LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

TRACEABILITY OF TRANSGENIC SOYBEAN FROM FORAGE TROUGH ANIMAL TISSUE TILL THE FOOD PRODUCT

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Summary

In recent years, there has been a notable concern on the safety of genetically modified (GM) foods/plants, an important and complex area of research, which demands rigorous standards. Molecular methods of GMO detection in food products are based on a short DNA sequence identification. Those sequences can be found in any type of product more or less processed. Even though it was demonstrated by numerous studies that those transgenic sequences will be found in the tissue of any animal that is fed with and were it does not have any metabolic function, there is a lack of studies concerning them crossing in the alimentary products.

The European legislation outlines labeling as GMO any food or feed product that contains equal or over 0.9 % GMO material. The presence of remaining DNA sequences in the tissues could easily contribute in reaching and even overcoming of this threshold. Starting from those suppositions, different types of tissues becoming from pigs that were fed with transgenic soybean, were analyzed. GMO detection was accomplished in respect of current specific legislation, following the European standards that were implemented in laboratory, by using PCR type analysis. In the frame of this experiment, sequences of transgenic DNA were identified in fresh but also after a simulation of processing tissues in order to prove the detection all along the technological process. This study emphasizes the necessity of considering those aspects in the process of legally labeling GMO products. Also, there is a need of a better communication for informing the producers and consumers concerning the detection methods and the labeling legislation.

Key words: GM soybean, GM food, GM feed, detection, farm pig

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

MOLECULAR FINGERPRINTING BASED ON DNA MARKERS AS A METHOD OF BROWN BEAR (*URSUS ARCTOS*) EXEMPLARS IDENTIFICATION - A CASE STUDY

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Summary

The population size of Brown bear in Romania represents about 40 % of total number from Europe, except Russian territory, reaching to about 6000 individuals so being the biggest European population of this species, according to IUCN report "Brown Bear Conservation Action Plan for Europe" - 1999. An approved limited harvesting quota for this species is needed in order to control the level of possible damages that can be caused. The hunting is allowed only for certain bears in conditions, places, and periods, and with the means established by the law. Hunting of brown bears is done only in the limit of the maximum number of individuals allowed by the law. Deduction of this maximum number of individuals for each administrator and game management unit is approved by ministerial order of the specific central public authority. Even so, it is estimated that the real number of brown bear individuals is lower than the proposed one. Annually, a bigger number of exemplars is declared in order to obtain an increased number of hunting permits that are illegally sold to foreign hunters. Up to date in our country, there is no program of brown bear identification and even the existing traceability methods can be easily defeated by poachers. In this study we propose a DNA molecular markers based system of fingerprinting that can lead to a data base construction. The DNA fingerprint is impossible to defeat and can be traced even in small remains of the body. Based on brown bear biological samples, by using the PCR technique and two types of molecular markers ISSR (inter-simple sequence repeat) and DAMD (Direct amplification of minisatellite DNA) a set of DNA fingerprints was established. Those fingerprints are accurate in distinguishing individuals and reproducible in time.

Key words: Brown bear, molecular markers, DNA fingerprinting

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

ASPECTS REGARDING TO RESPIRATORY DISEASES IN POULTRY FARMS FROM ROMANIA DURING 2013 - 2014 DETERMINED BY ELISA AND PCR ASSAYS

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Summary

In the Department of Diagnostic within NS Pasteur Institute, Bucharest, during January 2013 - September 2014 there were carried out 5386 serological tests by ELISA and 373 molecular tests by PCR assay for six specific agents associated with respiratory diseases in poultry (chicken and / or turkey): infectious bronchitis virus (IBV), infectious laryngotracheitis virus (ILT), avian pneumovirus (AMPV), *Ornithobacterium rhinotracheale* (ORT), *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), and in addition, *E. coli* (APEC) strains were pathotyped by PCR assays.

Samples were from chicken industrial farms (commercial) (parents, broilers, layers) and turkey farms, located in 9 counties in the central and southern Romania. The applied vaccination schemes were different from farm to farm but also depending on the age of poultry. They included in a non unitary manner vaccinations for IB prophylaxis (mostly with strains derived from CR / FR / Qx pathotype), applied to youth and adults poultry, ILT, ART (AMPV subtype B) applied to youth and adults poultry, and mycoplasmosis with MG and MS applied to adults.

The ELISA tests were performed with commercial kits (Affinitech, Pasteur, CIVTest Hipra and Biocheck) and PCR assays, real-time or classic, were made in house and with primers selected from the literature or on-line designed, based on the genetic sequences registered in GenBank.

For IBV there were recorded very high antibody titers in adult poultry, and PCR tests revealed the circulation of strains derived from CR / FR / Qx pathotype and the absence of Mass or It-02 strains in all chicken ages.

ILTV was present in unvaccinated layers and broilers of 42 days old, as the ELISA and PCR tests were positive. The molecular analysis registered negative results for broilers of 31 days old.

For avian pneumovirus (AMPV/ART/TRT) the serological analysis showed positive results for broilers, chickens of 8 weeks and layers. AMPV type A was present in young turkeys of 61 days old, types A and C were found simultaneously in layers of 31-36 weeks old, whereas the types B and C were concomitantly detected in broilers of 42 days old.

The ORT presence was showed both by ELISA and PCR in broilers of 42 days old, and adult hens and turkeys of all ages.

MG and MS were present in farms without specific vaccination, both in chicken and turkey of all ages, but also in farms with vaccinated poultry, but reached at higher ages.

The APEC strains were present in young chicken, starting from one day old, and also in sanitation control samples and, curiously, at a swan from a hunting area in southern Romania.

The PCR tests proved to be appropriate for the diagnosis of avian diseases, reporting the presence of strains of infectious bronchitis virus, *Mycoplasma synoviae* and *M.gallisepticum*, avian metapneumovirus, types A, B and C, and those of *Ornithobacterium rhinotracheale* and *E.coli* APEC epidemic clones in chicken industrial farms frorm Romania.

The ELISA kits used proved to be highly reliable to control the efficiency of vaccines, and in combination with PCR assays, to detect infectious agents with respiratory tropism in poultry.

Key words: respiratory avian diseases, ELISA, PCR

RESEARCH REGARDING THE RESISTANCE PHENOTYPES IN STAPHYLOCOCCI ISOLATED FROM BOVINE MASTITIS

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Summary

In cattle, mastitis is produced both of positive and negative coagulase staphylococci, with a correlation between progressive clinical forms and the pathogenicity level of strains.

Local treatments improperly made, have induced the multiple resistance to antibiotics phenomenon, which is transmitted by R plasmids intraspecific and interspecific. Antibiotic resistance created difficulties in mastitis therapy, which requires the necessary antibiogram at each isolate.

A total of 64 of positive and negative coagulase staphylococci strains were tested by Kirby-Bauer disk diffusimetric method using biodiscs with 15 antibiotics from several groups and the results were interpreted according to the standards.

Antibiotic resistance to β-lactams varied between 7.81% and 64.06%, and resistance to methicillin was 57.81%, indicating a high proportion of strains carrying the *mec* gene.

The resistance phenotypes of the tested strains, to the other groups of antibiotics, had a variable frequency, between 4.68% and 70.31%.

Key words: antibiotic, bovine, mastitis, phenotype, resistance

RESEARCH ON MOBILE SEROVARS FREQUENCY OF *SALMONELLA SPP*. AT *GALLUS GALLUS* SPECIES IN ROMANIA

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Summary

The globalizatin of trade with poultry material associated with changing technologies increase with the emergence of mobile *Salmonella* strains, contributed to the spread serovars in flocks of broiler chickens, breeding hens and chickens for egg consumption.

The epidemiologic research, in the outbreaks of paratyphoid, proved the existence of vertical and horizontal transmission direct and indirect inside farms and between farms. Also, the resaerch have shown that birds and poultry products are a huge reservoir of mobile serovars with a pronounced zoonotic risk.

In Romania was implemented the National Monitoring Program, Control and Eradication of Zoonotic Salmonellosis in *Gallus gallus* species, since 2009, and the study was carried out in 2009-2012.

The research was carried out in order to establish the prevalence of mobile *Salmonella* serovars in the three types of farms and to follow the objectives established by Community law.

Key words: mobile *Salmonella*, paratyphoid, prevalence

RESEARCH ON THE FREQUENCY OF STAPHYLOCOCCAL SPECIES ISOLATED FROM SUBCLINICAL MASTITIS PRIMIPAROUS BOVINES

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Summary

In the last years, several species of coagulase-negative staphylococci (CNS) have been isolated from animals but also from humans, producing different localized infections of which are distinguished in frequency and importance the subclinical mastitis of dairy cattle.

Considering these aspects, research followed: cultural, morphological, tinctorial characters and biochemical profile of the isolates regarding the typification. These characters were also researched by classical methodology with API Staph system multitest.

The isolated staphylococci formed on 5% defibrinated sheep blood agar, haemolytic characteristic colonies, with partial haemolysis, unhaemolytic, with different intensities of yellow pigment and white pigment.

Chapman medium allowed the differentiation of positive and negative mannitol strains, while Baird-Parker medium and Difco agar with 1% maltose and blue bromocresol allowed rapid differentiation of *S. aureus subsp. aureus* strains.

Bound coagulase was present in 18.75% of the isolates, while free coagulase was present in 15.63% of the isolates.

The API Staph multitest was used for biochemical differentiation of 64 strains of isolated staphylococci from bovines with subclinical mastitis and, based on the results of this system, 19 species of staphylococci were identified.

Key words: dairy cattle, coagulase-negative staphylococci, subclinical mastitis

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

A THEORETICAL APPROACH OF THE IMMUNE SYSTEM OF DANUBE HUCHEN (*HUCHO HUCHO*)

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Summary

The Danube huchen (*Hucho hucho*) represents the flagship of the *Salmonidae* family. The species is severely fragmented within the Danube drainage, and it is included in the IUCN Red List of Threatened Species. It is almost impossible to identify if any stocks are self-sustaining. In spite of its popularity among fisherman during the early '80s, to date, there is almost no scientifically supported information on this species. The aim of the paper is to establish, based on a comparison between trout and huchen in terms of habitat, water temperature, size and food type, a scheme of the immune system and its functioning in the latter. Since anthropogenic habitat alterations and pollution are major concerns affecting huchen populations, a better understanding of their immune system and a step by step approach to various immunological techniques could have a significant impact over the fish-orientated scientific community and species conservation. Furthermore, studying the immune system of huchen, a highly sensitivity species, would provide data that could make them the perfect sentinel, an indicator for the ecosystem health and for the conservation of running mountain waters, along with the species.

Key words: Danube huchen, *Hucho hucho*, immune system, conservation

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

VACCINATION FAILURE IN CHICKENS: POOR VACCINE QUALITY, HUMAN ERROR, OR VARIABLE INDIVIDUAL IMMUNE RESPONSE?

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Summary

Although vaccination against certain infectious diseases in chickens represents a widespread practice, it continues to contribute to major economic losses attributed to infectious diseases by low antibody titers, and subsequently, atypical cases or even disease outbreaks. In spite of being considered an improvable procedure from which significant solutions are expected, vaccination was and still is one of the most successful ways to prevent infectious diseases, mainly on chicken farms. Since vaccines are most often incriminated for immunization failure, the aim of the paper is to present other possible factors and scenarios that could interfere with the success of the procedure and therefore need to be envisaged. Factors such as the vaccine itself, vaccination technique, individual immune response, the influence of maternal antibodies, MHC variation from a generation to another, the health status or nutrition of the chickens can lead to vaccination failures. To improve the outcome of the vaccination on chicken farms, it is utmost important to identify, describe and select the critical control points where the operators could intervene to better control the results and thus diminish the economic losses.

Key words: chicken, vaccination, immune response, failure

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

IMPACT OF SOWS IN THE CARRIER STATE OF *SALMONELLA* SPP. IN PIGLETS

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Summary

Infection with *Salmonella* spp. in pig farms is usually endemic and largely asymptomatic. It is desirable to identify and eliminate this organism in an early stage of the production phase, starting from the examination of the sows before and after gestation, piglets after birth and weaning, fattening pigs before slaughter, and from the risk factors with important role in the transmission of *Salmonella* spp.

In this study we observed the transmission of *Salmonella* during the technological flow from the farm through fecal sampling from sows and their piglets until the age of weaning, and the importance of respecting disinfection between different stages of the flow.

Identification and isolation of *Salmonella* was performed by the method: EN ISO 6579: 2002 / AC: 2007 and for the isolation of *Salmonella* serovars API 20E method was used.

The prevalence of *Salmonella spp.* in fecal samples after examination of each farm under study, showed that the farm A were 50% sows and piglets carriers and in farm B, were found 27% positive samples.

Key words: Identification, methods, *Salmonella*, piglets, sows

ROLE OF SOWS (PREGNANT AND LACTATING) IN THE TRANSMISSION OF *SALMONELLA* SPP.

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Summary

Due to the significance of pigs shedding *Salmonella* spp. in contamination of carcasses, namely the introduction of this microorganism the food chain should begin by analyzing each stage of the production chain. It is important to consider the role of sows in the epidemiology of *Salmonella* spp.

The purpose of this study is to determine the prevalence of *Salmonella* spp. in pigs at different stages of breeding status by analyzing samples taken before and after calving, respectively after weaning piglets.

For the identification of *Salmonella* were taken (n=120) samples of feces of pigs in the two farms (A, B). All samples were analyzed by classical method SR EN ISO 6579: 2003 /AC: 2007 and API 20 E method.

Analyzing the samples of the two farms in the study were found different results, as before birth prevalence of *Salmonella spp.* ranged from 50% (farm A) and 63% (farm B), after birth until day 10 of lactation, there was a reduction of load between 33% (farm A) and 50% (farm B), but there was a slight increase in the number of sows that were excreted microorganisms of the genus *Salmonella,* before and after weaning piglets, reach in a load of 53% (farm A) and 55% (farm B).

After analyzing samples by API 20 E method, it was found that the most common serovars isolated from feces of sows were: *S.* typhimurium at a rate of 61.15%, followed by *S.* choleraesuis at a rate of 38.84%.

Results of this study indicate that sows constitutes a source of infection in swine herds being considered further source of contamination of carcasses after slaughter them.

Key words: Identification, faeces, *Salmonella*, serovars sows.

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

THE PREVALENCE OF POSITIVE ESBL *E. COLI* AND *KLEBSIELLA PNEUMONIAE* STRAINS IN THE DOGS IN THE IAȘI PADDOCK

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Summary

International organizations (WHO, OIE) warn about the circuit of cross-transmission of microbial strains multiresistant to drugs and the irrish on the public health. In Romania, the issue of stray dogs represents a regional particularity. The population of these free animals is in a continuous growth and their institutionalization in paddocks allows an easy assessment of the infectious riskfor the public health. The aim of this study was to detect the presence of *E. coli* and *Klebsiella pneumoniae* strains producing extended-spectrum beta-lactamases in the stray dogs in the Iaşi paddock and determining the antibiotic resistance of isolates. The antibiotic resistance by producing extended-spectrum beta-lactamases (ESBL) is a phenomenon that creates significant therapeutic problems in the whole medical world. In January 2015, 58 faecal samples were collectedby means of sterile buffers from approachable and clinically healthy dogs from the paddock. Of the total of samples studied, 15 (25.86%) were positive, the confirmation of certainty being achieved by means of PCR. The phenotypic confirmation of ESBL positive strains was achieved according to the protocol recommended by CLSI. This prevalence draws the attention tothe risk of cross-transmission of ESBL strains in the animal-human-animal circuit. The animals studied were not treated with antibiotics and could be a natural reservoir of positive ESBL E.coli and Klebsiela pneumoniae.

Key words: ESBL, *Enterobacteriaceae*, antimicrobial resistance

RESEARCH REGARDING IMMUNOSUPPRESSION INDUCED BY OCHRATOXIN

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Summary

The aim of this study was to establish the immunosuppressive effect of ochratoxin, using mononuclear cells separated from mice' spleens. 30 white miced were used, divided in three groups: control group (A), experimental group 1 (B, treated with *Mycoplasma* immunogen) and experimental group 2 (C, treated with ochratoxin and *Mycoplasma* immunogen). After two treatments (two weeks) spleen was harvested from all mice, and mononuclear cells were separated and cultured. The following paramenters were determined on the cells cultures: proliferation factor, with and without stimulation of cell cultures with phytohaemagglutinin (PHA), concanavalin (ConA) and *Mycoplasma* immunogen (My), and interleukin-2 synthesis in cell cultures. The results obtained showed normal values for proliferation factor in control group. The cell proliferation indexes showed changes depending on the mitogen used (3.2 - 4.0), and the cell proliferation factor was 2.6 in unstimulated samples. It was also found that stimulation with *Mycoplasma* immunogen, the synthesis of IL-2 was maximal. Con A and PHA had a moderate action. In cultures obtained from group C animals (treated with ochratoxin), IL-2 was absent, regardless of the mitogen used to stimulate the cells.

Key words: ochratoxin, immunosuppresion, *Mycoplasma* immunogen, cell culture

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHANGES DURING RIPENING OF TELEMEA CHEESE

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Summary

The research material was represented by 39 samples of ripened telemea cheese (30 day) collected between February and July 2014, from a diary processing plant, located in Cluj County. In this study we aimed at the following objectives: dynamic assessment, during maturation process, of compositional parameters from ripened telemea cheese and the evaluation of microbiological risk represented by *E. coli* and *Staphylococcus aureus*. All the cheese samples were analyzed using standardized methods. Specific colonies of *E. coli*, respectively *Staphylococcus aureus* were tested regarding enterotoxigenity capacity, using specific primers. Based on the statistical interpretation of the results, in case of compositional parameters, nonconformities have been found regarding the chemical components of cheese for the proteins, solids and fat parameters. During the process of ripening a descending evolution for pH value and proteins has been found, the fat revealing a uniform evolution reported at total solids and an ascending evolution has been found for fat, total solids, salt and titrable acidity parameters. In the isolates analyzed, the PCR testing for toxicity specific genes (stx, stx2, sea, sec, sep) didn't reveal any positive amplification. The microbiological risk represented by the presence of *E. coli* and *Staphylococcus aureus* is reduced. Although the microbiological load was relative low, the presence of *E. coli* and coagulase-positive staphylococci denote deficiency regarding strict compliance of good hygiene practice.

Key words: telemea cheese, chemical composition, ripening, microbiological risk

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GENERAL PRINCIPLES REGARDING RISK ANALYSIS OF THE LOW ACIDITY CANNED FOOD MANUFACTURING

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Summary

The main risk associated with low acidity canned food is the presence of *Clostridium botulinum* and its toxin, botulina, which causes botulism. After the confirmation of two cases of botulism in the 80's, the worldwide low acidity canned food manufacturers and importers have started to use the HACCP system for keeping under control the technological process, and in particular the heat treatment.

The legislation of different producing canned food countries specifies own critical limits for joining boxes and for other canning parameters and the sampling plans used for products testing.

Most of the manufacturers believe that altering of canned food and the presence of botulism are related to the survival of bacteria due to inefficient heat treatment and to the re-contamination that occurs after processing. Using of HACCP system, by identifying critical control points, in the low acidity canned food manufacturing has the main objective the prevention of botulism.

Key words: HACCP system, low acidity canned food, *Clostridium botulinum*

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

OFFICIAL CONTROLS ON TRADITIONAL PRODUCTS OF ANIMAL ORIGIN SUBJECTED TO INTRACOMUNITARY EXCHANGE

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Summary

Since 2007, Romania, as an EU State Member, is obliged to full application of European legislation on veterinary certification and official controls on products of animal origin, including traditional ones, which are subject to intracomunitary exchange.
Products must come from approved establishments for intracomunitary exchange, to move freely in the Community accompanied by commercial documents and to undergo checks on origin and destination by official veterinarians. They must carry out an identity check of each shipment to ensure that the products correspond to the information given in the accompanying certificates or documents and also to fulfil a physical inspection of each batch to ensure that products meet the requirements of Community law and national and are in a form, proper to be used for the purpose specified in the accompanying certificate or document.

Physical control or any other control is performed to verify compliance with food law, when required in case of suspicion, uncertainty or doubt, upon notification by the Rapid Alert System for Goods and Feed or actions based on the National Surveillance, prevention and control of animal diseases, those transmitted from animals to humans, animal protection and environmental protection Program.

To ensure traceability, operators engaged in intracommunitary exchange operations with animal origin products, including traditional products, must enroll in a special register comprehensive data on consignments of products of animal origin subject to intracommunitary exchange in order to be available to veterinary services when requested.

Key words: official control, traditional products, intracomunitary exchange

IDENTIFICATION OF PLANT EXTRACTS AS POTENTIAL ALTERNATIVE THERAPEUTIC MEANS FOR DAIRY COWS DIAGNOSED WITH SUBCLINICAL MASTITIS

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Summary

Since bovine mastitis is an economically impacting disease of dairy animals worldwide, majority of the investigations focused on defining epidemiological indicators and also control measures. As part of the control programs, the most popular therapeutic measures in clinical and subclinical mastitis in dairy cattle is the antibiotic treatment, less and less efficient under the pressure of increasing antibiotic resistance of inducing bacteria.

The aim of this study was to search for the alternative therapeutic potential of plant extracts such as *Calendula officinalis, Echinacea angustifolia, E. purpurea, Hippophae rhamnoides, Urtica dioica, Allium sativum, Salvia officinalis, Mentha piperita*) found on most of the Romanian pastures. The research was carried out on a group of 27 dairy cows, divided in two groups, healthy (n=10) and diagnosed with subclinical mastitis (n=17). Blood samples collected by puncturing the jugular vein were diluted with RPMI 1640 (1:4) and dispensed in 96-well plates. Duplicates of alcoholic vegetal extract treated variants (1.5 μl/well) were compared to cultures treated with PHA and LPS standard mitogens (1.0 μl/well). Glucose concentrations were measured by means of an orto-toluidine colorimetric test and stimulation indices (SI%) were calculated. The spontaneous SI was lower in the subclinical mastitis group (48.03±10.23) than in the healthy animals' group (53.44±9.34). There was no increase of SI in mastitic cows under the influence of plant extracts, that acted inhibiting (SI% from 17.54±13.97 to 39.90±7.89), when compared with the spontaneous (SI% 48.03±10.23) and alcohol induced (SI% 40.06±8.57) ones and the effects obtained in healthy animals (SI% garlic extract 56.71±12.91, SI% sage 58.26±12.31, SI% mint 54.60±15.97). The results indicated that the plant extracts used according to the described protocol failed to restore the *in vitro* cell-mediated immune response, suggesting the continuation of the research to establish the adequate dosage for these extracts.

Key words: plant extracts, subclinical bovine mastitis, alternative therapy

QUANTIFICATION OF NONSPECIFIC IMMUNE SYSTEM MEDIATORS IN A POPULATION OF DAIRY COWS PREVIOUSLY DIAGNOSED WITH SUBCLINICAL MASTITIS

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Summary

Bovine mastitis is one of the most important causes of economic loss in dairy industry with a severe human health impact. The bacterial etiology of mastitis exerts its impact on both the local and systemic immune effectors, thus quantification of the immune status of the animals can help in predicting the outcome of the disease. The investigations aimed to monitor the changes in nonspecific systemic humoral and cell-mediated immunity in Romanian Spotted and Holstein animals, aged 3 to 8 years, diagnosed with sublinical mastitis, by the use of precipitation techniques to quantify circulating immune complexes and total immunoglobulins levels from whey. Furthermore, the neutrophile/lymphocyte ratios were used to estimate the stress levels in the diseased animals and the carbon particle inclusion test to monitor the phagocytosis. The study revealed lower values of circulating immune complexes in whey of mastitic cows (0.002±0.010 optical density units, ODU) when compared with the healthy ones (0.004±0.027 ODU), while the total IgG levels were reversed (0.36±0.13 and 0.29±0.13 ODU, respectively). Stress index (N/L) values and phagocytosis were significantly higher in mastitic animals when compared to the healthy ones (N/L 1.24±0.69 and 0.48±0.15, $p<0.05$ and phagocytosis 1.78±0.21 and 0.44±0.23, $p<0.01$). The results indicated a higher clearance of the immune complexes, higher levels of stress and phagocytosis in cows with subclinical mastitis, suggesting the strong involvement of both non-specific humoral and cell-mediated systemic immunity despite the subclinical course of the disease.

Key words: dairy cows, subclinical mastitis, circulating immune complexes, total immunoglobulins, N/L index

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

ANTIBIOTIC RESISTANCE - AN OVERVIEW OF HORIZONTAL GENE TRANSFER MECHANISMS

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Summary

Antimicrobial agents are indispensable in the control of bacterial infections in humans, animals and plants. During the past decade, antibiotic microbial resistance (AMR) has become a world-wide problem in both human and veterinary medicine. WHO warns on the dawns of a post-antibiotic era. It is generally accepted that extensive and often irrational use of antibiotics represents the main risk factor for the augmenting antibiotic resistance. Under selective pressure imposed by the use of antimicrobial agents, bacteria, having developed resistance mechanisms, can multiply and expand, while the rest of bacteria are inhibited or destroyed. The resistance genes could be passed from one bacterium to another by horizontal gene transfer (HGT). The donors and the recipients can belong to different bacterial species and genera, located in various ecosystems, human and/or animal hosts, situation reflected by the „One Health" concept. Nowadays, a shift in perception on the traditional paradigm of antibiotics and AMR is emerging. Antibiotics are beginning to be perceived as inter-microbial signaling agents and even as sources of nutrition to microorganisms, rather than means to fight the pathogens. The present review focuses mostly on the mechanisms of antibiotic resistance and horizontal gene transfer.

Key words: Antibiotics, resistance, resistome, horizontal gene transfer.

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

AN OVERVIEW OF THE MOLECULAR EPIDEMIOLOGY OF THE MEAT – BORNE PATHOGEN *TOXOPLASMA GONDII* IN PORK AND WILD BOAR– A MINI REVIEW

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Summary

Toxoplasma gondii is considered one of the most important protozoa with zoonotic character, which can be acquired through consumption of the raw or undercooked meat. In veterinary practice of meat inspection, the presence of the tissue cysts, which if ingested by human consumer produce the diseases namely toxoplasmosis, regularly is not diagnosed. The aim of this work was to review the molecular epidemiology of the meat – borne pathogen *Toxoplasma gondii* in pig (*Sus scrofa domesticus*) and wild boar (*Sus scrofa*), processing twenty PubMed retrieved representative studies carried out over the last 10 years, in different geographical regions of the world. In foreground, the infection prevalence in different countries, and especially the *T. gondii* isolation rate from seropositive animals, risk factors providing the most reliable and accurate risk assessment for the consumer, isolation source of the pathogen and identified genotypes are presented. This update should be useful for the epidemiologists, public health workers and veterinarians.

Key words: prevalence, meat, *Toxoplasma*, epidemiology, PCR

BIOCIDE EFFECT ON BIOFILM-INTEGRATED BACTERIA ISOLATED FROM MEAT CARCASSES

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Summary

The aim of this paper was to evaluate the effectiveness of four disinfectants against planktonic and integrated in biofilm bacteria isolated from the meat surfaces. Disinfectant products were selected from commercial preparations commonly used for decontamination in food processing plants namely P3-oxysan and P3-topax 66 (Ecolab®), hydrogen peroxide and sodium hypochlorite. Biocide effect was evaluated against various species of *Enterobacteriaceae* and *Pseudomonas* isolated from the bacterial biofilm on meat surface (pork, beef and poultry) and against some bacterial associations from carcasses.

P3 topax-66 inhibited growth of all bacterial strains and associations, both as planktonic (0.025%) and integrated into the biofilm matrix (0.05%) at the lowest concentrations tested. P3-oxysan expressed bactericidal properties starting at 0.06% on *Escherichia coli* isolated from pig carcasses. The most resistant strain was *Enterobacter cloacae* and the association between *E. coli* and *Serratia liquefaciens*, which were resistant even at concentrations of 0.15%. Microbial biofilm eradication concentration was found 0.12% product against *Pseudomonas putida*, but for most bacterial strains tested and associations of these effects occurred at concentrations twice as high (0.25%).

The *Serratia* isolated strains and their associations included in biofilm were more resistant to the action of hydrogen peroxide compared to planktonic cells (1500 ppm). Bacteria in monoculture biofilms and mixt biofilms, with one exception (*E. coli*), were 10 times more resistant to disinfectants concentrations (15000 ppm).

Microbicidal effect of sodium hypochlorite was expressed at lowest concentration tested, 75 ppm for planktonic cells and 150 ppm for the bacterial biofilm.

Key words: microbial biofilm, meat, disinfectant effects, Calgary biofilm device

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IDENTIFICATION OF ANTIBODIES AGAINST *RHODOCOCCUS EQUI* IN HORSES BY USE OF THE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) - A REVIEW

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Summary

Rhodococcus equi is an emerging pathogen witch causes clinical signs in young foals, from 1 to 6 months of age, expressed by pneumonia and enteritis. Foals are unable to produce their own IgG against *Rhodococcus equi* until 10 or 12 weeks of age, so they depend on the mare antibodies, if the mother was vaccinated before foaling. Due to clinical diagnostic difficulties, researchers have developed different serological tests to evaluate the incidence and prevalence of the disease in equine. To identify the antibodies in equine and sick foals different serological assays were used, no commercial one being available. The most used immunological method is the enzyme-linked immunosorbent assay (ELISA). Nevertheless, all of the developed testes are "*in house*", based on other appropriate commercials ELISA assays, such as *Corynebacterium*. In these tests the authors have used different strains as antigens. The goal of this review is to describe and evaluate the different types of "*in house*" ELISAs used in the diagnosis of equine rhodococcosis.

Key words: ELISA, *Rhodococcus equi,* rhodococcosis, serology, equine

IDENTIFICATION OF ANTIBODIES AGAINST *RHODOCOCCUS EQUI* IN HORSES FROM DIFFERENT REGIONS OF ROMANIA USING AN IN HOUSE ELISA. FIRST REPORT

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Summary

Rhodococcus equi infection occurs worldwide and is one of the major causes of foal mortality during the first six months of life. The use of serogical tests in diagnosing equine rhodococcosis is limited; however, they play crucial role in immunological studies and potential control of the outbreaks. The objective of this study was to investigate the presence and levels of the antibodies against *Rhodococcus equi* in equine sera in different points of Romania, using an in house ELISA test. For that, a bacterial cell lysate was used as antigen. The technique involved in establishing the anti-*Rhodococcus equi* antibody levels in sera from Romanian horses was previously standardized in Poland on 175 sera. The positive and negative control sera were used to establish the concentration of the antigen on the plates. Subsequently, sera from 435 horses located in different regions of Romania were tested. The tests were carried out at SGG Warsaw, Poland between May and July 2013. The results were calculated in ELISA units, as percentages of the difference between the positive and negative controls. Most of the horses tested positive (74.59%). The results supported the presence of antibodies against *Rhodococcus equi* in Romanian horses at different levels. This is the first alert that indicates the presence and individual variability of anti-*Rhodococcus equi* antibodies in equine in Romania.

Key words: ELISA, *Rhodococcus equi,* rhodococcosis, serology, equine

RESEARCH REGARDING THE ANTIBACTERIAL ACTIVITY OF ACID-SOLUBLE CHITOSAN

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Summary

The paper presents the results of the antimicrobial activity of chitosan. Chitosan is a copolymer of glucosamine and N-acetylglucos- amine prepared from chitin by deacetylation. It is a natural and non toxic product that is widely-used in medicine and food industry.

The antimicrobial effect was tested against the representative Gram-positive bacteria: *Staphylococcus aureus* and *Bacillus cereus* and Gram-negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa* and one specie of yeast: *Candida albicans.*

Results obtained proved that chitosan exhibited a good antimicrobial effect on tested microorganisms.

Key words: chitosan; antimicrobial activity

CLASSICAL CHARACTERIZATION OF *E. COLI* ISOLATED FROM LAYING HENS

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Summary

Outbreaks of *Escherichia coli* infection in poultry are reported worldwide and the main feature of these pathologies is represented by significant economic losses associated with high morbidity and mortality, elevated costs for antimicrobial therapy and control measures. In case of laying hens, the egg production may diminish, but the most important consequence refers to the irreversible lesions affecting both the genital tract (salpingitis) and other internal organs. This paper was aimed to isolate, identify and characterize avian pathogenic *Escherichia coli* (APEC) responsible for two severe outbreaks of mortality in two related laying hens farms from North Transylvania, that reported sudden death (from 1 to 5% daily during one week) in spite of two distinct antimicrobial treatments using broad spectrum antimicrobials administered with the drinking water. The dead carcasses presented severe lesions of fibrinous polyserositis, perihepatitis, pericarditis, salpingitis and vitellin peritonitis, used as samples for the isolation of *E. coli* strains on selective agar (Brilliance™ E. coli/coliform Selective Agar, Oxoid). After the biochemical properties testing, the isolated strains were serotyped and the antimicrobial *in vitro* susceptibility was evaluated using Kirby-Bauer method according to CLSI guidelines. The results indicated the presence of two serotypes: O2:K1 and O1:K1, both with an extreme level of antimicrobial resistance exhibited towards: amoxicillin, amoxicillin and clavulanic acid, colistin, tetracycline, doxycycline and enrofloxacin. Susceptibility was recorded only in case of florfenicol and lincomycin with neomycin.

The existence of multiple antimicrobial resistance underlines the importance of rational use of antibiotics.

Keywords: *E. coli*, laying hens, serotyping, multiple antimicrobial resistance

E. COLI SEROTYPES AND THEIR *IN VITRO* SENSITIVITY TO COMPLEMENT IN FARMED ANIMALS

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Summary

E. coli represents one of the most widespread pathogens worldwide, causing serious episodes of enteric or non-enteric diseases. Such episodes are very difficult to overcome due to the fast course of the disease and also delayed changes in the adaptive immunity. Thus, the importance of non-specific, immediately acting innate immune factors becomes important. This study was aimed to investigate the resistance/sensitivity of *E. coli* strains isolated from calves with clinical signs of diarrhea (n=10), piglets showing edema disease (n=9) and coligranulomatosis of hens (n=11) to the complement. Microbial samples were subjected to classical isolation techniques using MacConkey and a selective agar (Brilliance™ *E. coli*/coliform Selective Agar, Oxoid). The strains were confirmed and further characterized by serotyping. Their sensitivity/resistance to complement was evaluated *in vitro* by a serial dilution technique (1/10, 50µg of complement to 1/80, 6.25µg of complement), using freeze dried Guinea pig complement. All the tubes were inoculated with 35 µl of a 24h bacterial culture of each serotype and the optical densities were determined spectrophotometrically (d=0.5, λ=535 nm) in 96-well plates. The sensitivity of the isolated strains varied with the serotype and concentration of the complement, rather than the species of isolation. The highest efficacy of the complement was observed versus strains O1:K1 isolated from hens (0.107 optical density units, ODU at dilution 1/40) and O8:K25 (0.126 ODU) and O8:K28 (0.110 ODU) isolated from calves with diarrhea, at its highest dilution rate. The highest resistance was recorded in strains isolated from edema disease (O141(H4), 0.522 ODU at the dilution rate of 1/80). The results supported an increased resistance to complement, stressing the enhanced pathogenic potential in the isolated *E. coli* strains.

Key words: *E. coli*, complement, neonatal diarrhea, edema disease, coligranulomatosis

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THE STUDY OF THE MICROBIAL BIOFILM STRUCTURE IN VARIOUS BACTERIAL SPECIES

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Summary

Bacteria embedded in biofilm are in various stages of growth depending on the location, depth and concentration of nutrients. Limited access within biofilm nutrients reduces the growth rate, which can affect susceptibility to antimicrobials. The aim of this study was to evaluate the characteristic of some bacterial strains and quantification and characterization of microbial biofilm structure utilizing specific programs. Stages of bacterial biofilm formation on surfaces with different levels of roughness, by bacterial strains were studied, by confocal microscopy, achieving both characterization and quantification of microbial biofilm formed through specific Comstat programs. The most suitable substrate for biofilm formation was the unpolished 304 stainless steel type, followed by 304 polished stainless steel, unpolished stainless steel 316, polished 316 stainless steel, PVC, glass and copper, respectively. The amount of biofilm formed is dependent on the type of micro-organism growth and the type of culture: in monoculture or mixed biofilm. A great variability in the three-dimensional architecture of microbial species analyzed was observed. This aspect can have an essential role in the colonization of substrate tolerance substances with antimicrobial role and especially the persistence of these microorganisms in the environment. Using confocal microscopy and image analysis software offers numerous possibilities obtained thorough qualitative and quantitative analysis of biofilm in particular its architecture.

Key words: microbial biofims structure, laser confocal microscopy,

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INFLUENCE OF CLIMATIC FACTORS ON THE HYGENIC QUALITIES OF MILK OBTAINED IN THE SUBCARPATHIAN MOUNTAIN REGION

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Summary

There is a growing interest by the dairy industry in exploiting the biodiversity of the mountain grassland as the cheese obtained from the milk produced in these regions has some specific characteristics. It is thought that the specific mountain climate provides higher quality milk that is the basis of traditional dairy products. A significant role in obtaining traditional dairy products has the mountain geo-climate that greatly influences the health of the animals and the milk's composition. During September 2011- August 2013, ample research has been made on the influence of climate on the hygienic quality of the milk processed in the south-western region of the Oriental Carpathians. Data on temperature, humidity, atmospheric pressure and rain has been provided from a local meteorological station. The milk samples hygiene were tested by determining the total number of germs (NTG) and number of somatic cells (NCS), using *Soleris and Ekoscope*. Data was analyzed using *MedCalc*, *SPSS version 20*, and *GRAPH Pad Prisma* and the connection between these two parameters has been determined with *Pearson r* coefficiency correlation. Statistical analysis of the correlation between climate factor-NTG and climate factor-NCS revealed some significant results. Synthetic analysis of these correlations shows that temperature-humidity index has a great influence over NTG and NCS. NTG and NCS were greater with rising temperatures (heat stress) and low humidity. Temperature and humidity are the most important climate factors that influence biodiversity on mountain grasslands and has also a positive effect on the milk produced.

Key words: mountain region, milk, NTG, NCS.

THE EFFECTS OF CLIMATIC FACTORS ON FAT AND NON-FAT DRY MATTER CONTENT OF RAW MILK OBTAINED FROM CATTLE RAISED IN CONDITIONS OF A SUBCARPATHIAN MOUNTAIN RANGE

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Summary

Health, welfare and production ofcattle are significantly influenced by geo-climatic conditions, which can act either directly on livestock, or indirectly on forage sources biodiversity. It is well known that climatic factors exert a major impact on the expression of productive potential in lactating cows, and especially on the quality level of milk. In this study, focused on investigation of raw milk, we propose evaluating the influence of the main climatic factors (temperature, humidity, atmospheric pressure, precipitation) on the seasonal evolution of fat and non-fat dry matter content of raw milk, obtained in the conditions of a subcarpathian mountain range.The research was conducted on the raw milk processed, from December 2011 to July 2013, in a commercial unit in southwestern part of Eastern-Carpathians. In collaboration with a weather station in the area, it was monitored the seasonal dynamics of temperature, humidity, atmospheric pressure and rainfall. Analysis of these data was the basis for quantifying the influence of the main climatic factors on fat and non-fat dry substance content of raw milk that was submitted to physicochemical testing with the *Ekomilk M* semiautomatic analyzer. For statistical analysis of data obtained were used *MedCalc* software and the *Pearson r.* correlation coefficient.From statistical analysis of the values obtained from the evaluation of variables climatic factors-fat/non-fat dry matter content, there were established the following correlations, relevant to the objectives pursued: fat content and non-fat dry matter content increased in inverse proportion with the decrease of air temperature (10.7°C) and proportionally with the increase of humidity (76%) in autumn season.We considerthat, the evolution of temperature andrelative air humidity representthemain environmental factors that provide biodiversity of mountain pastures and positively influence the overall composition of milk, especiallyitsfat and non-fat dry matter content.

Key words: raw milk, fat, non-fat dry matter, mountain range.

ATTEMPT TO INDUCE IMMUNOLOGICAL TOLERANCE IN CHICKEN EMBRYOS USING INOCULATION OF ALLOGENEIC CELLS INTO CHORIOALLANTOIC VESSELS

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Summary

The purpose of this study was the induction of intraembryonic tolerance to allogeneic antigens using mixed hematopoietic chimeras without recipient' conditioning. The experiments were performed on 180 embryonated eggs (Cobb 500 hybrids), obtained from S.C. AVE IMPEX SRL, Romania, and 6 donor adult birds (Rosso hybrids). Allogeneic blood and bone marrow mononuclear cells were obtained from processing of blood and bone marrow samples by centrifugation on Ficoll-density gradient and labeled with PKH2 dye and were inoculated into chorioallantoic vessels (days 9, 11 and 13 of incubation). After hatching, the following aspects were evaluated: hematopoietic mixed chimerism, donor-recipient compatibility and distribution of T cells subsets, by flow cytometry. The status of haematopoietic chimera could not be confirmed for any of the recipient birds, and mixed lymphocyte reaction shows a partial compatibility with donor of allogeneic cells only in the group obtained after inoculation of bone marrow mononuclear cells in the 9^{th} day of incubation. Evaluation of lymphocyte profile in birds from groups obtained after blood mononuclear cells inoculation in days 9, 11 and 13 and control groups shows a higher representation of naive T cells, effector and memory subsets being less represented. In contrast, in the birds from group obtained after inoculation of bone marrow mononuclear cells in the 9^{th} day of incubation, effector and memory phenotypes were better represented than the naive ones

Key words: immunological tolerance, allogeneic cells, chorioallantoic vesels, birds

CELLS INVOLVED IN ALLOGRAFT REJECTION

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Summary

In this paper there are described all the cells involved in the allografts rejection - antigen presenting cells (APCs), endothelial cells, T cell, B lymphocytes, NK cells, eosinophils and monocyte-macrophage system - both in mammals and birds. For individual cells are presented the origin, surface molecules and mechanisms by which they induce rejection. Most of these cells participate actively in these mechanisms, but some, such as endothelial cells of the allografts, are only targets of cellular effectors. Of all these cells, APC, T lymphocytes and monocyte-macrophage system are the most important for allotrasplant immunology.

Key words: allografts, rejection, cells

RESEARCH REGARDING IMMUNOSUPPRESSION INDUCED BY T-2 TOXIN

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Summary

The purpose of this research was to describe the immunosuppressive effect exerted *in vitro* by T-2 toxin. 30 white miced were used, divided in three groups: control group (A), experimental group 1 (B, treated with *Mycoplasma* immunogen) and experimental group 2 (C, treated with T-2 toxin and *Mycoplasma* immunogen). After spleen harvesting and mononuclear cells separation, the following paramenters were determined: proliferation factor, with and without stimulation of cell cultures with phytohaemagglutinin (PHA), concanavalin (ConA) and *Mycoplasma* immunogen (My), and interleukin-2 synthesis in cell cultures. The results show that in cell cultures of group A (control), cell proliferation takes place within normal parameters, while in group B, cell proliferation was moderate in absence of mitogens, and higher after stimulation with PHA. The data obtained regarding the IL-2 show that treatment with mitogens has effects on the synthesis of this interleukin, but this influence varies among groups, depending on *in vivo* treatment. Thus, in control group, the active dilution for the stimulation with PHA was with 1/3 lower than in stimulation with My. In group B, synthesis of interleukin-2 was higher when the stimulation was carried out with *Mycoplasma* extract. In group C, consisting of mice receiving T-2 toxin and *Mycoplasma* extract, synthesis of interleukin 2 was canceled, showing that T-2 toxin has a significant immunosupresive effect.

Key words: T-2 toxin, immunosuppresion, *Mycoplasma* immunogen, cell culture

CHANGES IN CELLULAR COMPARTMENT OF THE IMMUNE SYSTEM ASSOCIATED WITH ENZOOTIC BOVINE LEUKOSIS

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Summary

Enzootic bovine leukosis (EBL) is a viral disease of adult cattle caused by bovine leukaemia virus (BLV) and is accompanied by a series of changes in cellular and humoral compartments of the immune system. This review describes the most important cells affected by viral infection cells B cells, T cell immune response, as well as the kinetics, turnover, recirculation, and homing of lymphocyte populations. Cellular changes induced by BLV, taken together, are important for elucidating the pathogenic mechanisms underlying EBL, for and early and certain diagnosis of this viral disease and also for establishing the most efficient therapeutic strategies.

Key words: enzootic bovine leukosis, B cells, T cells

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

THE PREVALENCE OF *YERSINIA* SPECIES IN SLAUGHTERED PIGS

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Summary

This study follows the isolation frequency of *Yersinia* germs from different organs and carcasses samples collected from slaughtered pigs. The study presents a high importance because it reveals the probability of contamination with bacterial species of *Yersinia* genus, which are pathogen for human beings (especially for infants) through alimentary toxic infections, manifested by austere diarrhea syndrome. To determine the isolation prevalence of *Yersinia bacteria*, a total number of 7.258 organs and muscle samples were harvested and processed through microbiological assays; the study was developed during a period of 3 years (2012-2014). The identification-confirmation stage was realized by using API 20E galleries: the API 20E system offers the possibility of identification of *Yersinia germs* in 24 h, as well as other enteric bacteria.

The obtained results demonstrated a low incidence at portage of bacteria belonging to *Yersinia* genus (maximum 0.21 %), but these low values can be determined by the inhibition of *Yersinia* bacteria by preferential development of other bacteria which populate the intestine. The highest isolation frequency was observed at the level of serous surfaces (pleura and peritoneum), this specific observation demonstrating the carcass post-mortem contamination either because of slaughtered pigs evisceration in improper conditions (evisceration in improper conditions and minimal techniques) or because of the carcass faecal contamination. The statistical analysis on 3 years period continues a previous study. The obtained results establish a total prevalence of 0.321. The annual variations of the isolation frequency were situated between relatively low values (between 0.19% and 0.54%).

Key words: *Yersinia*, pig, prevalence, isolation.

EVALUATION OF AFLATOXIN M1 INCIDENCE IN RAW MILK AND RIPENED CHEESE RETAILED IN A TRADITIONAL MARKET

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Summary

Aflatoxin M1 is a metabolite of aflatoxin B1 which can occur frequently in milk and dairy products obtained from cattle that have been fed with contaminated forages. The study aimed to evaluate the incidence of aflatoxin M1 in the milk and dairy products sold on a traditional market from Transylvania area. All 60 samples of milk and 50 samples of ripened cheese were purchased during the period of September 2013 and March 2014. For the detection of M1 toxin we used Elisa method. We have found that 4 samples (8%) of milk have exceeded the European Commission regulation standard (50 ng/kg). None of the ripened cheese products have exceeded this accepted limit. Our results show that raw milk has a higher prevalence of contamination compared to processed products. In conclusion, the area studied should be monitored more carefully and the assessment of the mycological quality of raw milk obtained in small scale farms to be mandatory.

Key words: Aflatoxin M1, milk, cheese, market

LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ VOL. XLVIII(2), 2015, TIMIŞOARA

STUDY OF TOTAL AEROBIC MESOPHILIC BACTERIA DYNAMICS IN A LAYING HENS HOUSE, REARED ON DEEP LITTER

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Summary

This paper presents the results obtained by studying the air's load of aerobic mesophilic bacteria from a hall with laying hens reared on permanent litter over the whole breeding cycle and exploitation period.

The air\s bacterial load from the cargo hall exceeded the recommended value for our country, as well as those recommended by researchers from abroad, 2.5 x 10^5 CFU / m^3. The mean total aerobic mesophilic bacteria number or the total plate count (TPC) value increased from 9.07 x 10^4 CFU / m^3 in the first month of exploitation, to 1.05 x 10^6 CFU / m^3 in the last month, noting that this growth was achieved slowly since the second month until the ninth month of growth and exploitation.

Key words: total aerobic mesophilic bacteria, dynamics, hens house, deep litter

STUDY OF THE FUNGI DYNAMICS IN A POULTRY HOUSE WITH PERMANENT LITTER

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Summary

The paper presents the results of a study done on the fungi load dynamics in the air from a hall for hens reared on permanent litter, over the cycle and exploitation.

Fungal load in the air has reached high level values. Mean of TNF increased from 1.43 x 10^4 CFU / m^3 in the first month of operation to 9.99 x 10^4 CFU / m^3 in the last month. This growth was achieved slowly since the second month until the last month of hens growing period.

Key words: fungi, dynamics, hens' house, deep litter