of feed manufacturers in countries outside the EU is generated from the EU demands for harmonization of national with the European legislation. Regulation 1831/2003 on additives for use in feed replaced the former Directive 70/254 as the main legal text for feed additives. This Regulation divides all additives into categories and then into functional groups. There are four categories – technological, sensory, nutritional and zootechnical. Each of these is divided into functional groups.

At the same time, there are ongoing changes made by appropriate legislative structures of the European union related to the use of feed supplements. They are based on the results of monitoring, as well as documentation requested for the renewal of registration of feed supplements, and could lead to potential abolition of licenses for the use of, till then, some important additives. As these substances are added to feed, the international procedures for determining their efficacy and safety for health had to be established. By monitoring the impact of feed on the safety and quality of animal origin food, the parameters are obtained, which are used as the basis for defining the standards of the international programme for food safety (Codex alimentarius).

**Keywords:** feed supplements, legislation

## QUALITY CONTROL AND FEED SAFETY SYSTEMS IN FEED MILLS

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Reaction the food industry has tightened quality control procedures and traceability to reduce health risk and satisfy consumer demands for safer food. Agriculture is an integral part of the food chain. The market driven attitude of the food industry regarding food safety has been driven back to the agriculture sector from primary breeding, to live production and live haul, to feed, and integral part of the new food safety laws. HACCP is internationally recognized and ac-

cepted by the World Trade Organization. So far HACCP is not mandatory throughout the agricultural chain but will most likely become mandatory if companies are to continue to do business locally and internationally. The potential for the introduction of contaminants (microbiological, chemical, or physical) to eggs, beef, poultry, and pig meat from feed is real. Two most recent examples are in Canada (medicated feed was fed to pigs before slaughter) and the Benelux (dioxin in feed). Traditionally feed mills successfully implemented Good Manufacturing Practices (GMPs) to produce feed clean of contaminants thus protecting raw foods of animal protein. However, to increase the safety margin in the production of raw animal proteins and to regain consumer trust, HACCP implementation in feed mills is becoming a necessity. The feed mill management indicated that the implementation of HACCP improved and centralized their records, improved their confidence in recall procedures if needed, and gained customer confidence. In the long run they believe that production efficiency will improve because better maintenance records will lead to less breakdown. Changing government policies in response to consumer demand for safer food will most likely change the way agribusiness companies conduct their activities. Food processors are demanding high quality safe animal proteins from agribusiness suppliers. To meet these demands, we have to implement procedures such as HACCP to improve our ability safe raw inputs that ensure consumer satisfaction and trust.

**Keywords:** feed, mills, HACCP, ISO 9001, ISO 22000

## BENEFITS OF MILK AND MILK ALTERNATIVES IN PIGLET NUTRITION

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Milk contains not only nutrients, but also bioactive substances that are necessary for normal development and health of piglets. Due to relative undeveloped digestive tract presents weaning stress for piglets a big challenge. The weaning stress is not characterized only by social stress and change of physical environment but also by completely different nutrition: feed from feeder instead from the udder, separate consumption of water



and nutrients, change in texture, test and smell of feed, lower nutritive value of nutrients in feed compared to milk, removal of bioactive substances, presence of antigens, toxins and other antinutritive substances in feed. As a consequence weaning syndrome occurs. It is characterized by reduced feed and water intake, atrophy of the gut, bacterial translocation and disbiosis, maldigestion and malabsorption. This leads to reduced productional parameters, infection, diarrhea. The consequences might be reduced by appropriate management that includes also favourable feed composition. This article describes the properties, function and importance of milk constituents for the development and integrity of the gut. It is very hard to find equivalent replacement for the sow's milk at the time of weaning. The production of piglets per sow per year is growing in every farm, while management systems force the farmers to wean earlier, dedicating extra attention for: feed intake before and after weaning and how to take care of the young, small piglets. The end goal is to reach a good weaning weight, make the start after weaning more easy and get a better and healthy growth. Good feeding program should accomplish to: stimulate the digestive tract as a preparation on the feed intake after weaning, stimulate the acid and pepsin secretion and the pancreas secretion, development of the villi, 'Boost' of the growth after weaning and less diarrhoea problems after weaning.

**Keywords:** milk, feedstuff, piglet

## THE ETHIC BEHAVIOR TOWARDS ANIMALS

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The basic ethical question: how to achieve an integrated and a happy personality, one that can live in harmony with oneself and the environment (people, nature), presumes the re-examination of the human relationship towards all manifestations of life, including human behavior towards the animals. The motif of ethic behavior towards the animals is treated since the ancient philosophy systems, western and eastern as well (Empedocles, Pythagoras, Socrates, Buddha, MahavirJina etc. ) Contemporary movements for *animal welfare* and their 'rights' are contradictory to the way animals are 'integrated' in developed societies. By reviewing the ethic attitudes of the main philosophers (and some scientists) the aim of this study is to determine the connection between animal welfare and that of human beings. We expect that this work will open

new perspectives to clarify some paradigmatic beliefs and practices and that it will contribute to improving the treatment of animals in general.

**Keywords:** ethical behavior, animal welfare, developed societies, contradictory

## EVALUATION OF HAEMATOLOGICAL RADIO TOXICITY OF RHENIUM-188 HYDROXYETHYLIDENE DIPHOSPHONATE IN ANIMAL MODEL

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Bone seeking radiopharmaceuticals like rhenium-188 hydroxyethylidene diphosphonate (<sup>188</sup>Re-HEDP) are used for palliative treatment of bone metastases, and as target radionuclide therapy in patients with inoperable osteosarcoma, in addition to the external radiation therapy. The aim of our study was to evaluate hematological radiotoxicity of <sup>188</sup>Re-HEDP in canine animal model. Radionuclide <sup>188</sup>Re was obtained from <sup>188</sup>W / <sup>188</sup>Re generator. The procedure for the preparation of <sup>188</sup>Re-HEDP was conducted under the aseptic conditions and described protocol. The radiochemical purity was determined using thin-layer chromatographic method. As animal model we used an adult dogs of various breeds and of both sexes. The main parameters for monitoring of bone marrow toxicity were the number of leukocytes and platelets, followed before and during the six weeks after application of <sup>188</sup>Re-HEDP, once a week. Exclusion criteria were white blood cell (WBC) counts <6 10<sup>9</sup>/l and platelet counts <200 10<sup>9</sup>/l. Statistical data processing was performed using the software system STATISTICA for Windows XP Professional and STATISTICA 6.0 StatSoft. Group results were expressed by mean and standard deviation.

Radiochemical purity showed that over 98% of radio-active  $^{188}\text{Re}$  was in the form of  $^{188}\text{Re}$ -HEDP complex. In terms of haematological radio toxicity, doses that were used in the study had no significant effect on the number of leukocytes and platelets. The greatest reduction in the number of leukocytes and platelets in most animals was noted in the second week after the application of the radiopharmaceutical. After the sixth week of treatment, the values of the tested blood units reached the value before treatment. Animal model in our study allowed monitoring radio toxicity of  $^{188}\text{Re}$ -HEDP. The haematological radio toxicity of doses used is negligible and transitory. These results suggest that  $^{188}\text{Re}$ -HEDP is an attractive radiopharmaceutical for palliative treatment of primary and metastatic bone tumours.

**Keywords:** *rhenium-188, hydroxyethylidene diphosphonate, animal model, radio toxicity, bone metastases*

## FATTY ACID COMPOSITION OF ASPARAGUS OFFICINALIS OILS

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Vegetarians have no direct sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (long chain omega-3 fatty acids) in the diet, hence they must convert alpha-linolenic acid to EPA and DHA in the body. Human beings evolved on a diet that was balanced in the omega-6 and omega-3 polyunsaturated fatty acids (PUFA), and was high in antioxidants. Edible wild plants provide alpha-linolenic acid and PUFA. Today, we know that omega-3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer. Analysis of fatty acids was performed with GC-FID. Comparison of obtained results from analysis of fatty acids showed that, Asparagus oil has high nutritional value because it contains 45,62 linoleic acid and 8,84 % linolenic acid. Therefore, we can conclude that *Asparagus* oil are edible and have good nutritive values.

**Keywords:** *Vegetables, Fatty acids, GC-FID, Asparagus officinalis.*

## PRESENCE OF DIROFILARIA. REPENS IN PROFESSIONAL DOGS IN THE REGION OF SKOPJE DIAGNOSED WITH THE KNOTT MODIFIED TECHNIQUE

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In the period of March through April, 2010 blood was analyzed from 39 professional dogs without visible clinical symptoms and skin abnormalities. The aim of this research was to determine the presence of filarial nematodes in professional dogs older than 1 year in the region of Skopje – R. Macedonia and to identify present species. Dogs were kept in separate cages, in good hygienic conditions, treated with praziquantel every three months. For detection of microfilaria in blood a modified Knott technique was used. Differentiation of present microfilaria was done on the base of morphological characteristics and micrometry. Out of 39 dogs, 8 dogs (20,5%) were positive for the presence of *D. repens* with average length of 354,94 µm and width of 6,59 µm. This represent a first report of *Dirofilaria repens* determination in dogs in R. Macedonia which live in the area of high level underground water, making the ideal conditions for reproduction of the mosquitoes as a vector for this disease.

**Keywords:** *Dirofilaria repens, official dogs, Knott technique, micrometry;*

## ASSESSMENT OF COMPLIANCE OF RAW MILK SAMPLES FROM REPUBLIC OF MACEDONIA FOR RESIDUES OF TETRACYCLINES ACCORDING TO METHOD PERFORMANCE REQUIREMENTS LAID DOWN IN THE COMMISSION DECISION 657/2002/EC

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As antimicrobials tetracyclines are widely used at food producing animals for prevention and treatment of infection diseases caused by bacteria or as a feed additives to improve feed efficiency and growth. The presence of residues of these drugs might have various adverse effects as direct toxicity and allergic reactions in some hypersensitive individuals. Even more important long-time intakes of low-doses of antibiotics in foodstuffs can cause problems related with drug persistency at the microorganisms. The maximum residue limits (MRL's) for tetracycline according to Commission Regulation 37/2010/EU [1] are set at 100 µg/ kg. The same MRL's are in force in Republic of Macedonia according the national legislation [2]. Analytical methods that are employed for detection of residues of tetracyclines must be able to detect levels which are under and around the MRL's. The most widely used screening methods for detection of residues of tetracyclines in food are microbiological [3] or immunochemical [4]. As proscribed in the Commission Decision 657/2002/EC [5] each positive result must be confirmed with other validated methods that have to include spectrometric detection [6]. Decision limit (CC $\alpha$ ) and detection limit (LOD) are the validation pa-

rameters which are important for assessment of compliance of samples regarding the presence of residues of veterinary drugs in food.

In this paper the obtained results from the analysis of 480 samples of raw milk for the residues of tetracyclines will be presented and assessed for compliance regarding the obtained validation parameters during from the in house validation. The employed confirmatory method is High-Performance Liquid Chromatography (HPLC) with Diode Array Detection (DAD). Sample extraction was performed with McIlvaine buffer and the clean-up with OASIS HLB solid-phase extraction cartridges [6]. The samples were considered for non-compliant if the individual concentrations for tetracyclines exceeded the determined 131,1 µg/ kg, 121,1 µg/ kg, 116,8 µg/ kg and 124,5 µg/ kg for oxytetracycline, tetracycline, chlorotetracycline and doxycycline, respectively. The determined concentrations lower than 10,4 µg/ kg for oxytetracycline, 12,0 µg/ kg for tetracycline, 16,8 µg/ kg for chlorotetracycline and 20,7 µg/ kg for doxycycline were considered to be under the limits of detection of the employed method.

**Keywords:** tetracyclines, maximum residue limits, raw milk, HPLC

