COMPARATIVE STUDY BY MALDI TOF MASS SPECTROMETRY OF SOME SPECIES OF THE GENUS *BACILLUS*, FAMILY *BACILLACEAE*

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Summary

In recent years, mass spectrometry MALDI TOF (Matrix Assisted Laser Desorbtion Ionization - Time of Flight / desorption ionization matrix laser and calculation of mass by measuring the time of flight) was used for the rapid identification of microorganisms in clinical microbiology laboratories. The germs of the genus *Bacillus* are ubiquitous bacteria, because the spores they confer increased resistance to environmental factors.

The cultures of *Bacillus* spp studied (*Bacillus anthracis, Bacillus subtilis, Bacillus megaterium*) were seeded via loop prick on the simple agar and blood agar, in Petri plates in order to obtain isolated colonies. Bacterial colonies obtained were processed for 16S ribosomal proteins extraction, by the "*Microorganisms Profiling*" extraction trifluoroacetic acid (TFA 80%) procedure and analyzed with Microflex® LT 20 equipment.

Spectra analysis of the genus *Bacillus* biological agents representing the polypeptide fragments of the 16S ribosome revealed a mass range between 800 and 12000 Daltons. The characteristics average peaks has been included between: 1100-1300 Daltons; 3550-3650 Daltons; 4600-4950 Daltons; 5400-5900 Daltons; 6300-7300; 9300-10300 Daltons; 11500-12000 Daltons; 10500 to 11500 Daltons.

Mass spectra obtained were identified as belonging to the tested bacterial species and further confirmed by classical bacteriological methods. Analyzing the results of studied strains, obtined by MALDI TOF technology of studied strains, we can conclude that they are comparable in terms of structure and composition of 16S ribosomal subunit; thus demonstrating a high degree of phylogenetic relatedness.

Keyword: *Bacillus spp.,* diagnosis, MALDI TOF

MICROBIOLOGICAL MONITORING OF LACTOSE USED IN THE PREPARATION OF BIOLOGICAL AND DRUG PRODUCTS

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Summary

Lactose is a disaccharide derived from galactose and glucose used in the production of biologicals and medicinal products.

Microbiological tests done to monitor lactose are counting mesophilic bacteria and fungi that may grow under aerobic conditions (the determination of TAMC of casein soya bean digest agar, the determination of TYMC of Sabouraud-dextrose agar) and determine the presence of *E. coli* (subculture on a plate of MacConkey agar and confirmation by biochemical tests).

The tests are designed primarily to determine whether a substance or preparation complies with an established specification for microbiological quality.

Key words: Lactose, *E. coli,* biochemical tests, TAMC, TYMC

IDENTIFICATION OF SALMONELLAS BY COMPARING CLASSICAL METHOD WITH THREE RAPID METHODS

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Summary

Thanks to an innovative technology involving bacteriophage recombinant proteins, the test VIDAS® UP *Salmonella* (SPT) (Biomerieux), allows the specific detection of *Salmonella* in food, environmental and material from primary production samples.

The present study was done comparing the classical method SR EN ISO 6579: 2003 with the polymerase chain reaction (PCR), immunoenzymatic enzyme (miniVidas) and impendance (µ-Trac), through sampling of faeces from pigs and sanitation tests on the surface of carcasses.

Analyzing the results obtained by the classical method and rapid methods (PCR miniVidas and µ-Trac) a number of of 43.13% positive samples resulted by PCR, followed by miniVidas method, which obtained 41.17% positive samples, compared to the bacteriological and inductance method (µ-Trac), where where obtained only 35.29% positive samples**.**

Key words: Identification, method, samples, *Salmonella*

MOLECULAR BIOLOGY RESEARCH REGARDING LYOPHILIZED *FRANCISELLA TULARENSIS* REVITALIZED AFTER 40 YEARS

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Summary

Francisella tularensis is a highly contagious Gram-negative bacteria that causes tularemia or "rabbit fever" and it is contagious to humans. There are four known subspecies of *Francisella tularensis,* two of them are the most studied: *A Type strain* that is the more virulent (found in North America) and *B Type* (subspecies *holarctica*, also referred to as *palearctica* strain, found in Europe) that is the less virulent. The two other non-virulent subspecies are: *mediasiatica*, found in central Asia and *novicida*, of which is not know very much.

Our researches have been based on the revitalization of lyophilized strains of *Francisella tularensis* in order to obtain the positive controls required for the in hause real time PCR kit for CCHFv and TBEv and *Francisella tularensis* and *Borrelia burgdorferi s.l*. agents transmitted by ticks.

Lyophilized strains were rehydrated in nutrient broth, cultured in *Francisella tularensis* specific medium (CHAB-PACCV) and passed on nutrient medium. Microbiological diagnosis (including optical microscopy) was confirmed by immunoassay (Tularemia biothreat Alert kit, Tetracore) and molecular tests: Real Time PCR with TaqMan *Francisella tularensis* detection kit, Applied Biosystems for two genes (*fop*A and *tul*4) and TicKitqPCR (in house kit) for one target insertion sequence-like element (IS*Ftu*2).

The methods have confirmed the presence of *Francisella tularensis* strain in revitalized samples after 40 years of storage.

Key words: *Francisella tularensis*, identification, Real Time PCR, lyophilized, revitalized

SENSORIAL AND QUALITY PARAMETERS ASSESSMENT IN SOME TRADITIONAL MEAT PRODUCTS FROM ROMANIA

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Summary

Nowadays, traditional meat products are a well-consumed foodstuff in Romania, so the assessment of the sensorial and quality parameters is very important for food safety.

The traditional meat products samples (peasant file - 15, peasant neck -12 and peasant chest – 10) were collected from a large meat processing plant in Romania.

For all samples, sensorial assessment (shape and dimensions, general aspect, aspect on section, smell and taste) was performed. Using the Corona 45 Visnir analyzer, water, fat, total protein and NaCl content were determinate.

The sensorial assessment revealed normal parameters. The results ranged from 57.9 to 70.2% for water content, 5.1 to 17.4% for fat content, 15.5% to 19.8% for total protein content and 2.4 to 3.2 % for salt content.

All results were within the limits established by legislation, respectively maximum 5% for NaCl and minimum 15% for total protein (10). For water and fat content, there are not legislative limits, but the results were according to the product specification.

From this limited study, it can be considered that there is no risk for human health linked to the consumption of traditional meat products, but a continuous assessment of quality parameters should be performed, in order to ensure good products for consumers and the traditional meat products quality remains an exciting subject which has a stake in the near future.

Key words: traditional meat products, sensorial assessment, quality parameters

RESEARCHES REGARDING AIR CONTAMINATION WITH MESOPHILIC BACTERIA IN POULTRY HOUSE REARED ON ENRICHED CAGES

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Summary

The results of a study done on air load aerobic bacteria from a house for hens reared on enriched cages over one breeding cycle are presented in this paper.

The mean value of total number of mesophilic bacteria from hens house presented large oscillation from , from 3.61 x 10^4 CFU / m^3 of air in the first month, to 1.94 x 10^5 CFU / m^3 air in the sixth month, when it was recorded the maximum value, just to slightly decrease after, but still remaining at values of around 10^5

For this type of rearing hens, the total number of aerobic mesophilic bacteria ranged, for a long period of time, under the recommended values as some researchers referenced.

Key words: hens house, air, aerobic mesophilic bacteria, dynamics, enriched cages

MOLECULAR DETECTION OF TICK BORNE ENCEPHALITIS VIRUS IN FIELD COLLECTED TICKS FROM ROMANIA

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Summary

Tick-borne encephalitis virus (TBEv) is an member of the family *Flaviviridae* and is transmitted by the bite of infected ticks or occasionally by ingestion of unpasteurized milk. In Romania the geoclimate and ecological conditions allow the maintenance and circulation of TBEv in natural foci, situation that caused the outbreak of 1999 in Sibiu county.Ticks are of considerable medical and veterinary importance and tick-borne encephalitis infections are emerging diseases in humans.

We are analyzed pools of the adult ticks (*Ixodes spp.*, collected by flaging technique from the vegetation and the *Hyalomma spp.*, collected from spur tortoise *(Testudo greaca)*. The sites were randomly selected and the ticks were preserved in alcohol or RNAlater® (Ambion®, Applied Biosystems) solution until identification.The RNA extraction was performed with Trizol methods and amplification reaction was done after quantification of genetic material with specific nanodrop system.The molecular detection of TBEv was achieved by reverse transcription PCR, using specific primers for 3'UnTranslated Region (UTR) of the RNA genome of the virus (included in the TicKitqPCR kit concept).

The positive results have demonstrated the potential implication of ixodide species (*Ixodes ricinus* and *Hyalomma aegypti*) in transmision of Tick Borne Encephalitis virus in Romania.

Key words: 3'UnTranslated Region (UTR),TBE virus, *Ixodes spp., Hyalomma spp.*

DETERMINATION OF SERUM IMMUNOGLOBULINS IN NONSPECIFIC STIMULATED DAIRY COWS

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Summary

Determination of serum immunoglobulins (IgA, IgG, IgM) concentration allows the assessment of the health status of lactating cows, including in experimental conditions. In the present experiment we used a group of seven lactating cows, clinically healthy, in the first 3-4 months of lactation with high milk production, to determine the level of IgA, IgG and IgM in their serum before and after inoculation with an immunomodulatory phytoextract (patent pending).

According to the experimental protocol, cows' blood was collected before s.c. administration of the phytoextract, then at 24 hours, 4 days, 7 days and 14 days from the time of inoculation. Blood samples were processed for hematological and serological analysis. Hematological examination showed a significant hyper-leukocytosis even at 24 hours after phytoextract inoculation which lasted up to 14 days; increased neutrophils level at 24 hours and significant decrease in lymphocytes that rise gradually to normal level after 14 days.

For the determination of circulating immunoglobulins, serological tests using ELISA kits were carried out. Comparing to the mean values (expressed in mg / ml) registered prior to the experiment, at 24 hours after phytoextract inoculation, a marked decrease was recorded for all parameters, after which each type of immunoglobulin turned to a different direction. The main values of IgA, with special role in mucosal protection and IgM, the main immunoglobulinic opsonin in serum were slightly varying during the 14 days experimental period (plateau level); IgG showed a significant gradual decline even at 14 days after experiment (probably because the body's defense response was taken by phagocytes).

Key words: hyperleukocytosis, immunomodulation, neutrophilia, immunoglobulins

METABOLIC STATUS OF PEKIN AND DOMESTIC DUCKS GROWN IN MICRO FARMS

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Summary

Web-footed poultry are not the solution for the "worlds hunger", they are an important niche în meat production chain, being the only poultry that use food mostly ignored, such as: the grass growing aside the roads, moats, swamps. From ducks people use: meat, eggs, pouf, grease, eventually inferior members (flippers-for gelatin aș well aș carpenters clay); ducks are household poultry, resistant to disease, temperature variations etc. The biological material for this research is represented by the ducks from two micro farms (Tăticu- Hărman Farm which grow domestic ducks, Brașov County; Luisa Savu Frumușani Micro farm, Călărași County which grow pekin ducks). În our country, în 2016, it is not known the existence of any intensive growing farm for web-footed. The evaluation of the heard ducks health was achieved by assessment of the metabolic status based on biochemical and hematological determinations.

Key words: domestic ducks, pekin ducks, metabolic status

IDENTIFICATION OF ORAL MICROFLORA IN DOGS WITH DIAGNOSED DENTAL DISORDERS

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Summary

Out of over 300 species of bacteria present in the oral cavity in companion carnivores, some are present only at certain times (transient, nomadic), but most of them are permanent residents of the dental plaque and may induce severe periodontal diseases.

Gingival, parodontal and dental plaque samples were examined by classical microbiological techniques and identified by API chromogenic test.

The results indicated that *Staphylococcus spp.* was found in all patients, but was present only in gingival isolates (32.25%). Microorganisms of the genus *Streptococcus* accounted for 8% of the bacteria isolated from the dental biofilm.

The genus *Porphyromonas* held the highest overall prevalence (18.18%), followed by *Corynebacterium spp*. (17%), *Eikenella (Bacteroides) corrodens* (12.12%), *Actinomyces canorus* (7.7%) and *Prevotella intermedia* (7.57%) of the entire bacterial population. *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum* were found in 7% and 6.06% of the total number of oral microorganisms, respectively.

According to this study, the plaque in companion dogs was not a homobacterian aggregate, but a biofilm composed of several bacterial species. The larger the number of dental biofilm bacterial species, the more serious should be the clinical development of the dental disease.

Key words: dental plaque, microflora, *Porphyromonas spp.*, *Corynebacterium spp*.

THE IMMUNOLOGICAL VALUE OF DIFFERENT INACTIVATION METHODS IN OBTAINING DESENSITIZING PRODUCTS FROM STAPHYLOCOCCAL STRAINS

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Summary

Clinical observations carried out on some canine patients suffering from atopic dermatitis with concurrent staphylococcal infection led to the conclusion that the bacteria could cause hypersensitivity. Intensely pruritic inflammatory lesions tending to expand suggested an allergic reaction. In these cases, immunotherapy is carried out with gradually increasing doses of allergen over a period of time. The study aimed to compare the value of different preparatory techniques to obtain desensitizing allergens for dogs with staphylococcal dermatitis.

Different inactivation methods (heating to 80ºC for 10, 20 and 30 min, repeated freezing-thawing) were tested to prepare various antigenic extracts from two staphylococcal strains, *S. aureus* and *S. intermedius*, isolated from dogs with bacterial dermatitis. Sera sampled from similarly diseased dogs were tested for the presence of anti-staphylococcal antibodies and possibilities for further use in describing the antigenic properties of the staphylococcal preparations, by rapid agglutination and agar gel diffusion against a *S. aureus* strain. Rapid and slow agglutination, as well as double immune diffusion tests were then used to define the antigenic properties of the staphylococcal extracts.

The diagnostic value of the preparation and usefulness of different inactivation techniques in obtaining antigenic products are discussed.

Key words: *Staphylococcus aureus, Staphylococcus intermedius*, antigenic structure, inactivation

COMPARATIVE ANALYSIS OF FAT GLOBULES OF MILK FROM ALPINE AND CARPATHIAN GOAT

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Summary

Fats represent the basic morphological characteristics of milk. It is the most variable component present as globules or microspherules with diameter between 1,6 µm and 10 µm. They are different from one species to another, within the same species can vary depending on breed, the animal's health and stage of lactation. The purpose of the study is the comparative analysis of spherules of fat and of the conglomerates between them in goat milk and colostrum originated from two different races. Therefore morphophysiological investigations were conducted on samples of milk and colostrum obtained from clinically healthy Alpine (n = 12) and Carpathian goats (n = 12) during March to July 2015, using the Squash technique on vital preparations colored panoptic (Dia-Quik-Panoptic) and (Gill's Haematoxilin Romvachrom testing). Microscopic examination revealed the presence of small spherules of fat and a more evident agglomeration in both compared races' colostrum. At Alpines, quantitatively, there is an obvious agglomeration of microspherules fat, which leads us to argue that in terms of percentage this breed's milk is more concentrated in fat. It is not recommended the staining technique (Gill's Hematoxylin Romvac Rom) because after examination preparations are deteriorating rapidly. We recommend using Dia Quick Panoptic because through this technique preparations are of better quality and highlight much better the morphological and physiological characteristics of the spherules' fat.

Key words: goat milk, colostrum, fat spherules

STUDIES ON FUNGI AIR CONTAMINATION IN POULTRY HOUSE REARED ON ENRICHED CAGES

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Summary

The results of a study done on the fungi load and dynamics in the air from a house for hens reared on enriched cages, over the cycle and exploitation are presented.

The mean of TNF increased from $14.47 \times 10^3 \pm 1.63 \times 10^3$ in the first quarter of operation cycle to $1.93 \times 10^4 \pm 0.36 \times 10^4$ in the last of breeding.

The increase of fungi in air load hall caged hens was very slow in the first three months of the operating cycle. The concentration of fungi found in hen's house taken in this study may present a health risk factor for people working in this environment

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

HIGHLIGHTING THE CROSS-REACTION BETWEEN WEST NILE VIRUS AND OTHER FLAVIVIRUSES BY IMMUNOFLUORESCENCE

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Summary

West Nile virus (WNV) belongs to the genus *Flavivirus*, family *Flaviviridae*. Flaviviridae family include a large number of pathogenic viruses to humans and animals. Due to their morphological, structural (common polypeptides) and genetic similarity and physicochemical properties, the different species of *Flaviviridae* express common antigenic and serological reactions. Since 1996, the major outbreak in Romania, the virus has become a problem of public health and veterinary surveillance in Romania, Europe, the Mediterranean basin and later in the US.

Yellow fever virus (YFV) is highly pathogenic to humans, it is considered as a type-species of the genus *Flavivirus*.

Using an indirect immunofluorescence (IF) assay (BIOCHIP® Technology, EUROIMMUN) we observed the existence of cross-reactions between YFV, WNV and Tick Borne Encephalitis virus (TBEV). To demonstrate the presence of antibodies specific for every virus we used ELISA (IgM antibody capture ELISA and ELISA IgG).

Despite the cross-reaction wich occur IF is an analytical method recommended when it is too difficult or expensive to prepare specific reagents required by other methods (eg. ELISA). The IF test can contribute as a valuable screening test for flaviviruses detection.

Key words: *Flaviviridae,* West Nile virus, yellow fever virus, immunofluorescence reaction, enzyme immunoassay reactions

HISTOMORPHOMETRICAL AND HISTOARHITECTURAL ASPECTS OF THYMUS DEVELOPMENT IN COBB 500 EMBRYOS

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Summary

The aim of this study was the histomorphometric assessment and characterization of embryonic thymus development in Cobb 500 hybrids. 45 fertilized eggs of Cobb 500 hybrids were used, and five embryos or cervical region of 5 embryos were collected in the day 4, 6, 8, 10, 12, 14, 16, 18 and 20 of incubation and were fixed in ethanol 80^{o}. Histological sections were stained by hematoxylin-eosin method and were assessed aiming the morphological aspects and the following parameters: the area and perimeter of the lobules, the thickness of the cortex and the perimeter and area of the medulla. The earliest hystological record of the lymphoepithelial structure of the thymus in Cobb 500 embryos was obtained in the 8th day of incubation. This appearance of the thymus was preserved until day 10 of embryonic development, when it was also notices the formation lobules. Thymus of 18-20 days old Cobb 500 embryos resembles the thymus of young birds, showing that this organ is fully developed before hatching.

Key words: Cobb 500, embryo, thymus

PHENOTYPE OF THE T CELLS INVOLVED IN IMMUNOLOGICAL TOLERANCE IN POULTY

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Summary

Immunological tolerance represents a complex of phenomena characterized by the absence of the specific responses to antigens. It is one of the fundamental properties of the immune system and is associated with its ability to discriminate between own antigens (self antigens) and foreign antigens (non-self antigens). The purpose of this study was to establish the major T cell populations involved in aquired immunological tolerance to xenogeneic antigens. The experiment was carried out on 90 embrionated eggs of Cobb 500, to which the immunological tolerance was induced by inoculation of xenogeneic blood and bone marrow mononuclear cells at 6^{th} day of incubation. At the age of three weeks, the compatibility assessment and the analisys of the T cells phenotype was performed. The result showed that the memory T cells and particually $CD25^+$ memory T cells are present in a higher proportion in experimental groups.

Key words: imunological tolerance, poultry, $CD25^+$ T cells

CLIMATE FACTORS EFFECTS ON THE EVOLUTION OF THE MAIN PHYSICAL PARAMETERS OF RAW MILK OBTAINED ON THE BACKGROUND OF A SUBCARPATHIAN MOUNTAIN AREA

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Summary

The biodiversity of the mountain pastures is in good part insured by the specifics of the climate factors and it is the ground for obtaining traditional dairy products, with a high quality flavor and texture. These qualities significantly increase the opportunities of reevaluating milk and dairy products obtained in mountain side regions. In this study we intend to evaluate the influence of climate factors on the seasonal evolution of the main physical parameters (freezing point, density and pH) of raw milk produced in the climate conditions of the sub-Carpathian mountain region of Valea Gurghiului. The studies were performed on samples of raw milk collected and processed by a commercial society in the south-western part of the Eastern Carpathians. The research consisted in monitoring the seasonal dynamics of temperature, humidity, atmospheric pressure and precipitations levels, in collaboration with a meteorological station in the area, in order to evaluate the influence of these climatic factors on the physical properties of freshness of raw milk. Sample testing was peformed with the Ekomilk M semiautomatic analyzer and the statistic analysis of the obtained data was made using the MedCalc program and the Pearson r. correlation coefficient. The overall analysis of the obtained data underlied the evaluation of the freshness of raw milk, by quantifying the influences the climatic factors had on the freezing point, density and pH of the milk. The statistic analysis of the data values obtained from evaluating the variables in the climatic factors – freezing point/density/pH of the milk relation has provided relevant correlations for the purpose of this study. These correlations have revealed a high degree of freshness of the raw milk, confirmed by medium levels of the freezing point (-0.566°C), density (28.24-28.92 g/L) and pH (6.68), obtained in average conditions of a temperature of 11.5-21°C, humidity of 66-83% and atmospheric pressure of 962.9 mb. We consider that the air's temperature and relative humidity and their evolution are environmental factors that have a major impact on the biodiversity of the mountain pastures and that favorably influence the overall composition of raw milk, especially the evolution of the specific physical characteristics for determining the degree of freshness of the milk.

Key words: freezing point, density, pH, raw milk, mountain biodiversity

ANTIFUNGAL INDICES OF SOME STRAINSOF MICROMYCETES AGAINST MYCOTIC INFECTIONS OF *APIS MELIFERA*

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Summary

The propose of study had served 21 strains of micromycetes from the National Collection of Non-pathogenic Microorganisms from Institute of Microbiology, Academy of science of Moldova and two pathogenic strains – *Aspergillus flavus* and *Aspergillus niger*. The tested strains were isolated from soil samples taken from the central zone of Moldova. As nutrient medium for isolation, of micromycetes served malt agar, Czapek, Sabouraud-Amidono and ammonium. Isolation of strains was performed in thermostat at temperature 28^{o}C, during 14 days. As a result of screening were selected two strains SP.62 and SP. 97 with potential antifungal increased against *Aspergillus flavus* pathogen with higher antifungal actions against petrified offspring of bees. The diameter of inhibition zone *of Aspergillus flavus* was on the strain SP. 62 - 30 mm, and of the strain p. SP. 97 - 35 mm.

Key words starins, micograma, însemination, screening, micromycetes.

RESEARCH REGARDING THE EPIDEMIOLOGY OF BLUETONGUE DISEASE IN TIMIS COUNTY

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Summary

The purpose of this study was to establish the evolution, spreading and prevalence of Bluetongue disease in sheep and cattle in the Timis County during 2014. A total of 1390 susceptible animals (134 sheep and 1256 cattle) from 42 localities of Timis County were examined using the competitive ELISA. The immunoenzymatic assay confirmed the presence of antibodies against VP7 protein of Bluetongue virus for 9 cattle and 74 sheep. The prevalence of Bluetongue disease in Timis County, for a total of 44,046 cattle and 826,603 sheep was 0.02%, respectively 0.008%. Bluetongue disease evolution was one centered on two major outbreaks, on the Eastern and Southern area of the County, noting that most of the positive cases were recorded in Southern outbreak (6 cattle and 51 sheep).

Key words: Bluetongue disease, ELISA, prevalence, Timis County

IMMUNOMODULATORY EFFECT OF A *PLANTAGO MAJOR* WATER EXTRACT IN MICE

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Summary

Plantago major L. is a species in the family *Plantaginaceae*, well known for its wound healing, antiinflamatory, antioxidant, antibiotic, antitumoral, modulator of the cell-mediated immunity, hematopoietic effects, being used in traditional medicine for centuries. The aim of this study was to demonstrate the immunomodulatory effect of *Plantago major* water extract after vaccination against *Pasteurella multocida* and the experimental infection with a *Pasteurella* strain pathogenic for mice. Inoculation of the Plantago major water extract before experimental infection led to a reduced mortality and stimulation of the leukocyte proliferation, reversing the neutrophils-lymphocytes ratio.

Key-words: *Plantago major*, immunomodulation, mice

IN VITRO BLAST TRANSFORMATION AND LEUKOCYTE MIGRATION INHIBITION TESTS AND THEIR DIAGNIOSTIC VALUE IN BOVINE TUBERCULOSIS

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Summary

Rapid and accurate diagnosis of bovine tuberculosis (BT)represents one of the most important feaatures of the disease allowing epidemiological control and elimination of zoonotic risk. As well known, cell-mediated immunity is the background of the immune response, therefore one would expect alterations of blast transformation and leukocyte migration in infected animals.

The investigations were carried out on a BT positive farm, classified based on tuberculinic skin test, on blood samples collected on heparine (50 IU/ml), from both positive and negative animals. The blood was subjected to the *in vitro* leukocyte blast transformation and leukocyte migration inhibition tests. The leukocyte blast transformation involved the dilution of the blood sample 1:4 with RPMI 1640 and subsequent cultivation in 96 well plates, using aditionally PHA M, bovine and avian tuberculin and incubation time of 62 h at 37^0 C. The cell growth was monitored by orto-toluidine glucose consumption test. For the leukocyte migration inhibition, the blood was centifuged in density gradient at 1500 rpm, the leukocytes were washed in RPMI 1640 three times by centrifugation (800 rpm) and the final deposit was harvested, diluted with RPMI and placed in capilary tubes. The leukocytes were allowed to migrate for 18h at 37^0 C in migration chambers against RPMI with addition of bovine and avian tuberculin and migration area diameters were measured.

The spontaneous blast transformation index (%) was significantly ($p<0.05$) increased (20.23±11.08) in the positive group, when compared to both bovine (0.886±13.62) and avian (3.12±10.86) tuberculins, with a very high variability of the individual values. There were no statistically significant differences between the BT positive (1.06 ±0.881mm) and negative (1.65±1.36) groups for the leukocyte migration inhibition test.

These results rather suggest the complementary value of the two laboratory tests then their certainty diagnostic value.

Key words: bovine tuberculosis, blast transformation, leukocyte migration inhibition diagnosis

THE DIAGNOSTIC VALUE OF THE SIMULTANEOUS USE OF DELAYED TYPE HYPERSENSITIVITY AND GAMMA INTERFERON QUANTIFICATION TESTS IN BOVINE TUBERCULOSIS

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Summary

The main agent of tuberculosis (TB) in mammals is *M. bovis*. Its broad host range also includes humans, and *M. bovis* caused TB in the latter cannot be differentiated from the M. tuberculosis induced disease. Thus, the importance of the accurate differentiation by laboratory methods of the causative agent is utmost important for the epidemiology of human cases. The study aimed to comparatively investigate the diagnostic value of cutaneous cell-mediated reactivity (ST) versus *in vitro* gamma interferon response (GIFN) towards avian and bovine tuberculin (APPD and BPPD, respectively). Out of a total of 29 Romanian spotted dairy cows, aged from five to nine years, ten tested positive for both techniques, 11 were ST pozitive, GIFN negative, three responded doubtful to ST and were negative for GIFN while negative for both tests were five animals. The GIFN optical density values (OD) recorded in GIFN test were higher for the ST positive animals than for the doubtfull and negative ones. Similarly, there were statistically significant ($p<0.05$) differences between the OD values red for the specific BPPD (0.362±0.355 units) versus the non/specific APPD (0.186 ±0.103 units) in the ST –GIFN positive animals, which were higher than those recorded in ST positive – GIFN negative animals (BPPD – 0.121±0.029 units and APPD - 0.111±0.019 units). In both ST doubtful and ST negative-GIFN negative categories, the values recorded for the specific BPPD were lower (0.150±0.06 units) than those recorded for the non-specific APPD (0.170± 0.075 units). In ST negative GIFN negative animals the BPPD and APPD values were close to each other (0.125± 0.017 and 0.127± 0.016 units, respectively). These results suggested that there was a parallel disgnostic course of the two tests, with high values for specific and low values for non specific sensitisation, respectively.

Key words: *M. bovis*, tuberculosis, diagnosis, delayed type hypersensitivity, gamma interferon

FLUORESCENT ANTIBODY TEST IN RABIES DIAGNOSIS: TIPS, PITFALLS AND TROUBLESHOOTING

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Summary

Rabies is a fatal zoonotic viral infection of the central nervous system with the causative agent Rabies virus capable of infecting all mammal species. The most widely used test for rabies diagnosis is Fluorescent Antibody Test (FAT), which is recommended by both WHO and OIE. The FAT is sensitive, specific and cheap. The sensitivity of FAT depends on the specimens, equipments, reagents and on the proficiency of the diagnostic staff. In this paper we would like to review the literature and to highlight the major critical points that occur when the method is performed in order to get reliable results.

Key words Rabies, diagnosis, fluorescent antibody test, microscope.