Institute of Food Safety, Animal Health and Environment „BIOR"

3rd International Conference

LABORATORY DIAGNOSTICS IN VETERINARY MEDICINE, FOOD AND ENVIRONMENTAL SAFETY

15-16 September 2011
Riga, LATVIA

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3rd International Conference on Laboratory Diagnostics in Veterinary Medicine, Food and Environmental Safety

Riga, 15–16 September, 2011

Venue: Institute of Food Safety, Animal Health and Environment „BIOR" Riga, Lejupes iela 3

WEDNESDAY, 14.09.2011

Afternoon. Arrival of participants

THURSDAY, 15.09.2011

8.30 Transport (bus) from Hotel „Radi un Draugi" to the Conference Venue

9.00 – 10.00 REGISTRATION. COFFEE

PLENARY SESSION I

Chairman ***Dr. Aivars Bērziņš***

10.00–10.15 Opening of the Conference.

Zanda Matuzale, Ministry of Agriculture, Head of Veterinary and Food Department

Rafaels Joffe, Director of Institute of Food Safety, Animal Health and Environment „BIOR"

10.15–10.45 Epidemiology of Foodborne Yersiniosis. **Professor, Dr. Maria Fredriksson-Ahomaa (FINLAND)**

10.45–11.15 Food-borne zoonoses: fish parasites. **Dr. Muza Kirjušina (LATVIA)**

11.15–11.35 COFFEE BREAK. **POSTER SESSION I**

11.35–12.05 *Clostridium botulinum* – food safety and public health aspects.

Professor, Dr. Miia Lindström (FINLAND)

12.05–12.35 Interdisciplinary research in biology: our experience. **Professor, Dr. Inese Kokina (LATVIA)**

12.35–12.45 **DISCUSSION**

12.45–13.45 **LUNCH**

PLENARY SESSION II

Chairwoman Professor, Dr. Maria Fredriksson-Ahomaa

13.45–14.05 Antibiotic resistance of *E. coli* from the farm animals in Estonia. **Dr. Moonika Musting (ESTONIA)**

14.05–14.25 The definitive hosts of *Toxoplasma gondii* in Finland: the role of wild Eurasian lynx. **DVM Pikka Jokelainen (FINLAND)**

14.25–14.45 *Echinococcus multilocularis* in the wild canids in Latvia. **Dr. Guna Bagrade (LATVIA)**

14.45–15.10 COFFEE BREAK. **POSTER SESSION II.**

15.10–15.30 Parasites in the endangered Finnish population of Eurasian lynx (*Lynx lynx*). **Gunita Deksne (LATVIA)**

15.30–15.50 Intestinal parasites of the Eurasian beaver (*Castor fiber*) in Latvia. **Zanda Bērziņa (LATVIA)**

15.50–16.00 **DISCUSSION**

16.00 Departure to Hotel „Radi un Draugi" (Old Town of Riga)

16.00–18.00 Free time in Old Town of Riga

18.00 Departure from Hotel „Radi un Draugi" to CONFERENCE DINNER

18.30–22.00 CONFERENCE DINNER AND SOCIAL EVENT (Park of the Institute „BIOR").

Dress code: Smart Casual.

22.00–22.30 Departure to the Hotel and old town (SOCIAL EVENT may continue!)

FRIDAY, 16.09.2011

9.30 Transport (bus) from Hotel „Radi un Draugi" to the Conference Venue

10.00–10.30 **REGISTRATION. COFFEE.**

SESSION III

Chairwoman Professor, Dr. Miia Lindström

10.30–11.00 A report on the EHEC outbreak May to July 2011 in Germany. **Professor, Dr. Bernd Appel (GERMANY)**

11.00–11.20 Food Safety – The Role of Veterinary Service in Russia. **Professor**, **Dr. Panin Alexander (RUSSIA)**

11.20–11.40 Simple and sensitive isolation method of *Eimeria bovis* oocysts from soil samples. **Triin Lepik and Dr. Brian Lassen (ESTONIA)**

11.40–12.00 MRSA in veterinary medicine. **Dr. Jatzek Szwedowski (GERMANY)**

12.00–12.15 **DISCUSSION**

12.15–12.35 **COFFEE BREAK. POSTER SESSION III.**

SESSION IV

Chairman ***Dr. Vadims Bartkevičs***

12.35–12.55 Preparation of samples for determination of fat soluble vitamins by HPLC. **Dr. Roman Lilleorg (ESTONIA)**

12.55–13.15 Lesions in tissues of Aleutian disease seropositive minks using different feed supplements in feed. **Inga Pigiņka (LATVIA)**

13.15–13.35 Prevalence of *Staphylococcus aureus* and its enterotoxins in fermented dairy products. **Liene Straupe (LATVIA)**

13.35–13.55 Occurrence of *Dirofilaria* spp. in dogs in Latvia. **Linda Stepanjana (LATVIA)**

13.55–14.05 **DISCUSSION**

14.05–14.30 COFFEE. SNACKS. DEPARTURE.

PLENARY LECTURES

AND

ORAL PRESENTATIONS

EPIDEMIOLOGY OF FOODBORNE YERSINIOSIS

BY M. FREDRIKSSON-AHOMAA

Department of Food Hygiene and Environmental Health, P.O. Box 66, 00014 Helsinki University, Finland

Yersiniosis is a zoonotic bacterial disease with a high incidence among humans especially in Europe. It is the third most frequently reported bacterial enteric disease after campylobacteriosis and salmonellosis in most European countries. Foodborne yersiniosis is caused by *Yersinia enterocolitica* or *Y. pseudotuberculosis*. Most of the reported cases are caused by *Y. enterocolitica*. However, *Y. pseudotuberculosis* infections are probably under-recognised because this pathogen is not routinely tested in many countries and the clinical diagnosis can be challenges. All *Y. pseudotuberculosis* strains are considered to be pathogenic but *Y. enterocolitica* includes strains of diverse pathogenicity. Accurate identification is necessary to discriminate harmless environmental *Yersinia* spp. from enteropathogenic *Y. enterocolitica* and *Y. pseudotuberculosis*. Infection with enteropathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* can cause a variety of symptoms depending on the age of the person infected. Common symptoms are fever, abdominal pain and diarrhea. Diarrhea is a more dominant symptom in *Y. enterocolitica* infections, while abdominal pain and fever are more frequent in *Y. pseudotuberculosis* infection. Occasionally, typically among adults, complications such as joint pain (reactive arthritis) and skin rash (erythema nodosus) may occur. Most reported cases of yersiniosis are sporadic and outbreaks are uncommon. In recent years, however, *Y. pseudotuberculosis* has emerged as an outbreak associated organism in Finland and Russia. Infections are primarily acquired orally through contaminated foods or water. In particular, pork and pork products have been implicated as the major source of human *Y. enterocolitica* infections. In the reported outbreaks of *Y. pseudotuberculosis*, fresh produce and untreated surface water have been implicated in the illness. Animals have long been suspected of being reservoirs for pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis*. Fattening pigs have shown to be the main reservoir of human pathogenic *Y. enterocolitica* strains because of the high prevalence of these strains in the tonsils and the high similarity of pig and human strains. The principal reservoir host of *Y. pseudotuberculosis* is believed to be wild animals, especially rodents and birds. However, the transmission routes of these pathogens from animals to humans are still mostly unknown. Using PCR, pathogenic *Y. enterocolitica* has frequently been detected frequently, especially on pig tongues, but also on the surface of freshly slaughtered pig and poultry carcasses, in minced pork and fermented sausages. Only a few studies have been conducted to investigate the prevalence of *Y. pseudotuberculosis* in meat products. Although several studies on the prevalence of enteropathogenic *Yersinia* in non-human sources have been conducted, a lot of questions remain to be solved using sensitive and specific detection and characterization methods in the future.

FOOD-BORNE ZOONOSES: FISH PARASITES

BY M. KIRJUŠINA

Institute of Systematic Biology, Daugavpils University, 13 Vienibas street, Daugavpils, LV-5401, Latvia;
Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, Riga, LV-1076, Latvia

Aquaculture is currently one of the fastest growing food production sectors in the world. Fish and crustaceans are generally regarded as safe and nutritious foods, but products from aquaculture have sometimes been associated with certain food safety issues, as the risk of contamination by chemical and biological agents is greater in freshwater and coastal ecosystems than in open seas. There are many different methods of farming fish, ranging from intensive commercial operations to extensive small-scale or subsistence systems, and food safety hazards vary according to system, management practices and environment (WHO Technical Report Series 883, 1999). There are numerous of harmful fish parasites for human health is known. Some of them are highly pathogenic and cause diseases, some – different gravity of allergic reaction. Hosts of zoonotic parasitic agents involve many fish species from various waters: fresh, brackish and marine. Fish-born human zoonoses are widespread and principal human diseases are trematodiases, cestodiases and nematodiases. Main course of human infection is the consumption of raw or inadequately cooked fish.

Fish-borne trematodiases are spread diseases in many areas of the world. According with the World Health Organization (1995) data the number of people infected with transmitted from fish trematodes exceeds 18 million. Agents from two genera *Opisthorchis* and *Clonorchis* are more important for human health. Liver flukes cause serious diseases in certain countries.

For humans flatworm infections from the consumption of fish are not common and are not very pathogenic. Diphyllobothriasis is the major human cestodiasis.

Anisakiasis is caused by larval stages of nematodes whose normal definitive hosts are marine mammals. Humans are accidental hosts, and the parasites almost never develop in human gastrointestinal tract. The most common species causing disease in humans is *Anisakis simplex*, in North America, Europe and Japan there are additionally other species *Pseudoterranova decipiens* and *Contracaecum* spp. Clinically human anisakiasis can be take a number of forms depending on the parasite location and lesions caused by the larvae.

The prevalence of fish-born human zoonoses in endemic areas is related to the cultural eating traditions, geographical location, population movements, economical situation, environmental degradation and growing international market. Resources to fish-born parasitic zoonoses are generally handicapped by the shortage of sufficient data on health and economic impact.

Therefore, hazard assessment and designing effective prevention and control programs are investment process.

INTERDISCIPLINARY RESEARCH IN BIOLOGY: OUR EXPERIENCE

BY I. KOKINA

Institute of Systematic Biology, University of Daugavpils, 13Vienības Str., LV-5401, Latvia

The interdisciplinary researches in biology become more popular in recent years. The results provide significant achievements both in biology and other sciences, including medicine, pharmacy, engineering, etc. Daugavpils University Institute of Systematic Biology in cooperation with Innovative Microscopy center currently provides two interdisciplinary researches, implementing the ESF and ERAF projects.

ERAF project "Plant breeding technology development on the biocompatible microchips" (2010/0321/2DP/2.1.1.1.0/10/APIA/VIAA/144) speeds up new prospects for the acquisition of new plant forms for breeding needs. There is development of nano- and microchips ongoing for this purpose, using nanolithography technology, observation of plant cell and tissue cultures, using *in vitro* methods, its transfer to developed chip prototypes, exploration of intensification and properties. Also, molecular analyses are widely used, defining on biocompatible surfaces obtained plant-regenerants' genetic features.

As the result of the research using the developed nano- and microchips, a technology will be made that will help to intensify the transport of different substances (eg. vitamins, antioxidants, phytohormones) in plant cells, thus significantly speeding up the acquisition of new breeding material *in vitro*. It is important, that the research includes a number of agricultural crops important for economy of Latvia (alfalfa, red clover and flax).

ESF project „Interdisciplinary research group in Bio-irisation" is realized as the interdisciplinary research in Zoology, Material Physics, Optical and Laser Physics, Spectroscopy. Questions related to animal body surface color and its various aspects have been generally studied a long time ago.

Finding out this mechanism is very important in bionics, because this knowledge can be used for creation of new strategically important materials. One of the processes which are connected with color of living organisms is irisation. Our research novelty and the main difference from others is a new integrated approach – interdisciplinary research of bio-irisation process in the aspect of biosystematics. This will allow comparing processes of irisation for various taxons, creating a fundamentally new method for identification of species, as well as in parallel, using computer modeling, to increase knowledge of using bionics irisation for creation a new type of holograms and reflective materials.

The research is done owing to financial means from the ESF project
No 2009/0206/1DP/1.1.1.2.0/09/APIA/VIAA/010 and
from the ERAF project No 2010/0321/2DP/2.1.1.1.0/10/APIA/VIAA/144

CLOSTRIDIUM BOTULINUM - FOOD SAFETY AND PUBLIC HEALTH ASPECTS

BY M. LINDSTRÖM

Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Finland

Clostridium botulinum is an anaerobic spore-forming bacterium that produces the most potent natural toxin, botulinum neurotoxin, during its vegetative growth. When in human or animal body, the neurotoxin blocks cholinergic nerves and causes flaccid paralysis, botulism, which may lead to death upon respiratory collapse. The classical foodborne botulism is an intoxication that follows ingestion of preformed neurotoxin with food or drink. Other forms of botulism include intestinal toxaemia and wound botulism, which are a consequence of spore germination and outgrowth into a toxic culture *in vivo*.

C. botulinum strains are divided into four physiological groups. Groups I and II strains produce neurotoxin types A, B, E, and F that cause botulism in humans. Group I strains are proteolytic mesophiles, while Group II strains are nonproteolytic and mainly psychrotrophic. The different physiological properties between Groups I and II and a wide strain variation set challenges for the laboratory diagnostics of botulism and control of the pathogen in the food chain.

The presentation discusses general properties of *C. botulinum* strains, food safety and public health questions regarding the risk of botulism, and the laboratory diagnostics of botulism, aiming to highlight recent epidemiological and bacteriological work in the field.

ECHINOCOCCUS MULTILOCULARIS IN THE WILD CANIDS IN LATVIA

BY G.BAGRADE[1,2], G. DEKSNE[3], J. VARFOLOMEJEVA[3]& J.OZOLIŅŠ[2]

[1]*Natural History Museum of Latvia, K. Barona iela 4, LV-1050 Riga, Latvia;*
[2]*Latvian State Forest Research Institute "Silava", Rigas iela 111, LV-2169, Salaspils, Latvia;*
[3]*Department of Microbiology and Parasitology, Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, Riga, LV-1076, Latvia*

The data presented here is part of the research project done on helminth fauna of wild canids with an emphasis on intensity and extensity of invasion of *Echinococcus multilocularis* in wildlife in Latvia. Carcases of 62 wolves, 97 foxes and 52 raccoon dogs were examined according to conventional helminthological methods. The research material was collected throughout territory of Latvia. Red fox is the main definitive host for *E. multilocularis* in wildlife in Latvia with the prevalence of infected animals of 27.8% and the intensity of infection of 1–4200 parasites per animal. *E. multilocularis* was detected also in wolves and raccoon dogs. In wolves the parasites are found in 3.2% of cases with an intensity of 62–380 parasites per animal and in raccoon dogs – in 5.8% of cases with an intensity of 1–815 parasites per animal. More frequently infection with *E. multilocularis* in foxes is found in the Western rather than Eastern part of Latvia (dividing line is river Daugava; sample material is equal from both parts). Infected foxes were detected in Vaiņodes, Priekules, Ventspils, Saldus, Talsu, Bauskas, Ozolnieku, Jelgavas, Ādažu, Kokneses, Jēkabpils, Krāslavas, Madonas, Smiltenes, Apes municipalities; raccoon-dogs in Ugāles, Saldus and Jelgavas municipalities, and wolves – in Ugāles and Dobeles municipalities. Red fox is the principal definitive host of *E. multilocularis* and responsible for the contamination of the environment, disseminating parasite eggs with feces. Foxes are major factor in maintenance of the parasite life-cycle and the main source of infection for intermediate hosts and subsequently for other susceptible definitive hosts. Therefore special interest should be paid to the Ventspils municipality where all three tested canid species were infected; and Saldus and Talsu municipality where the highest prevalence of infected foxes is registered to this date. The tapeworm *E. multilocularis* is one of the most pathogenic zoonotic parasites in Europe, leading to alveolar echinococcosis in humans. The qualitative features of fox population, as well spreading of fox population to many urban areas may represent major risk factors for human infections. The research is supported by the Latvian State Forest Research Institute "Silava" and Institute of Food Safety, Animal Health and Environment "BIOR".

INTESTINAL PARASITES OF THE EURASIAN BEAVER (*CASTOR FIBER*) IN LATVIA

BY Z.BĒRZIŅA[1,2], M.GACKIS[3] & G.DEKSNE[1,4]

[1]*Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, Riga, Latvia;*
[2]*Department of Zoology and Ecology, Faculty of Biology, University of Latvia, 4 Kronvalda Bulv., Riga, Latvia;*
[3]*Forest Faculty, Latvia University of Agriculture, 11 Akadēmijas, Jelgava, Latvia;*
[4]*Institute of Systematic Biology, Daugavpils University, 13 Vienibas street, Daugavpils, Latvia*

Since 2004, after Latvia joined the EU, the Eurasian beaver (*Castor fiber*) became strictly protected according to the EC Habitats Directive, Appendix IV (Directive 92/43/EEC). Beaver population in Latvia has increased every year since reintroduction. Despite the increase in the beaver population in recent years in Latvia, there is little information on helminth diversity and of its epidemiological importance. From conservation perspective, endoparasite infection might thus hamper population recovery or decrease the efficiency of conservation actions.

In this study, the prevalence and diversity of beaver intestinal parasites in Latvia was investigated by examination of visceral organs for endoparasites (n=13). The samples were collected during fall hunting season in 2010 and spring hunting season in 2011 from four largest river basins in Latvia – Gaujas, Daugavas, Lielupes and Ventas. The method used for organ investigation was visual examination and adapted sedimentation method of organ scraping. Identification of parasites was based on morphological key characteristics.

Parasitological analyses revealed adult helminthes of one nematode family Trichostrongylidae (prevalence 69%) and one trematode species *Strichorchis subtriquetrus* (100%). Both parasites were observed in high burden – *S.subtriquetrus* 87 trematodes and nematodes from Trichostrongylidae family 478 individuals per animal. The highest level of infection (800.5 parasites per animal) was observed in basin of river Daugava which is also the biggest river basin in Latvia. Meanwhile, second highest infection level (317.3) was observed in Lielupes river basin which is the smallest river basin in Latvia.

Despite low parasite diversity, prevalence of observed parasites in beaver in present study was high. This is first study of beaver endoparasites in Latvia and provides new knowledge about distribution and prevalence of beaver parasite population.

PARASITES IN THE ENDANGERED FINNISH POPULATION OF EURASIAN LYNX (*LYNX LYNX*)

BY G. DEKSNE[1,2], J. LAAKKONEN[3], P. JOKELAINEN[3], A. LAVIKAINEN[4], A. NÄREAHO[3], K. HOLMALA[5], I. KOJOLA[5] & A. SUKURA[3]

[1]*Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, LV-1074, Riga, Latvia;*
[2]*Institute of Systematic Biology, Daugavpils University, 13 Vienibas street, LV-5401 Daugavpils, Latvia;*
[3]*Department of Veterinary Biosciences, Faculty of Veterinary Medicine, University of Helsinki, Agnes Sjöbergin katu 2, FI-00014 University of Helsinki, Helsinki, Finland;*
[4]*Haartman Institute Department of Bacteriology and Immunology P.O.Box 21 FI-00014 University of Helsinki, Finland;*
[5]*Finnish Game and Fisheries Research Institute, Viikinkaari 4, FI-00790 Helsinki, Finland*

In this study, we screened lynx in Finland for endoparasites by analysis of faecal samples, by investigating their intestinal contents and lungs. The samples were collected during winter hunting season 2010-2011. Approximately 13% of the lynx population living in Finland was screened for endoparasites in this study.

The method used for faecal samples was quantitative MgSo4 flotation (FLOTAC® technique). Parasite species identification was based on egg morphology. Identification of adult worms was also based on morphological key characteristics. Lungs were examined macroscopically for parasites and anomalies. Tissue samples of each lobe were fixed in 10 % buffered formalin to produce standard histological sections (5µm) which were stained with hematoxylin-eosin (H&E) and Grocott's modification of Gomori's methenamine silver (GMS)

Eggs and oocysts of a total of six different endoparasites were identified with species richness 3.2 species per animal and adult forms from four different species with species richness 2.2 species per animal. *Toxocara cati* were identified with prevalence 71.4% in faecal samples and – 93% in intestines; *Taenia* spp. – 28.6% in faecal samples and 68.2% in intestines; *Diphyllobothrium* sp. - 5% in faecal samples and – 2% in intestines. Only eggs were detected for Capillaridae-like nematodes and *Uncinaria stenocephala* (prevalence 45.7% and 0.6% respectively). There were also protozoan *Isospora* sp. oocysts (0.6%). Meanwhile for *Mesocestoides* sp. cestode, only adult forms were detected. All lungs examined appeared normal on gross examination and no parasites were detected. No parasites or major histopathological changes were seen in any of the tissue sections. As animals were frozen before sample collection, most of *Taenia* spp. adult forms were without rostellar hooks and the structure of the

proglotids was. Only 16 *Taenia* tapeworms from ten animals were identified to the species level by morphology, as *Taenia laticollis*. *Taenia* tapeworms having obviously longer and wider strobila than that of *T. laticollis* were observed in four lynx. Two individuals were identified as an unknown species of *Taenia* by mitochondrial DNA sequences (the partial cytochrome c oxidase 1 and complete NADH dehydrogenase 1 genes).

This is the first large-scale, systematic investigation of parasites in of lynx in Finland. The observed prevalence and diversity of endoparasites in the present study was high. This study provides new knowledge about distribution and prevalence of lynx parasite populations.

THE DEFINITIVE HOSTS OF *TOXOPLASMA GONDII* IN FINLAND: THE ROLE OF WILD EURASIAN LYNX?

BY P. JOKELAINEN[1], G. DEKSNE[2], K. HOLMALA[3], A. NÄREAHO[1], I. KOJOLA[3] & A. SUKURA[1]

[1]*Department of Veterinary Biosciences, Faculty of Veterinary Medicine, P.O. Box 66 (Agnes Sjöbergin katu 2), FI-00014 University of Helsinki, Helsinki, Finland;*
[2]*Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, LV-1076, Riga, Latvia, and Institute of Systematic Biology, Daugavpils University, 13 Vienibas street, Daugavpils, LV-5401, Latvia;*
[3]*Finnish Game and Fisheries Research Institute, Viikinkaari 4, FI-00790 Helsinki, Finland*

The Felids living in Finland comprise approximately 800 000 domestic cats and a small, but growing, population of about 2 500 free-ranging Eurasian lynx (*Lynx lynx*). This setting, one domestic and one wild definitive host species of *T. gondii* is intriguing from the epidemiological point of view: Cats are known to be important hosts that can shed *T. gondii* oocysts to the environment, also in Finland – but how about the lynx? We investigated samples from lynx shot during the hunting season of 2010-2011 in Finland. We detected *T. gondii* specific IgG antibodies in 290 (86.1%) of 337 lynx sera. The method used was a direct agglutination test, and samples positive at dilution 1:40 were defined as seropositive. We also examined 336 fecal samples with a quantitative $MgSO_4$ flotation technique and none of them tested positive for the presence of *T. gondii*-like oocysts. The majority of the lynx examined had serologic evidence of natural exposure to *T. gondii*, while no ongoing contribution to the environmental oocyst burden was detected. This may be due to age distribution of the animals hunted and examined. Shedding might occur mainly in association with the primary infection, as could be extrapolated from cat studies, whereas the measurable antibody response indicates previous encounter with the parasite. Lynx are part of the host range of *T. gondii* in Finland and commonly encounter the parasite. Nevertheless, the domestic cats outnumber the lynx and thus probably play the bigger role in the epidemiology of toxoplasmosis. (These preliminary results have been a part of presentations presented at the NWDA meeting, June 2011, Oravi, Finland, and at the 11th International Congress on Toxoplasmosis, June 2011, Ottawa, Canada).

SIMPLE AND SENSITIVE ISOLATION METHOD OF *EIMERIA BOVIS* OOCYSTS FROM SOIL SAMPLES

BY B. LASSEN & T. LEPIK

Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62, 51014 Tartu, Estonia

The intestinal parasite *Eimeria* has a direct life cycle requiring an external phase when shed in faeces to the environment to become infective. Despite this crucial part of the parasite life cycle, available methods lack the ability to effectively detect and quantify oocysts from the soil. We developed a simple method to isolate oocysts from soil samples. The groups used were: 1) negative controls, 2) positive controls without soil, and 3) with or 4) without 1 ml faeces material on 10 gram soil. The soil was weighed in a 60 ml plastic syringe and 20 ml of sugar-salt solution was added prior to adding a piston and pushing the liquid to the tip in approximately 20 degree angle from horizontal leaving an air bubble in the narrowing part of syringe. The bubble was then used to gather the oocysts by gently rotating the syringe periodically for 30 min at the same angle. The syringe was then placed vertically for 15 minutes tip upwards. Then, approximately 3 ml of the contents was filtered through gauze to a 14 ml centrifuge tube. The piston was pulled back and the steps repeated from after adding the sugar-salt solution. The filtered liquid was mixed with a pipette and transferred to a 0.6 ml reading chamber to count the oocysts in vertical 3 lanes equal to 0.0702 liter. The total number of oocysts present in the sample was calculated according to the sample volume in the 14 ml tube. Positive control samples contained 42.7±5.5% of the original number of oocysts added (50.000), while soil samples without faeces contained 20.8±4.4%, and with faeces 21.9±3.4%. Comparing with recently published study of retrieving *Toxoplasma* oocysts from soil (Lélu et al. 2011) our method seems as good as the best method tested and simpler. This method was sensitive enough to detect oocysts numbers as low as 14 oocysts/gram soil. Sensitivity of the method is thus relevant considering that very few oocysts are needed to infect an animal. We believe this method is applicable to determining soil contamination with oocysts of specific areas as well as for other experimental purposes.

PREPARATION OF SAMPLES FOR DETERMINATION OF FAT SOLUBLE VITAMINS BY HPLC

BY R. LILLEORG & V. NÕMM

Veterinary and Food Laboratory, Kreutzwaldi 30 Tartu 51006 Estonia

The method we are using for determination fat soluble vitamins (D, E and A vitamins) is based on standards EVS EN 12821:2000, EVS EN 12822:2000 and EVS EN 12823:2000, that respectively describe vitamin D, vitamin E and vitamin A determination in foodstuffs by HPLC. The principle of method is that the homogenized test sample is saponified by KOH solution and after this procedure fat soluble vitamins are extracted from solution by appropriate solvent. The extract thereafter is evaporated and residue is dissolved in ethanol. Obtained solution is ready for HPLC analysis. A and D vitamins are detected by UV detector and E vitamin is detected by fluorescence detector. In the beginning, when the method was taken into the use, the saponification of sample and extraction of vitamins was done as it is described in corresponding standard method. That means that for saponification was taken up to 25 g of sample, then was added antioxidant and 50 - 150 ml of KOH in methanol and water solution. For extraction was used petroleum ether and diethyl ether solution 1:1. Vitamins were extracted three times from the reaction solution with 50 - 150 ml amounts of solvent. Solution containing vitamins was washed with water and separated using separating funnel. Obtained solution was evaporated with rotary evaporation system. Residue was dissolved in exact volume of ethanol and analyzed by HPLC. As we can see this method needs respectively big amounts of reagents and solvents per sample and takes a lot of time. We have improved given method. Now we take 0.2 – 1.0 g of sample (depends on vitamins content), add antioxidant, 2 ml of water and 10 ml of 2M KOH in methanol. After saponification in nitrogen atmosphere we extract fat soluble vitamins with 10 ml of hexane. Solution with added hexane is centrifuged and exact volume is taken from upper hexane layer. Next step is same as it is described in standard method, solution is evaporated and residue is dissolved in exact volume of ethanol. Ethanolic solution is ready for HPLC analysis. Analysis results show that improved method works as good as standard method, it is friendlier to the environment (smaller amounts of reagents and solvents are used) and takes less time. Therefore our laboratory changed the saponification and extraction procedure of test sample.

ANTIMICROBIAL RESISTANCE OF *ESCHERICHIA COLI* ISOLATED FROM THE FARM ANIMALS IN ESTONIA

BY M. MUSTING & L. HÄKKINEN

Veterinary and Food Laboratory, Kreutzwaldi 30, 51006 Tartu, Estonia

Escherichia coli on the farm animals is considered an important reservoir of antimicrobial resistance. The aim of our study was to determine the resistance patterns of *E. coli* strains isolated from faecal samples of apparently healthy cattle and pigs and from the pathological material of calves and suckling or weaned piglets in 2006 – 2010. The isolation and identification of *E. coli* was done by using selective media and several biochemical tests. A total 366 *E. coli* strains were collected over 4-year period and tested by the microdilution Vet MIC method (National Veterinary Institute, Uppsala, Sweden) to determine the MICs of 13 antimicrobials. In general the occurrence of the resistance of *E. coli* isolated from cattle was lower than among *E. coli* isolates from pigs. In 2010 the resistance to quinolones was observed in *E. coli* indicator strains. Multidrug resistance (resistance to three or more unrelated antimicrobials) was detected in *E. coli* isolated from pathological material of pigs, nearly all of which were resistant to ampicillin, tetracycline, sulphamethoxazole, trimethoprim. 2010 was the first year when the resistance to cefotaxime was observed in *E. coli* indicator strains isolated from cattle and from the pathological material of pigs. The following conformation analyses for AmpC and ESBL were performed respectively. The results show that *E. coli* strains isolated from farm animals are important reservoirs of resistance to the older-generation antimicrobials and the resistance to quinolones and third generation cephalosporins is evolving. More restricted use of antimicrobial agents in food animal production should be implemented.

LESIONS IN TISSUES OF ALEUTIAN DISEASE SEROPOSITIVE MINKS USING DIFFERENT FEED SUPPLEMENTS IN FEED

BY I. PIGIŅKA, D. KRASTIŅA & R. GRANTA

Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, Riga, LV-1076, Latvia

Aleutian disease is widespread and cause loses for minks' farms. Aleutian disease is chronic immunosuppressive disease with immune complex deposition, lymphocyte and plasma cells infiltration in tissues. Aleutian disease has not specific treatment or successful vaccination program. Therefore decelerate development of lesion in tissues from Aleutian disease hope with using complete and balanced feed. Aim of study was investigate lesions in tissues of Aleutian disease seropositive minks using different Supplements in feed.

Study was carried out from 2010 to 2011. Two groups of weanling mink-puppies were formed in minks' farm in July 2010: experimental (n=21000) and control (n=31800) groups. Both groups included brown and sapphire minks. During five months (July 2010 to November 2010) for experimental group minks were using feed with Supplement-A, and for control group – with Supplement-B.

At totally 200 slaughtered mink-puppies were randomly selected for blood collection in November 2010 and were investigated by countercurrent-immuno-electrophoresis (CIEP) test for Aleutian disease virus antibody detection. Blood and tissues samples of 100 animals from these selected slaughtered mink-puppies were tested by conventional polymerase chain reaction (PCR) for Aleutian disease virus genome detection and by classical histology. Carcasses' measures data were collected for there 100 slaughtered mink-puppies. Blood samples were taken for CIEP investigation to Aleutian disease virus antibody present at July 2010 from 100 randomly selected minks-females, whom puppies were used for formation of control and experimental group. Research dates were comparing between experimental and control group's brigades and statistically confirmed. In statistical analyses was used $\acute{\alpha} = 0.01$. The chi-square test was used for dates of serological, PCR results and amount of dead minks. Differences in incidence were evaluated by Fisher's formula. The median test was used to analyse minks' body measures.

Study results showed, in group of brown mink-puppies using Supplement-A in feed, had significantly lower ($p<0.01$) amount of minks with Aleutian disease virus antibody.

Brown mink-puppies using feed with Supplement-A, had significantly lower ($p<0.01$) ADV seropositive causes than females of their puppies.

In group of brown and sapphire mink-puppies using Supplement-A in feed, had significantly lower ($p<0.01$) mortality than in group of mink-puppies, which using Supplement-B.

Developing of Aleutian disease lesion associated by interstitial pneumonia was decelerated in group of brown mink-puppies using Supplement-A in feed. In group of brown mink-puppies using Supplement-A in feed, had significantly lower ($p<0.01$) amount of minks with interstitial pneumonia and lesion intensity than in group of brown mink-puppies, which using Supplement-B.

In conclusion, evident difference of Aleut disease antibody presence in minks, intensity of interstitial pneumonia and amount of dead minks between experimental and control groups statistically confirmed, that can depended from difference compositions in Supplement-A and Supplement-B.

OCCURRENCE OF *DIROFILARIA* SPP. IN DOGS IN LATVIA

BY L. STEPANJANA

Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, Riga, LV 1076, Latvia;
Institute of Systematic Biology, University of Daugavpils, 13 Vienibas street, Daugavpils, LV 5401, Latvia

Dirofilaria repens is zooanthroponosis disease and it is widespread. However a date absent about *Dirofilaria repens* distribution in animal and human population in Latvia. Therefore our aim of study is investigate *Dirofilaria* spp. presence in dogs' blood in Latvia.

Work was during four years from 2008 to 2010. In general were investigated 2227 samples of domestic dogs' blood, but positive was only 72 domestic dogs. Blood samples were received from Rigas regions of Latvian. All samples were analysed by following parameters: results of parasitological investigation of bloods smears, year of investigation, season, Rigas region of Latvia, age of animal, sex of animals and breed of dog. Parasitological investigation was included two different tests for *Dirofilaria* spp. larva detection. First parasitological test was microscopy of not centrifuged EDTA blood sample when microscopy a blood smears. Second method was KNOTT test including centrifuged EDTA blood sample microscopy. KNOTT test was used first time in Latvia and was adapted in laboratory. KNOTT test is the general method for nematode - *Dirofilaria* spp. detection.

D. repens was detected in 72 samples of domestic dogs blood, including 39 samples from male, 23 – from females and 10 - from unknown sex. Highest invasion of *D. repens* was in 2008 (35 infected dogs). Lower invasion of *D. repens* was in 2009 (26 infected dogs) and in 2010 (11 infected dogs). Incidence of *Dirofilaria* spp. in dogs decreased from 2008 to 2010. Basically, *D. repens* was detected in male, large size dogs from 6 to 9 years old. *Dirofilaria repens* was mostly found in the dogs' blood in the spring and autumn, when the mosquito feeding activity is high.

THE PREVALENCE OF *STAPHYLOCOCCUS AUREUS* AND ITS ENTEROTOXINS IN FERMENTED DAIRY PRODUCTS

BY L. STRAUPE, G. TUPE, R. GRANTA, A. BĒRZIŅŠ & R. JOFFE

Institute of Food safety, Animal Health and Environment "BIOR", 3 Lejupes Street, Riga LV-1076, Latvia

The bovine milk and milk products often are the source of staphylococcal intoxication. *Staphylococcus aureus* produces a wide variety of enterotoxins with emetic activity and are able to cause illnesses if ingested with contaminated food (Le Loir et al. 2003). A total 177 samples of sour cream (n=39), cottage cheese (n=98) and cheese (n=40) from 13 different manufacturers were collected from Riga farmers market. Isolated coagulase positive *S. aureus* was tested for enterotoxin encoding genes. In total 14% of samples (24/ 177) were positive for enterotoxin presence. The most common enterotoxin types were SED (84%) and SEA (33%). SEC was not detected. The cottage cheese was a most contaminated product with a 50% prevalence of *S. aureus*. Moreover, cottage cheese contained the highest diversity of enterotoxin genes, including SEA, SED, SEE and SER. The prevalence of enterotoxigenic samples in sour cream and cottage cheese was 43% and 41%, respectively. The prevalence of coagulase positive *S. aureus* in cheese was 10%. *S. aureus* is present in all tested fermented product types. Prevalence of *S. aureus* enterotoxins encoding genes in 60 *S. aureus* samples was SED – 27%, SEA – 13%, SEE – 5%. SEB was found only in cheese with prevalence 2%.

MRSA. NEWS ON THE VETERINARY FIELD

BY SZWEDOWSKI JATZEK

Methicilin-resistant *Staphylococcus aureus* (MRSA) is an antibiotic-resistant bacterium and a major concern in both human and veterinary medicine.

Several cases of subclinical mastitis in animals were caused by MRSA and the strains were indistinguishable from MRSA isolated from a carrier working in close contact with animals. This suggests the transmission of these isolates between humans and animals. Different studies were done to evaluate whether animal MESA isolates are a possible source of human infections.

POSTER ABSTRACTS

THE INFLUENCE OF COW FEED CAROTENOIDS ON MILK ANTIMICROBIAL PROPERTIES

BY U. ANTONE[1], A. JEMEĻJANOVS[1], V. ŠTERNA[1], I.H. KONOŠONOKA[1] & J. ZAGORSKA[2]

[1] *Research Institute of Biotechnology and Veterinary Medicine 'Sigra' of the Latvia University of Agriculture, Institūta iela 1, Sigulda, LV-2150, Latvia;*
[2] *Latvia University of Agriculture, Department of Food Technology, 2 Lielā iela, Jelgava, LV-3001, Latvia*

Despite various roles of carotenoids in cow's feed and their ability to increase the immunity of animals, little attention has been paid to their influence on milk antimicrobial properties. The aim of the present study was to evaluate the influence of feed enrichment with different sources of carotenoids on concentrations of lysozyme and immunoglobulins (Ig) in milk, and to the growth of psychrotrophic bacteria (PB).

15 cows were selected and divided into 1 control (G1) and 2 experimental groups (G2, G3) that were as similar, as possible. The basic feed was equal in all groups, e.g. silage was fed to ad libidum and rapeseed animal meal – 2 kg per cow per day. The G1 feed was supplemented by rapeseed oil (100 g); the G2 – by rapeseed oil (100g), and carrots (7 kg); the G3 – by red palm oil "Carotino" (100 g) per cow per day.

Pooled milk samples of each cow group were obtained from afternoon milking 1 day before feed enrichment (D0), in days 7, 24, 35, 42 (during the feed enrichment), and 1 week after feed enrichment (D56), immediately cooled to 4--8 °C, and transported to the laboratory. The concentrations of immunoglobulins (IgA, IgG, IgM) and lysozyme were determined by turbodimetric method in the Petera Delles Food Processing laboratory of the Faculty of Food Technology of the Latvia University of Agriculture. The growth of PB during 5 day storage of milk samples in temperature of 4-6 °C was measured using the plate count agar in accordance with standard method in RI 'Sigra'.

Results show that during feed supplementation with carotenoid feedstuffs milk from both experimental cow groups was significantly more resistant to psychrotrophic bacteria growth. In day 7 (D7) milk samples PB count during 5 day storage increased 667 (G1), 242 (G2), and 19 (G3) folds, in day 24 (D 24) samples – 433 (G1), 69 (G2), and 36 (G3) folds, but in day 35 (D 35) samples – 600 (G1), 12 (G2), and 3 (G3) folds.

This positive effect can be explained by the increased amounts of such antimicrobial proteins as immunoglobulins and lysozyme. In milk of cows whose diet was supplemented with carrots, Ig concentration was significantly higher ($p<0.05$) in sampling days D24, D42 and D56, compared to control groups milk, but the lysozyme concentration – in D24, D35, D42, and D56. In milk of cows whose diet was supplemented with red palm oil, Ig concentration was significantly higher ($p<0.05$), in sampling days D7, D24, and D56, compared to CG, but the lysozyme concentration – in D7, D35, D42, and D56. The increase mechanism of the antimicrobial protein concentration is not fully understood, and carotenoids are not directly involved in the formation of their molecules, however the higher amounts of antimicrobial proteins in EGs milk can be derived from the feed enrichment with carotenoids thus strengthening cow health, as well there can be other reasons why PB increase is hindered.

PARASITOFAUNA OF FISH IN THE RIVER WEST DVINA AND RESERVOIRS OF ITS BASIN

BY E.K. SKUORAT[1], S.M. DZIAHTSIARYK[1], N.A. BENETSKAYA[1], A.I. GREBNEVA[1], A.N. LEMEZA[1], T.A. GOVOR[1], R.L. ASADCHAYA[2] & A.S. KOVTIK[1]

[1]RUE «Fish Industry Institute», Laboratory of Fish Diseases, Minsk, 22 Stebeneva Str., 220024, Minsk, Republic of Belarus;
[2]RUE «Institute of Experimental Veterinary Medicine named of S.N. Vyshelessky», Laboratory of Molecular Biology, Minsk, 28 Briketa Str., 220003, Minsk, Republic of Belarus

Lakes and rivers of Belarus where fishery is allowed are wonderful places for the rest of both Belarusians and foreigners. However, on the territory of the republic several dozens of fish parasites that can cause epizooties leading to great damage in fish industry have been registered. That is way it's necessary to conduct parasitological research of fish living in natural reservoirs.

For the last 10 years the researchers of Belarus have been monitoring the parasite fauna of fish of natural reservoirs. A special attention is paid to the identification of causative agents – diseases of helminthozoonosis (opisthorchiasis and tapeworm disease) which are dangerous for people and animals.

In 2010 a complete fish parasitological analysis was done in the River West Dvina (near Vitebsk and Beshenkovichy towns) and the lakes of its basin (Selyavskoye, Usvechie, Cherstvyadskoye, Solonets, Matyrino, Gretskoye, Bolshoye Yazenskoye, Cheress, Polonskoye, Bernovo, Plissa Bolshaya, Tserkovishche, Petrovshchina, Lisno, Isubitsa, Gorodno).

Fourteen species of fish (403 specimen) including chub – 1, silver bream -13, dace – 2, crucian carp – 32, goldfish – 30, rudd – 4, bream – 85, tench – 27, perch – 102, roach – 43, pikeperch – 25, European eel – 10, pike – 27, ide – 2 were examined.

Twenty two species of parasites belonging to different taxonomic groups were discovered:

Phylum **Ciliophora** - *Ichthyophthirius multifiliis;*
Class **Crustacea** - *Ergasilus sieboldi, Ergasilus briani, Argulus coregoni;*
Class **Monogenea** - *Dactylogyrus* sp.;
Class **Hirudinea** - *Piscicola geometra;*
Class **Nematoda** - *Anguillicola crassus, Camallanus lacustris, Philometroides sanguinea, Raphidascaris acus;*
Class **Acanthocephala** - *Acanthocephalus lucii;*
Class **Trematoda** - *Postodiplostomum cuticola, Diplostomum* sp., *Tylodelphys conifera, Tylodelphys podicipina, Rhipidocotyle illense, Paracoenogonimus ovatus;*

Class **Cestoidea** - *Ligula intestinalis, Khawia sinensis, Caryophyllaeus fimbriceps, Bothriocephalus claviceps*, and *Triaenophorus nodulosus*.

As a result, the highest invasion rate (IR) has perch from Bernovo Lake: infection with *Tylodelphys conifer* is 14-132 parasites per fish with invasion extensity (IE) of 75 %. Further in decreasing: *Posthodiplostomum cuticola* (IR - 1-123 parasites/fish, IE - 17-100 %), *Raphidascris acus* (IR – 18-123 parasites/fish, IE – 50 %), *Ergasilus sieboldi* (IR – 1-88 parasites/fish, IE – 20-100 %), *Khawia sinensis* (IR – 1-73 parasites/fish, IE – 10-70 %), *Camallanus lacustris* (IR – 2-64 parasites/fish, IE – 5-100 %), *Tylodelphys podicopina* (IR – 6-56 parasites/fish, IE – 33-100 %). Causative agents of helminthozoonosis are not discovered.

The basin of the River West Dvina is a picturesque area with outstanding natural beauty that attracts a lot of tourists year and year out. Thus, the study of fish health is a hot issue problem from the scientific and practical point of view, and it will be continued in the future.

CRAYFISH - REMARKS FOR RISK ASSESSMENT

BY I. BRIEDE

Daugavpils University, 13 Vienibas street, Daugavpils, LV 5400, Latvia

Crayfish are widely distributed in the whole world. They are found in water bodies that don't freeze fully and have shelter against predators. Most crayfish can't tolerate polluted water, although some species are hardier. Some crayfish have been found living as much as 3 meters underground; a few survive in saltwater. There are known more than 40000 crayfish species in the world, where from only four species are present in Latvia. They are the European species noble crayfish (*Astacus astacus*), narrow-clawed crayfish (*Astacus leptodactylus*), the North American signal crayfish (*Pacifastacus leniusculus*) and spiny–cheek crayfish (*Orconectes limosus*). With the growth of crayfish aquaculture the occurrence of disease outbreaks is likely to increase. The presence of pathogens in crayfish tissues does not necessarily result in an observable, detrimental effect on the crayfish. More than 50 viruses had been reported from crustaceans, particularly from Australian freshwater crayfish species that are now farmed semi-intensively around the world. Freshwater crayfish may be susceptible to infection by viruses important for other species, including finfish, and can act as carriers for these pathogens. Some viruses such as infectious pancreatic necrosis virus (IPNV) cause an acute disease in salmonids, but do not produce disease in crayfish. White spot virus complex (WSV) is highly pathogenic to freshwater crayfish. Both gram negative and gram positive bacteria have been isolated from crayfish haemolymph. The most frequently reported gram negative genera are *Pseudomonas*, *Aeromonas*, *Acinetobacter*, *Flavobacterium* and *Vibrio*. *Micrococcus* and *Staphylococcus* are the most often reported gram positive genera. Bacterial infections are important in intensive and highly artificial systems. Many bacteria are presumed to be secondary pathogens of crustaceans. Bacteria have frequently been reported in association with the exoskeleton, enteric tract or haemolymph of freshwater crayfish. Some bacterial pathogens, found in crayfish may cause diseases in freshwater fish. The gram negative organism *Yersinia ruckeri* is one such organism for which freshwater crayfish have been proposed as a reservoir or carrier of infection. Most of fungal infections cause devastating crayfish diseases. Sometimes crayfish act as intermediate host to a number of metazoan parasites including digenean trematodes, cestodes and acanthocephalans. The parasite insists in the tissues of the crayfish and remains in an inactive status until the crayfish dies or is eaten by a predator. The introduction of an exotic pathogen organism of serious disease cause dangerous risk to fish species and they should be taken into account in the risk assessment process.

WHITE BLOOD CELL COUNT AS METHOD TO IDENTIFY ALTERATIONS OF STRESS IN A WINTERING PASSERINE BIRD

BY D. CIRULE[1,2] & I. KRAMS[2,3]

[1]*Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, Riga, LV-1076, Latvia;*
[2]*Institute of Systematic Biology, Daugavpils University, 13 Vienibas street, Daugavpils, LV-5401, Latvia;*
[3]*University of Tartu, Ülikooli 18, 50090 Tartu, Estonia*

The nonspecific stress syndrome has some basic characteristics that are shared by many animals such as birds, mammals, reptiles, amphibians and fish. Environmental stress refers to physical, chemical, and biological constraints on their survival and reproduction. The primary response of animals to an environmental stressor is rapid, and it is evidenced as physiological changes by increases in arterial blood pressure, glycemia, respiratory frequency, muscle tone and circulating glucocorticoids. Measuring glucocorticoid levels has many applications in ecology and it has proven to be an invaluable tool to assess stress in animals. However, there are draw-backs associated with glucocorticoids. For example, levels of plasma corticosterone, the main glucocorticoid of birds, rise quickly immediately following capture of an individual, thus making it difficult to obtain baseline measurements in field situations. Furthermore, the increase in corticosterone produces cascading effects on other physiological systems, including the immune system. The increase of corticosterone produces a secondary response lasting for hours or even days. The latter responses are generally associated with chronic stress, which often makes it impossible to determine differences between chronic and acute stress.

Increases in glucocorticoid hormones cause characteristic changes in the leukocyte component of the vertebrate immune system that can be quantified. Growing evidence from the veterinary, biomedical and ecological literature and the consistent nature of the hae- matological response to stress, makes differential white blood cell counts from blood smears a reliable and popular method of measuring physiological stress. It also provides an assessment of innate immune function giving insight into an individual's immune status at the time of capture. In birds, this response is recognized, among other characteristics, by an increase in the number of heterophils (H) and a decrease in the number of lymphocytes (L) in the blood, immunodepression, and a reduction of body weight. The ratio H/L gives a reliable and widely used stress estimator in birds and reptiles. In birds, the H/L ratio is known to increase in response to a relatively long- lasting environmental factors and perturbations, including starvation, diseases, injuries (Vleck et al. 2000), territory quality (Mazerolle and Hobson 2002), urbanization (Ruiz et al. 2002), parasitic infection (Davis et al. 2004; Lobato et al.), radioactive

contamination (Camplani et al. 1999), psychological disturbance, and general handling and transport of birds (Parga et al. 2001; Scope et al. 2002; Groom- bridge et al. 2004; Davis 2005). The magnitude of change in leukocyte proportions depends on the intensity and persistence of the stressor (Averbeck 1992; Vleck et al. 2000).

With the widely used corticosterone response, it is well known that basal hormone levels must be sampled within 3 min of capture (Romero and Romero 2002) since levels of plasma corticosterone rise quickly immediately following capture of wild animals. H/L ratios have been shown to increase in several species following a 1- to 3-h transport (Parga et al. 2001; Scope et al. 2002; Groombridge et al. 2004). This fact has important implications for researchers, because it suggests that leukocyte profiles in birds can change within shorter time intervals. However, the results by Davis (2005) show that routine handling times up to 1 h do not affect H/L ratios in House Finches (*Carpodacus mexicanus*). This discrepancy raises an important question: what are the time intervals needed for leukocyte profiles to change significantly under conditions of stress? To answer this question, we captured adult male Great Tits (*Parus major*) and made blood smears from samples obtained from four groups of the birds 4, 30, 60 and 120 min after capture, to evaluate the effect of handling time on leukocyte profiles under conditions of acute stress. The Great Tit is a species widely used in ecology studies, and basic knowledge on the effect of capturing and handling on leukocyte profiles is important to interpret results obtained in the field.

As we known the increase in glucocorticoid hormones causes characteristic long-lasting changes in the leukocyte numbers, we tested whether stress-related handling of male Great Tits (*Parus major*) may cause rapid changes in their leukocyte profile. We found that handling stress significantly increased heterophil counts between 30 and 60 min after capture, while lymphocyte and eosinophil counts significantly declined between 60 and 120 min after capture. The increase in heterophil counts and reduction in lymphocyte counts caused an increase of the heterophil and lymphocyte ratio (H/L) between 60 and 120 min after capture. Overall, these results indicate that leukocyte profiles in wintering male Great Tits may change more rapidly than previously thought, reflecting acute stress of individual birds.

PRELIMINARY RESULTS OF DOGS AND CATS FAECAL SAMPLES INVESTIGATION IN DAUGAVPILS REGION

BY A. DAUKŠTE, M. KIRJUŠINA & A. ZDANKOVSKA

Institute of Systematic Biology, Daugavpils University, 13 Vienibas street, LV-5401,Daugavpils, Latvia

Nematodes are most frequent intestinal parasites of dogs and cats. Some species such as *Toxocara canis*, *Ancylostoma caninum* are pathogenic for humans. Therefore pets can made negatively influence on their owners' health. The aim of the study was to investigate dogs and cats parasites in Daugavpils region.

The study was carried out from January 2010 to May 2011. Faecal samples of 140 dogs and 61 cats were collected. All investigated animal were form Daugavpils region and kept in different conditions (dogs and cats from shelters, rural and village areas). There are stray animals – 61.4% of dogs and 44.3% of cats. Age of dogs was from 1 month to 18 years old, cats - from 3 month to 17 years old. Investigated animals consist 84 male and 56 female of dogs; and 26 male and 35 female of cats.

Faecal samples were examined by NH_4NO_3 flotation technique. Parasite species identification was based on egg morphological features. Prevalence of infection was calculated.

Prevalence of infected dogs is 18.6 %, mostly they are stray dogs (80.7%). Prevalence of infected cats is 24.6% (80% of them were stray cats).

For dogs registered 7 parasite species: *Isospora* sp. (Prevalence - 1.4%), *Toxocara canis* (P - 22.3%), *Toxoascaris leonina* (P - 4.3%), *Trichuris vulpis* (P - 5.6%), *Uncinaria stenocephala* (P - 4.2%), *Physaloptora* sp. (P - 2.8%), *Capilaria* sp. (P - 1.4%). Three nematode species were detected in domestic dogs – *T. canis*, *T. leonina* and *T. vulpis*.

In cats` samples were detected 3 species: *Isospora* sp. (P - 0.61%), *Toxocara cati* (P - 7.1%), *T. leonina* (P - 2.1%). Last two parasites species were found in domestic cats.

On end of May during castration procedure of 2 years old dog in Daugavpils shelter were found 2 adult nematodes, which were recognized as *Dirofilaria repens.*

During study were detested three pathogenic for human parasite species.

IMPAIRED IMMUNITY OF XYLOPHAGOUS INSECTS AND A LOW INFESTATION RATE OF SCOTS PINE UNDER COOLING EFFECT OF FOREST LAKES IN NORTHERN EUROPE

BY J. DAUKŠTE[1,5], T. KRAMA[1], R. CIBUĻSKIS[1], I. KIVLENIECE[1], M. ĀBOLIŅŠ-ĀBOLS[2], M.J. RANTALA[3] & I. KRAMS[1,4]

[1]Institute of Systematic Biology, University of Daugavpils, 13 Vienibas street, LV-5401, Daugavpils, Latvia;
[2] Department of Biology, Indiana University, Bloomington, IN 47405, USA;
[3]Section of Ecology, Department of Biology, University of Turku, FIN-20024 Finland;
[4]Institute of Ecology and Earth Sciences, University of Tartu, Estonia;
[5]Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, LV-1074, Riga, Latvia

We studied infestation rates of Scots pines *Pinus sylvestris* in relation to distance of the trees from forest lakes in Latvia, northern Europe. In summer of 2008 and 2009 we felled 72 pines of approximately 65 years age, and we incubated sections of them in insect emergence traps. It was found that the trees near lakes were significantly less infested by xylophagous insects than the trees sampled away from lakes. The insect numbers and densities were much higher in trees sampled away from lakes. We also tested the ability of *Tomicus piniperda*, the most abundant species of xylophagous insects in our samples, to resist the entomopathogenic fungus, *Beauveria bassiana*. The results show that beetles sampled near lakes were less susceptible to the fungal infection than individuals sampled away from forest lakes indicating that the beetles near lakes invested more in their immunity during larval phase. It was found that during the warmest days the maximum ambient temperatures were 2.32°C lower near lakes than away from lakes. Since increased temperatures not only trigger drought stress, but it may also cause temperature-sensitive mortality of conifers, the cooling effect caused by the lake micro-climate may make pines near lakes more resistant against attacks of xylophagous beetles indicated by impaired immunity of *T.piniperda* there. This is the first demonstration of a direct link between reduction of pine resistance and insect immunity-mediated increase in infestation rates by bark- and wood-boring insects in areas without the cooling effect of forest lakes.

SEROPREVALENCE OF TOXOPLASMA GONDII IN RED FOX (VULPES VULPES) AND RACCOON DOG (*NYCTEREUTES PROCYONOIDES*) FROM LATVIA

BY G. DEKSNE

Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, Riga, LV-1076, Latvia;
Institute of Systematic Biology, Daugavpils University, 13 Vienibas street, LV-5401, Daugavpils, Latvia

Toxoplasma gondii is widely distributed in wild and domestic animals. Infection in intermediate hosts is acquired by ingesting meat or carcasses contaminated with *T. gondii* cysts or by consuming contaminated food, water, or soil. *T. gondii* is unable to reproduce sexually in non felids, asexual stages infected tissues of intermediate hosts serves as a source of parasite transmission to other animals by carnivorism witch helps maintain enzootic *T. gondii* in the food chain. Foxes and raccoon dogs eat almost anything including garbage, soil, and plants therefore they are good indicators of *T. gondii* infection in the environment. Also toxoplasmosis in wild carnivores is clinically and epidemiologically important because clinical toxoplasmosis in some of these hosts simulates rabies. Population of red fox and raccoon dog are increasing over the last ten years in Latvia.

The aim of the present study was to investigate and compare the seroprevalence of *T. gondii* in red fox (*Vulpes vulpes*) and raccoon dog (*Nyctereutes procyonoides*) from Latvia using fluids from thawed hearts. Samples were collected in time period October - December, 2010. Animals were legally hunted during annual rabies oral vaccination program. Total 161 red fox and 107 raccoon dog's hearts were collected from all game management districts. The heart muscle were collected during necropsy and placed in a sterile plastic container. The samples were frozen and stored at -20°C. Fluids from hearts was collected from the thawed samples and stored at -20°C until analysis. Fluid samples were analyzed by means of an indirect in-house ELISA using a *T. gondii* tachyzoite (RH strain) protein extraction as the antigen and anti-dog IgG (whole molecule) peroxidase antibody produced in rabbit as the secondary antibody. Samples were diluted 1:100 and analyzed in duplicate.

The prevalence in red foxes was 18.6% which is significantly higher than the value of 4.7% found in raccoon dogs. Seropositive animals were detected in all almost all game management districts excluding areal of Riga. There were no significant differences in seroprevalence in different game management districts. These are just preliminary results and study is still going on.

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PRELIMINARY STUDIES OF *TRICHINELLA* INVASION IN WILDLIFE ANIMALS WITH THE DIFFERENT ANTI-RABIES ANTIBODY TITRE

BY M. DOBROSTANA[1,2], E. BAKASEJEVS[2,3] & M. KIRJUSINA[2]

[1]*Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, LV-1076, Riga, Latvia;*
[2]*Institute of Systematic Biology, Daugavpils University, 13 Vienibas street, LV-5401, Daugavpils, Latvia;*
[3]*Food and Veterinary Service, Border Control Department, BIP, Piekrastes 22, LV-5422, Daugavpils Latvia*

Rabies is an acute infectious viral disease of the central nervous system that affects all warm-blooded wild and domestic animals and humans. Vaccination is the key element in protecting animals and humans from Rabies infection. The method, which is widely used in worldwide practice to control and eradicate rabies distribution, is depositing vaccine baits containing an attenuated anti-rabies liquid vaccine throughout habitats of reservoir species (foxes, raccoon dogs, badgers). The animals' health status affect on the efficacy of vaccination against rabies has not been fully studied yet. However, it is known that parasitic invasion could affect the immune response to vaccination. *Trichinella* invasion is common in wildlife of Latvia.

The aim of this study is to investigate the *Trichinella* occurrence in wildlife animals with the different titre of anti-rabies antibodies (Ab).

For research purposes four raccoon dogs *(Nyctereutes procyonoides)* and eleven red foxes *(Vulpes vulpes)* were collected. Animals' necropsy was performed during 24 hours after animals' death. From each wild animal blood samples and muscles from foreleg were collected. Blood samples were serologically investigated with ELISA commercial kit. Muscle samples from foreleg of animals were tested by magnetic stirrer artificial digestion to detect *Trichinella* spp. larvae.

According to the ELISA results animals were divided into three groups:
I group – animals (n=5) with undetectable seroconversion (Ab titre < 0.125 IU/ml);
II group - animals (n=6) with insufficient seroconversion (0.125 IU/ml < Ab titre < 0.5 IU/ml);
III group – animals (n=4) with sufficient seroconversion (Ab titre ≥ 0.5 IU/ml).

Trichinella invasion was detected in one animal of the second group and in three samples of the third one. At this stage of the study the affect of *Trichinella* invasion on the development of anti-rabies antibodies has not been found yet. This study is still in progress.

SHEEP *PRNP* GENOTYPE PREVALENCE IN LATVIA

BY K. GRĀVE & R. GRANTA

Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, LV-1076, Riga, Latvia

Scrapie is fatal neurodegenerative sheep and goat disease, which belongs to transmissible spongiform encephalopathies group (TSE). Cause of scrapie is infectious cellular prion protein isoform, which is encoded by *PRNP* gene. When connection between *PRNP* gene and susceptibility to classical scrapie was discovered, European Commission started to work on creating genetically resistant sheep population to stop the distribution of this disease.

The aim of this work was to investigate diversity of *PRNP* gene and to determine predominant genotypes in prevailing sheep breed in Latvia – Latvian darkheaded (LD).

There were 222 LD sheep breed *PRNP* gene sequences analyzed regarding codons 136, 154 and 171. As for codons 136, 141, 154 and 171 there were 105 LD sheep *PRNP* sequences analyzed. These samples were sent to Institute of Food Safety, Animal Health and Environment "BIOR" within the National monitoring program of animal infectious diseases.

In Latvian darkheaded sheep there were eleven genotypes established regarding classical scrapie. Most prevalent were ARR/ARQ (50.0%), ARQ/ARQ (22.1%) and ARR/ARR (15.3%). In relation to atypical scrapie ten genotypes were detected. Most prevalent were ALRR/ALRQ (45.7%), ALRQ/ALRQ (21.9%) and ALRR/ALRR (21.9%). Considering this into account, we can conclude that Latvian native sheep breed is sufficiently resistant to classical scrapie.

Results obtained in this work can be used in future Latvian darkheaded sheep genetic diversity studies and for developing classical scrapie resistant sheep population as well as for atypical/Nor98 scrapie strain risk assessment in dominant Latvian sheep breed.

DETERMINATION OF DIBENZO-P-DIOXINS, DIBENZOFURANS AND DIOXIN-LIKE PCB'S IN FISH AND MEAT IN LITHUANIA

BY R. MAŠARAITĖ[1,2,3], J. PETRAITIS[1], I. JARMALAITĖ[1], E. NAUJALIS[3]

[1]*National Food and Veterinary Risk Assessment Institute, J. Kairiūkščio str. 10, LT-08409, Vilnius, Lithuania;*
[2]*Natural Science and Technology Center, Chemistry Institute, A. Goštauto str. 9, LT-01108, Vilnius, Lithuania;*
[3]*Natural Science and Technology Center, Semiconductor Physics Institute, A. Goštauto str. 11, LT-01108, Vilnius, Lithuania*

Fish, meat and their products play a significant role in dietary intake of PCDD/PCDF, that's why the analytical methods of dibenzo-p-dioxins, dibenzofurans and PCB's were developed by HR-GC/MS. Despite very small amounts of these congeners, their toxisity is very high, so sensitivity and selectivity of analytical methods are very important analysing samples with HR-GC/MS, because it is possible to achieve concentrations in the level of g^{-9} (ng) or even g^{-12} (pg).

Extraction and clean – up of the samples suppose to be the most important goal. As analytes are lipophylic so the fat content of the sample is determined firstly. Further analysis is based on the clean – up of fat, fractionating and is made with several columns filled with different sorbents such as silicagel, florisil, celite and carbon. The amounts of PCDD/F's are very small so the cleaning process should be as accurate as possible. Validation was carried out at the level of interest. Recoveries of internal standarts were achieved 40 – 130 % for fish and 60 – 120% for meat. Trueness and precision were evaluated on repeatability and reproducibility conditions (Table 1). Limits of quantification (< 1/5 of maximum limit) and other validation parameters fulfill requirements that are set out in COMMISSION REGULATION.

Table1. Validation data in fish and meat matrixes on reproducibility conditions (n = 11)

	SD, pg/g fresh weight	**RSD, (<15 %)**	**Trueness, (+/-20%)**
Upper bound PCDD/F's (Fish matrix)	3.18 +/- 0.4	12.8	- 6.5
Upper bound PCDD/F's, DL-PCB's (Fish matrix)	7.10 +/- 0.5	7.4	-12.2
Upper bound PCDD/F's (Meat matrix)	0.77 +/- 0.04	5.3	1.5
Upper bound PCDD/F's, DL-PCB's (Meat matrix)	3.46 +/- 0.3	7.9	1.3

Fish, meat and their products mostly contain congeners such as 2,3,7,8-tetrachlordibenzofuran, 2,3,4,7,8-pentachlordibenzofuran and polichlorbiphenyls 105, 118 in bigger amounts.

There were analysed 87 fish samples (Baltic herring, Baltic sprats, Baltic salmon, Baltic cod liver, carp and their products) and 24 animal fat samples (chicken fat, bovine fat, pork fat) 2005 – 2010 in NFVRAI. According the European Commission Regulation, 23 % of fish samples were found positive that does not meet the requirements concerning WHO-$TEQ_{(1998)}$-PCDD/F-PCB.

QUALITY OF FRESH CUT PEAR SLICES TREATED BY DIFFERENT COMBINATIONS OF ANTIBROWNING AGENTS

BY I. KRASNOVA[1], V. SURAKA[2], D. KARKLINA[3], & D. SEGLINA[1]

[1]*Latvia State Institute of Fruit Growing, Graudu Street 1, Dobele, LV 3701, Latvia;*
[2]*Institute of Food Safety Animal Health and Environment „BIOR", 3 Lejupes street, Riga, LV-1076, Latvia;*
[3]*Latvia University of Agriculture, Faculty of Food Technology, 2 Liela str., Jelgava, LV 3001, Latvia*

One of the main problems in food industry is fruit browning. It is deteriorating fresh cut fruit quality and affecting consumer acceptance of product. Microbiological safety and overall quality of fresh cut pear slices during the realization could be maintained using different antibrowning agent solutions for surface treatment. The aim of study was to evaluate different combinations of inhibitors on microbiological quality and antibrowning reactions of fresh cut pears. Pears (*Pyrus communis*, cultivar 'Belorusskaja Pozdnaja') were washed in running water and dipped for 30 min in prepared Natureseal® FS disinfectant. After fruits were peeled, cut into 1-1.5 cm pieces, and immersed in solution of antibrowning agents or in distilled water. Structure stabilizers (calcium chloride (CaCl) 0.5%; calcium lactate (CaLac) 0.5%), acidifiers (citric acid (CA) 0.5% + ascorbic acid (AA) 1.5%); cranberry juice (CJ) 20%) and sweeteners (sucrose syrup (S) 15%, fructose syrup (F) 15%) were used as inhibitors in eight different combinations. Sucrose and fructose syrups (both 15%) without inhibitors were taken as control solutions. Samples were stored for 12 days at temperature +4 ± 0.5 ˚C. Microbiological analyses (*E.coli*, total aerobic psychrophilic microorganisms (TAPM), molds, yeast physical analyses (colour and texture measurements), chemical analysis (vitamin C and total phenol content) and sensory evaluation were performed in every 3 days. Results showed that the best antibrowning effectiveness was obtained using browning inhibitor combination of sweeteners (F or S), acidifiers (CA, AA) and structure stabilizer (CaCl). During the storage number of mold and total aerobic psychrophilic microorganisms differed significantly regarding to the combination of browning inhibitors. Increase of microbiological contamination was reached after six days of storage. Molds were detected in a range from 3.78 to 2.02 log CFU g-1 and TAPM from 6.31 to 1.98 log CFU g-1 while yeast growth at sixth day was not detected or was lower compared to first day. Samples treated by combination of sweetener (S), structure stabilizer (CaLac) and acidifier (CJ) showed the lowest microbiological contamination what could be explained by antibacterial properties of cranberry juice. Evaluating CFU g-1 (colony-forming units) of samples during the experiment, recommendable storage time for fresh cut pear slices was 5 days at +4 ± 0.5 ˚C.

FIN CONDITION OF SALMON (*SALMO SALAR*) AS WELFARE INDICATOR

BY R. MEDNE[1,2], E. LIEPIŅŠ[2], I. RUTKOVSKA[1,2] & M. ZIŅĢIS[1]

[1]*Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, Riga, Latvia;*
[2]*Faculty of Veterinary medicine, Latvia University of Agriculture, 8 K. Helmaņa street, Jelgava, Latvia*

Condition of all fins was assessed in intensively cultured salmon (*Salmo salar*) in comparison with pond-reared salmon, natural spawning salmon smolt and adult salmon from Baltic sea. Salmon of both genders (male and female) and different ages were used in research. Fin necrosis and fin pathologies weren't observed in smolts reared in ponds and natural smolts. In many salmon smolts reared in tanks different fin pathologies were common: completely healthy fins, more or less damaged fin.

The highest fin necrosis index is for dorsal fin (2.19), but the lowest for ventral fin, accordingly 0.04 and 0.06 in salmon reared in tanks. The loss of pair fin in smolts has developed asymmetricaly and is observed in one or both fins.

Bacteriological examination of fin tissue shows that dominates *Aeromonas* spp. (more than 80%), more rarely bacteria from *Flavobacterium* spp. and *Pseudomonas* spp. only in some cases. It was observed that fin necrosis of salmon parrs has developed in the result of combination of trauma and bacteria.

IMPROVEMENT OF FOOD SAFETY MANAGEMENT SYSTEM IN CATERING SECTOR

BY A. MELNGAILE

Food and Veterinary Service, 30 Peldu street, Riga, LV-1050, Latvia

Provision of food safety nowadays is based on introduction of preventive procedure based on HACCP principles. Success of HACCP system depend on both the hazard identification and the introduction of adequate monitoring measures in critical control points of technological processes.

The aim of the research was to investigate the application of the methodology of microbiological risk assessment in catering establishments to ensure science-based proposals for improvement of food safety management system.

The results on microbiological testing of 17192 food samples and 17604 surface swab samples were analysed using methods of mathematical statistics. In order to perform mathematical analysis a grouping system of food and environmental objects was created: food samples were grouped into 14 identification classes, 31 groups and 142 types, taking into account the food main components and characteristic methods of technological processing; environmental objects were grouped into 16 identification classes, 90 groups and 187 types, taking into account the characteristic application of equipment, utensils, constructions and other objects; and methods of technological processing of foods were grouped into 18 groups taking into account the extent and way of technological processing, including characteristic sequence of technological processes.

The statistical data were analysed with help of software package SPSS 13.0 using Che-Square criterion and Variance Analysis ANOVA for mathematicl analysis of data.

Results of the research suggest that risk of total microbiological contamination of ready-to-eat foods, as well as the probability of presence of coliforms and *S. aureus* in foods is substantially dependent on the class, group and type of ready-to-eat foods due to the nature of main food ingredients ($p=0.000$), as well as due to method of technological processing ($p=0.000$). Risk of microbiological contamination of surfaces in the kitchen environment is substantially dependent on the class, group and type of environmental objects due to their role within course of technological processes ($p=0.000$). The higher probability of the microbiological contamination is proven for individual types of foods such as fried and braised meat and offal foods, especially fried poultry and fried minced meet foods, pasta foods with meat components, pancakes with meat or curd stuffing, rissole soup salads prepared from both the raw vegetables and cooked vegetables and certain sweets – dessert creams, mousse, sweet porridge, pastry foods with cream stuffing and muffins ($p=0.000$).

The results of the research suggest that microbiological risk assessment can be recommended for verification and improvement of self-control procedures. Microbiological risk assessment can be a helpful tool to reveal the characteristic trends in transmission of microbiological contamination and to ensure science-based guidelines for setting of priorities for purposeful control of food safety hazards.

CELLS MORPHOMETRIC PARAMETER OF LYMPH NODES IN PIG WITH PORCINE CIRCOVIRUS-2

BY I. PIGIŅKA[1] & E. BIRĢELE[2]

[1]*Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, Riga, LV 1076, Latvia;*
[2]*Preclinical Institute, Faculty of Veterinary Medicine, Latvian University of Agriculture, 8 K. Helmaņa street, Jelgava, LV-3004, Latvia*

Pig infected by porcine circovirus-2 (PCV2) has unspecific clinical sings and gross pathology, bet has specific histological lesion in lymphoid tissues. It is lymphocyte depletion and histiocytic cells proliferation in lymph nodes; as well lose visualisation of follicles in lymph nodes. Usually, intensity of lesions in lymph nodes caused by PCV2 is evaluated by score ranges from 0 to 3. This evaluation system was based on the tissues histological structure. Therefore aim of study was to investigate amount of cells of lymph nodes in pig with porcine circovirus-2.

Study was carried out at winter 2010. For research purposes were collected 5-15 weeks old five pigs carcases from farm. Pigs were selected by following pathological changes: wasting, enteritis or respiratory disease, pallor or jaundice and lymphadenopathy. Pigs' necropsy was performed during 12 hours after pigs' death. From each pig were collected blood samples and lymph nodes *lnn. inguinales superficiales sinister at dexter, traheobronchales* and *mesenterici craniale.*

Blood samples were serologically investigated for PCV2 antibody detection by ELISA Synbiotics, Serelisa PCV2 Ab Mono Blocking kits. Lymph nodes were investigated by classical histology with haematoxylin-eosin staining and immunohistochemistry for PCV2 antigen detection with Ingenasa PCV2 antibody. Lymph nodes were microscopically evaluated by score ranges from 0 to 3. Macrophages, lymphocytes, histiocytic and plasma cells were counted separately for cortex, paracortex and medulla area in lymph node. In each histological slide of lymph node was investigated by five fields 150x150 μm in cortex, paracortex and medulla area. Morphometric investigation for histological slides was carried out in Institute of Systematic Biology Daugavpils University and was used microscope Nikon Eclipse 90i with the NIS-Elements BR™ laboratory image analysis system.

In the result, all pigs were seropositive for PCV2 and PCV2 antigen were detected in all lymph nodes of pigs. Lymph nodes evaluated by score ranges 3 had average number of lymphocytes 102.5, macrophages 26.4 and histiocytic cells 14.7 in cortex. Lymphocytes located in periphery of follicle, but macrophages and histiocytic cells in germinal centre of cortex. Lymph nodes evaluated by score ranges 3 had average number of lymphocytes 72.4, macrophages 28.4, histiocytic cells 17 in paracortex and lymphocytes 23.8, macrophages 37.9, histiocytic cells 26.6 in medulla.

The results of study are the same us other researches dates concerning histiocytic cells, macrophages proliferation and lymphocytes depletion. Research is in process.

ON THE STUDY OF CARP (*CYPRINIS CARPIO*) PARASITOFAINA IN LATVIAN PONDS

BY M. KIRJUSINA[1,2] & M. SERŽANTS[1,3]

[1]*Institute of Systematic Biology, Daugavpils University, 13 Vienibas street, Daugavpils, Latvia;*
[2]*Institute of Food Safety, Animal Health and Environment "BIOR", 3 Lejupes street, Riga, Latvia;*
[3]*Food and Veterinary Service, 30 Peldu street, Riga, LV-1050, Latvia*

Common carp is the main object in Latvian aquaculture. Therefore, it is important to know carp health condition in all part of Latvia and in different types of fish farms.

The aim of the study was to investigate marketable size carp parasites and summarize data concerning carp parasite fauna in previous researches.

The parasite fauna of carp from fish farms was studied in different regions of Latvia mainly during spring - autumn seasons. For fish examination was used the total parasitological dissection method. Parasite species identification was based on morphological characteristics.

Sixty-six species of parasites belonging to following systematic groups have been found: Protozoa (23 species), Monogenea (9), Digenea (9), Cestoda (9), Nematoda (4), Acanthocephala (2), Hirudinea (1), Mollusca (1), Crustacea (1), were found.

Results of study show that more diversity of parasite species were detected from Protozoa, herewith pathogenic species for fish *Chilodonella piscicola*, *Goussia carpelli* and *Ichthyophthirius multifiliis.*

More frequently invasion with *Dactylogyrus extensus*, *D. achmerovi* were registered in carp gills and obtain 100% prevalence in some ponds. In high water temperature *D. extensus* observed in small quantities but prevail thermophilic *Dactylogyrus* species. Pathogenic for carp yearling *Gyrodactylus kathariner*i wasn't common species in ponds.

Two trematoda metacercaria species has been recorded in carp musculature – *Paracoenogonimus ovatus* and *Apharhyngostrigea conu*. These species appeared in carp last five years. Pathogenic species *Posthodiplostomum cuticola* and *Sanguinicola inermis* were found rarely.

Amount of larvae cestoda *Valipora campylancristrota* and *Paradilepis scolecina* in carp significantly increased last time, due to a large number of birds (definitive hosts of parasites) around ponds. Pathogenic *Bothriocephalus achejlognathi* was detected in small amount in fish fry.

Phillometroides cyprini is common species for carp in ponds and in some lakes.

In general differences of parasite fauna of carp between different regions of Latvia are negligible.

THE DINAMICS OF FINDING BACTERIA AND PHYSIOCHEMICAL PARAMETERS OF COLD-SMOKED SAUSAGES DURING RIPENING

BY I. SILIŅŠ & E. LIEPIŅŠ

Institute of Food and Environment Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture, Helmana str. 8, Jelgava, LV-3004, Latvia

Theoretically most of relevant food pathogen bacteria can be found in cold-smoked sausages. However, when fermentation process is not adequate, there is a potential microbiological risk – some food pathogens may survive and proliferate during ripening.

The five series of cold smoked sausages were investigated (total 105 samples) on presence and count of planed investigated bacteria, and the mean values of lg(cfu g-1) were estimated, in addiction to pH, salt concentration (%), and water activity (aw) changes at ripening time. For detection and enumeration of bacterial cultures standard microbiology of food and animal feeding stuffs ISO methods, adapted in Latvia were used. Salt concentration, pH, and water activity (aw) were measured on 0, 1st, 3rd, 5th, 7th, 14th and 21st days of maturation in each time with microbiological investigations. The results represent the mean ± standard deviations. Differences were considered statistically significant when $p < 0.05$.

It was observed that bacterial counts - total aerobic count (TAC) and *Staphylococcus aureus* increased from 5.72 to 9.41 lg and from 1.38 to 2.68 lg respectively. The count of detected *E. coli* decreased approximately by 1.5 logs (from 2.48 to 0.85 lg) during sausage ripening time of 21 days. *L. monocytogenes* was detected in one of five sausages series, but tested only during the first 5 days when the count decreased from 3.41 to 2.08 lg cfu g-1, and was not detected on days 7, 14, and 21of sausage ripening. *Salmonella* spp. was not detected at any time. In this study the reduction of *L. monocytogenes* count was lg 0.27 day-1, but for *E. coli* count a decrease rate was lg 0.08 day-1. The samples of cold smoked sausages had a mean initial pH value of 5.80 ± 0.04, and the final pH mean value of 4.64 ± 0.05. The mean value of initial water activity was 0.96 which decreased in the product from 0.963 ± 0.004 to 0.817 ± 0.006 in 21 days. The salt concentration increased from 2.94 ± 0.01 to 3.53 ± 0.03 content %. All measured physical and chemical parameters significantly ($p < 0.01$) correlate with the decreased *L. monocytogenes*, and *E. coli* count, at the same time with the increased TAC and *S. aureus* count in first 5 days. It is also seen that aw, pH value, and salt concentration changes did not affect the growth of *S. aureus* in ripening time in 21 days. In this study *S. aureus* growth rate was lg 0.10 every day.

The continual decreased changes of water activity and partly pH, and increasing salt concentration diminished possible initial count of some bacterial species, such as *L. monocytogenes* and *E. coli* that allows considering cold-smoked sausages being relatively safe and healthy meat product, under condition that initial contamination with *S. aureus* is minimal.

Water activity (aw), pH value, and salt concentration changes did not affect the growth of *S. aureus* in ripening time in 21 days.

PREVALENCE OF ANTIBODIES TO PRRSV IN WILD BOARS FROM LITHUANIA

BY J. BUITKUVIENE[1,2], R. CEPULIS[1], J. VALANCIUTE[1] & A. STANKEVICIUS[1]
[1]*Lithuanian University of Health Sciences, Tilžės str. 18, LT - 47181, Kaunas, Lithuania;*
[2]*National Food and Veterinary Risk Assessment Institute, J. Kairiūkščio str. 10, LT-08409, Vilnius, Lithuania*

PRRSV is endemic in most swine-producing countries, and is associated with major economic losses. Antibodies to PRRSV can be found in pig samples world-wide, however, positive wild boar samples were only occasionally found in some countries harbouring very dense swine and wild boar populations. Most likely wild boars there became infected by domestic swine as a result of seldom direct or indirect contacts. Majority of pig farms in Lithuania were positive for PRRSV, but recently some had depopulated and became PRRSV free. Since domestic pigs and wild boars have the same susceptibility to various infections there was major concern to monitor the epidemiological PRRSV situation in feral pigs.

The objective of the present study was to investigate prevalence and distribution of PRRSV antibodies in Lithuanian wild boars.

A total of 659 serum samples from wild boars from 42 locations throughout Lithuania were collected during autumn–winter hunting seasons 2008/2009 and 2009/2010. The wild boars sera were analyzed via different ELISA test systems, IDEXX PRRS 2XR Ab, IDEXX HERDCHEK PRRS X3 antibody test kits (Corporate Headquarters IDEXX Laboratories, Inc., USA), Ingezim PRRS Europe (Ingenasa, Madrid, Spain) according to manufacturer's instructions and ISO/IEC 17025:2005 standard accredited laboratory.

From 659 examined wild boar sera, 43 (6.5 %) were positive to PRRSV antibodies. Investigation of PRRSV antibodies with different ELISA kits did not show difference in detection positive serum samples ($p>0.05$). The results of serological analysis are summarized in Table 1. Antibodies to PRRSV were detected in all age groups, however seroprevalence was significantly higher in adult animals (Table 2). Wild boars serum samples from 31 locations out of 42 investigated were seropositive for PRRSV.

In spite of the fact that PRRSV is actively circulating in domestic swine of Lithuania, the seroprevalence in wild boars was only 6.5 %. This result indicates very low possibility of contacts between wild boars and domestic swine, which could present opportunity for PRRSV transmission. Similar results of PRRSV seroprevalence (8.92%) in feral pigs were reported in Croatia (3). However in reports from Italy the prevalence (37.7%) of PRRSV antibodies was quite high and it could be due to PRRSV transmission from domestic pigs to wild boars (2).

Table 1. The results of detection PRRSV antibodies in wild boars samples

Year	Number of investigated serum samples	Number of positive serum samples	% positive
2008	286	26	9.1
2009	274	15	5.5
2010	99	2	2.02
Total	**659**	**43**	**6.5**

Table 2. Prevalence of PRRSV antibodies in wild boars serum by age groups.

Age group	Number investigated	Number positive	% positive
Juveniles (up to 12 month)	227	8	3,5
Subadults (up to 24 month)	266	9	3,4
Adults (over 24 month)	166	26	15,7

This is the first report of serological evidence of PRRSV infection in the wild boar population in Eastern Europe. Interestingly, PRRSV antibodies in feral pigs were so far not detected in the neighboring countries such as Russia (1) or Poland.

Acknowledgement

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PATHOGENIC AIL-POSITIVE *YERSINIA ENTEROCOLITICA* IN SLAUGHTERED PIG TONSILS IN LATVIA

BY M. TERENTJEVA[1], K. GRĀVE[2] & A. BĒRZIŅŠ[1,2]

[1]*Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture, K.Helmaņa 8, Jelgava, LV 3004, Latvia;*
[2]*Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes iela 3, Rīga, LV-1076, Latvia*

Yersinia enterocolitica is a food-borne pathogen, which can cause yersiniosis in human. Pigs are the most important carriers of pathogenic *Y. enterocolitica* and pathogen was often isolated from pig tonsils in previous studies. Since majority of *Y. enterocolitica* strains, which are widely distributed in the environment are non-pathogenic, pathogenicity of *Y. enterocolitica* isolates should be assessed.

The aim of the present study was to confirm the pathogenicity of *Y. enterocolitica* isolates from pigs using PCR method.

A total amount of 20 *Y. enterocolitica* cultures, which were isolated from pig tonsil samples, were used for PCR.

Pig tonsil samples were collected in two slaughterhouses in Latvia during 2007-2008. Samples were tested applying the direct plating of sample material on CIN agar, the selective enrichment in ITC broth prior to plating and the cold enrichment method for 7, 14 and 21 days. Biotyping and serotyping were applied for *Y. enterocolitica*.

One of genetic loci encoding invasive ability of yersiniae is *ail* (attachment invasion loci) region *Y. enterocolitica*, respectively it is considered to be one of pathogenicity factors.

Pathogenicity of isolates was confirmed with PCR method, detecting the presence of *ail* gene.

All *Y. enterocolitica* cultures isolated from pig tonsils belonged to biotype 4, serogroup O:3. One of genetic loci encoding invasive ability of yersiniae - *ail* was confirmed in all *Y. enterocolitica* isolates.

The presence of *ail* gene in *Y. enterocolitica* isolated from Latvian slaughtered pig tonsils confirms that all isolates were human pathogenic and may have an impact on pork safety to consumers.

PARASITOFAUNA OF THE WINTER FISHERY CAUGHT FISH IN LAKES NEAR FROM RIGA AND IN THE GULF OF RIGA

BY M. ZIŅĢIS[1], R. MEDNE[1,2], I. RUTKOVSKA[1,2], & O. MAKSIMOVA[2]

[1]*Institute of Food Safety, Animal Health and Environment BIOR, 3 Lejupes street, Riga, LV-1076, Latvia;*
[2]*Faculty of Veterinary Medicine, Latvia University of Agriculture, 8 K.Helmaņa, Jelgava, LV-3004, Latvia*

There are many winter fishing places near from Riga. Fish were investigated from the Gulf of Riga and lakes near from Riga: lake Ķīšezers, lake Mazais Baltezers, lake Dūņezers. Fish were gained in the winter of 2010-2011. Parasitological investigation was held for fish caught during the research, ichtiopathological changes were determined and measurements of weight and length were made and gender was determined. Investigations were made for following species of fish: perch (*Perca fluviatilis*), ruffe (*Gymnocephalus cernua*), eelpout (*Zoarces viviparus*), bream (*Abramis brama*), roach (*Rutilus rutilus*), Baltic herring (*Clupea harengus membras*), rudd (*Scardinius erythrophthalmus*), pike-perch (*Stizostedion lucioperca*). The following parasites in these fish were found: *Thynnascaris adunca, Ligula intestinalis, Philometra* spp., *Triaenophorus nodulosus, Posthodiplostomum cuticula, Acanthocephalus lucii, Trypanosoma percae*.

LIST OF PARTICIPANTS

PARTICIPANT	INSTITUTION	COUNTRY	CONTACT
Antone Unigunde	Latvia University of Agriculture, "SIGRA"	LATVIA	sigra@lis.lv
Appel Bernd	Federal Institute for Risk Assessment	GERMANY	bernd.appel@bfr.bund.de
Asadchaya Raisa	RUE «Institute of Experimental Veterinary Medicine named of S.N. Vyshelessky»	BELARUS	asadchaya@mail.ru
Avsejenko Jeļena	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	jelena.avsejenko@bior.gov.lv
Bagrade Guna	Natural History Museum of Latvia	LATVIA	guna.bagrade@ldm.gov.lv
Bakasejevs Eduards	Daugavpils University, Food and Veterinary Service	LATVIA	edlasen@gmail.com
Bartkevičs Vadims	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	vadims.bartkevics@bior.gov.lv
Bērziņš Aivars	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	aivars.berzins@bior.gov.lv
Bērziņa Zanda	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	zanda.berzina@bior.gov.lv
Briede Inese	Daugavpils University	LATVIA	inesebriede28@inbox.lv
Bubuliene Ruta	National Food and Veterinary Risk Assessment Institute	LITHUANIA	rbubuliene@vet.lt
Buitkuviene Jurate	National Food and Veterinary Risk Assessment Institute	LITHUANIA	jbuitkuviene@vet.lt
Cīrule Dina	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	dina.cirule@bior.gov.lv
Grāve Kristīne	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	kristine.grave@bior.gov.lv
Daukšte Anžela	Daugavpils University	LATVIA	anzela.dauksteb@biology.lv
Daukšte Janīna	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	janina.dauksteb@bior.gov.lv
Deksne Gunita	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	gunita.deksne@bior.gov.lv
Dobroštana Marianna	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	marianna.dobrostana@bior.gov.lv
Dzerkale Anna	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	anna.dzerkale@bior.gov.lv
Eizenberga Inga	Latvia University of Agriculture, IFSAHE "BIOR"	LATVIA	inga.eiz@inbox.lv
Fredriksson-Ahomaa Maria	University of Helsinki	FINLAND	maria.fredriksson-ahomaa@helsinki.fi
Jarmalaite Inga	National Food and Veterinary Risk Assessment Institute	LITHUANIA	ijarmalaite@vet.lt
Joffe Rafaels	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	rafaels.joffe@bior.gov.lv

PARTICIPANT	INSTITUTION	COUNTRY	CONTACT
Jokelainen Pikka	University of Helsinki	FINLAND	pikka.jokelainen@helsinki.fi
Kirjušina Muza	Daugavpils University	LATVIA	muza.kirjusina@biology.lv
Kļavkalne Sanita	SIA Piensaimnieku laboratorija	LATVIA	sanita.klavkalne@pienslabs.lv
Kokina Inese	Daugavpils University	LATVIA	inese.kokina@biology.lv
Kooļa Jeļena	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	jelena.koola@bior.gov.lv
Konvisers Genadijs	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	genadijs.konvisers@bior.gov.lv
Kramarenko Toomas	Veterinary and Food Laboratory	ESTONIA	toomas.kramarenko@vetlab.ee
Krasnova Inta	Latvia State Institute of Fruit Growing	LATVIA	inta.krasnova@lvai.lv
Krastiņa Dita	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	dita.krastina@bior.gov.lv
Krūmiņa Daiga	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	daiga.krumina@bior.gov.lv
Kuus Merle	Veterinary and Food Laboratory	ESTONIA	merle.kuus@vetlab.ee
Lassen Brian	Estonian University of Life Sciences	ESTONIA	brian.lassen@gmail.com
Lepik Triin	Estonian University of Life Sciences	ESTONIA	triin.lepik@emu.ee
Lilleorg Roman	Veterinary and Food Laboratory	ESTONIA	roman.lilleorg@vetlab.ee
Lindström Miia	University of Helsinki	FINLAND	miia.lindstrom@helsinki.fi
Liparte Sandra	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	sandra.liparte@bior.gov.lv
Makarova Svetlana	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	svetlana.makarova@bior.gov.lv
Mamlejeva Dzhamila	Federal State Institution „Leningrad Interregional Veterinary Laboratory"	RUSSIA	general@vetlab.spb.ru
Matuzale Zanda	Ministry of Agriculture	LATVIA	zanda.matuzale@zm.gov.lv
Melngaile Aija	Food and Veterinary Service	LATVIA	aija.melngaile@pvd.gov.lv
Medne Ruta	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	ruta.medne@bior.gov.lv
Miņina Janīna	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	janina.minina@bior.gov.lv
Musting Moonika	Veterinary and Food Laboratory	ESTONIA	moonika.musting@vetlab.ee
Mäesaar Mihkel	Veterinary and Food Laboratory	ESTONIA	mihkel.maesaar@vetlab.ee

PARTICIPANT	INSTITUTION	COUNTRY	CONTACT
Ozollapa Inese	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	inese.ozollapa@bior.gov.lv
Panin Aleksandr	The-All Russia State Centre for Quality and Standardization of Veterinary Drugs and Feed (VGNKI)	RUSSIA	vgnki@vgnki.ru
Pau Ülle	Veterinary and Food Laboratory	ESTONIA	ylle.pau@vetlab.ee
Piginka Inga	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	piginka@inbox.lv
PridotkasGediminas	National Food and Veterinary Risk Assessment Institute	LITHUANIA	gpridotkas@vet.lt
Rodze Ieva	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	ieva.rodze@bior.gov.lv
Ruska Diāna	SIA Piensaimnieku laboratorija	LATVIA	diana.ruska@pienslabs.lv
Rutkovska Ilze	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	ilze.rutkovska@bior.gov.lv
Segliņa Dalija	Latvia State Institute of Fruit Growing	LATVIA	dalija.seglina@lvai.lv
Sergejeva Maija	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	maija.sergejeva@bior.gov.lv
Seržants Martiņš	Food and Veterinary Service, DU	LATVIA	martins.serzants@pvd.gov.lv
Siliņš Indulis	Latvia University of Agriculture	LATVIA	indulis.sil@inbox.lv
Streikiša Madara	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	madara.streikisa@bior.gov.lv
Stepanjana Linda	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	linducis21@gmail.com
Straupe Liene	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	wildkit@inbox.lv
Suraka Valerija	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	valerija.suraka@bior.gov.lv
Szwedowski Jatzek	SARSTEDT Ltd.	GERMANY	sarstedt@inbox.lv
Terentjeva Margarita	Latvia University of Agriculture	LATVIA	margarita.terentjeva@llu.lv
Valciņa Olga	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	olga.valcina@bior.gov.lv
Vedel Katrin	Veterinary and Food Laboratory, Tartu	ESTONIA	katrin.vedel@vetlab.ee
Viskuba Elita	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	elita.viskuba@bior.gov.lv
Ziņģis Mārcis	Institute of Food Safety, Animal Health and Environment "BIOR"	LATVIA	zingismarcis@inbox.lv

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