

Molecular Detection and Phylogenetic Relationships of Honey Bee-Associated Viruses in Bee Products

Delka Salkova, Ralitsa Balkanska, Rositsa Shumkova, Stela Lazarova, Georgi Radoslavov, and Peter Hristov

Abstract

In the last few years, the isolation and amplification of DNA or RNA from the environment (eDNA/eRNA) has proven to be an alternative and non-invasive approach for molecular identification of pathogens and pests in beekeeping. We have recently demonstrated that bee pollen and bee bread represent suitable biological material for the molecular identification of viral RNA. In the present study, we extracted total RNA from different bee products (pollen, n = 25; bee bread, n = 17; and royal jelly, n = 15). All the samples were tested for the presence of six of the most common honey bee-associated viruses—Deformed wing virus (DWV), Acute bee paralysis virus (ABPV), Chronic bee paralysis virus (CBPV), Sacbrood virus (SBV), Kashmir bee virus (KBV), and Black queen cell virus (BQCV)—using a reverse transcription polymerase chain reaction (RT-PCR). We successfully detected six records of DWV (10.5%, 6/57), four of ABPV (7.0%, 4/57), three of Israeli acute paralysis virus (IAPV) (5.3%, 3/57), and two of BQCV (3.5%, 2/57). Using ABPV primers, we also successfully detected the presence of IAPV. The obtained viral sequences were analyzed for phylogenetic relationships with the highly similar sequences (megablast) available in the GenBank database. The Bulgarian DWV isolates revealed a high homology level with strains from Syria and Turkey. Moreover, we successfully detected a DWV strain B for the first time in Bulgaria. In contrast to DWV, the ABPV isolates formed a separate clade in the phylogenetic tree. BQCV was closely grouped with Russian isolates, while Bulgarian IAPV formed its own clade and included a strain from China. In conclusion, the present study demonstrated that eRNA can be successfully used for molecular detection of honey bee-associated viruses in bee products. The method can assist the monitoring of the health status of honey bee colonies at the local, regional, and even national levels.

Keywords: honey bee-associated viruses; RT-PCR; phylogeny; honey bee products.

Clinical trial of the efficiency of three different compositions of acaricidal substances against varroosis in honey bee colonies

Delka SALKOVA, Kalinka GURGULOVA, Ivanka ZHELYAZKOVA

Abstract

This study aimed to evaluate and compare the varroacidal efficacy and mite mortality dynamic during autumn treatment of honey bee colonies in two experimental areas (Boychinovtsi Northwestern Bulgaria and Zlatiya- Northeastern Bulgaria), treated with three available veterinary medicinal substances. The clinical studies were conducted on the efficiency of the three acaricidal combinations (AC) - one, based on 3.6 mg flumethrin/strip (AC-1) and two contents of essential oils (first one with composition: 5 g thymol plus 2 g peppermint oil/lamellae (AC-2), and the second one with composition: 4 g thymol plus 2 g peppermint oil/ lamellae (AC-3), in the autumn of 2017. We used the product containing coumaphos and an additive with oxalic acid for the control treatment. Methods for establishing the levels of *Varroa destructor* infestation in bees and in brood were used according to OIE Terrestrial Manual. After 35 days of AC-1 exposure, 94.5% and 87.82% efficiency were achieved in the apiaries in Boychinovtsi and Zlatiya, respectively. Efficiencies of the combinations tested (AC-2 and AC-3) for 45 days were detected high only in the Zlatiya apiary (97% and 95%), also 91% and 80% in the Boychinovtsi apiary, respectively. The results of the experiments showed the absence of resistance to the tested substances.

Keywords: *Apis mellifera*, Resistance, *Varroa destructor* mite, Varroacidal efficiency

Molecular Detection and Phylogenetic Analysis of Deformed Wing Virus and Sacbrood Virus Isolated from Pollen

Ralitsa Balkanska, Rositsa Shumkova, Nedyalka Atsenova, Delka Salkova, Heliana Dundarova, Georgi Radoslavov and Peter Hristov

Abstract

Among many pathogens and pests, honey bee viruses are known as one of the most common cause of diseases in honey bee colonies. In this study, we demonstrate that pollen grains and bee bread are potential sources of viral DNA. We extracted DNA from 3 types of pollen samples: directly provided by beekeepers (n = 12), purchased from trade markets (n = 5), and obtained from honeycombs (bee bread, n = 10). The extracted DNA was used for molecular detection (RT-PCR analysis) of six of the most widely distributed honey bee viruses: deformed wing virus, sacbrood virus, acute bee paralysis virus, black queen cell virus, Kashmir bee virus, Israeli acute paralysis virus, and chronic bee paralysis virus. We successfully managed to establish only the deformed wing virus (DWV) and the sacbrood virus (SBV), with different distribution frequencies depending on the territory of the country. The phylogenetic analyses of Bulgarian isolates were performed with the most similar sequences available in molecular databases from other countries. Phylogenies of Bulgarian viral strains demonstrated genetically heterogeneous populations of DWV and relatively homogenous populations of SBV. In conclusion, the results obtained from the current study have shown that pollen is a valuable source for molecular detection of honey bee pathogens. This allows epidemiological monitoring of honey bee diseases at a regional and a national level.

Keywords: honey bee viruses; RT-PCR; pollen; phylogeny; epidemiology

Composition and diversity of bacterial communities associated with honey bee foragers from two contrasting environments

Stela Lazarova, Lyudmila Lozanova, Boyko Neov, Rositsa Shumkova, Ralitsa Balkanska, Nadezhda Palova, Delka Salkova, Georgi Radoslavov and Peter Hristov

Abstract

The honey bee is associated with a diverse community of microbes (viruses, bacteria, fungi, and protists), commonly known as the microbiome. Here, we present data on honey bee microbiota from two localities having different surrounding landscapes – mountain (the Rhodope Mountains) and lowland (the Danube plain). The bacterial communities of abdomen of adult bees were studied using amplicon sequencing of the 16S rRNA gene. The composition and dominance structure and their variability within and between localities, alpha and beta diversity, and core and differential taxa were compared at different hierarchical levels (operational taxonomic units to phylum). Seven genera (*Lactobacillus*, *Gilliamella*, *Bifidobacterium*, *Commensalibacter*, *Bartonella*, *Snodgrassella*, and *Frischella*), known to include core gut associated phylotypes or species clusters, dominated (92–100%) the bacterial assemblages. Significant variations were found in taxa distribution across both geographical regions and within each apiary. *Lactobacillus* (Firmicutes) prevailed significantly in the mountain locality followed by *Gilliamella* and *Bartonella* (Proteobacteria). Bacteria of four genera, core (*Bartonella* and *Lactobacillus*) and non-core (*Pseudomonas* and *Morganella*), dominated the bee associated assemblages of the Danube plain locality. Several ubiquitous bacterial genera (e.g., *Klebsiella*, *Serratia*, and *Providencia*), some species known also as potential and opportunistic bee pathogens, had been found in the lowland locality. Beta diversity analyses confirmed the observed differences in the bacterial communities from both localities. The occurrence of non-core taxa contributes substantially to higher microbial richness and diversity in bees from the Danube plain locality. We assume that the observed differences in the microbiota of honey bees from both apiaries are due to a combination of factors specific for each region. The surrounding landscape features of localities and related vegetation, anthropogenic impact and land use

intensity, the beekeeping management practices, and bee health status might all contribute to observed differences in bee microbiota traits.

Keywords: amplicon sequencing; *Apis mellifera*; bacterial microbiota; landscape structure; microbiome

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Molecular Detection of *Nosema* spp. in Honey in Bulgaria

Delka Salkova, Rositsa Shumkova, Ralitsa Balkanska, Nadezhda Palova, Boyko Neov,

Georgi Radoslavov and Peter Hristov

Abstract

Environmental DNA (eDNA) analysis is related to screening genetic material of various organisms in environmental samples. Honey represents a natural source of exogenous DNA, which allows for the detection of different honey bee pathogens and parasites. In the present study, we extracted DNA from 20 honey samples from different regions in Bulgaria and tested for the presence of DNA of the ectoparasitic mite *Varroa destructor*, as well as *Nosema apis* and *Nosema ceranae*. Only *Nosema ceranae* was detected, showing up in 30% of all samples, which confirms the widespread prevalence of this pathogen. All positive samples were found in plain regions of the country, while this pathogen was not detected in mountainous parts. None of the samples gave positive amplifications for the *Nosema apis* and *Varroa mite*. The obtained results from this study confirm previous observations that eDNA contained in honey is a potent source for effective biomonitoring of actual diseases in the honey bee.

Keywords: *Apis mellifera*; DNA analysis; health status; pathogens; molecular identification

Effect of *Nosema apis* and *N. ceranae* on honey bee *Apis mellifera* queen development

Sigmar Naudi, Risto Raimets, Margret Jürison, Egle S. Liiskmann, Marika Mänd, Delka Salkova, Reet Karise

Abstract

Nosema apis and *N. ceranae* are agents causing the disease called nosemosis in honey bee workers and queens. Few is known about the impacts of it on honey bee development. The royal jelly in queen cells was infected with *Nosema* spores to see whether and how it affects the development of honey bee queens. Seven groups of grafted honey bee larvae were established, and treated as follows: high and low concentrations of *N. ceranae* and *N. apis*, mixes of both species in both concentrations, and untreated control. After allowing nurse bees to fill the queen cells with royal jelly, an injection of 50 000 spores or 10 000 spores was added into the royal jelly. We found that only *N. apis* decreased the hatching rate of honey bee queens both in single and mixed treatment at high dosages, but we did not detect any morphological deviations in unhatched pupae.

Key words: honey bee *Apis mellifera*, *Nosema* spp., honey bee queen breeding, queen quality

**IMPACT OF THE PLANT-BASED NATURAL SUPPLEMENT IMMUNOSTART HERB
ON HONEY BEE COLONY PERFORMANCE**

Rositsa SHUMKOVA, Ralitsa BALKANSKA, Delka SALKOVA, Peter HRISTOV

Abstract

Winter is the season that poses the greatest challenges for honey bee colonies. Therefore, the main approach in beekeeping practice is aimed mainly at providing sufficient quality food supplies for bee colonies in early autumn. We conducted the present study to test the influence of the natural plant extract IMMUNOSTART HERB on population strength, stored pollen area, capped worker brood area, and honey yield. The experimental groups were supplied with IMMUNOSTART HERB 4 times at 7-day intervals, whereas sugar syrup was given to the control groups. The obtained results showed that the applied supplemental diet affected all investigated biological parameters, with the most noticeable effect after the second application. In all measurements, the honey bee colony parameters in the treated groups showed higher values in comparison to the control groups. These results highlight the potential of herbal supplements to effectively improve bee colonies' development during the period of scarce bee forage, as well as to provide suitable conditions for successful overwintering.

Key words: *Apis mellifera*; herbal extract; colony strength; overwintering

TICKS AND ASSOCIATED TICK-BORNE PATHOGENS FROM DOGS AND RED FOXES FROM BULGARIA

M. S. PANAYOTOVA-PENCHEVA, B. VICHOVA, V. I. DAKOVA & D. S. SALKOVA

Abstract

Climate changes in recent years led to a sharp rise in the tick population and an increase in the number of animals and people with tick-borne infections. The domestic and wild carnivores, especially the dogs, have a huge role for the distribution of ticks in certain areas. In this study 60 ixodid ticks collected from domestic dogs and red foxes from Bulgaria have been investigated for infection with *Ehrlichia canis*, *Hepatozoon canis*, *Babesia* spp., and *Rickettsia* spp. The results showed that the dogs were infected with two tick species - *Rhipicephalus sanguineus* (72%) and *Ixodes ricinus* (28%). The red foxes were infected with only one species - *I. ricinus*. Out of all *R. sanguineus* ticks, 43.6% were female and 56.4% male. The opposite was observed for *I. ricinus* - female specimens (86.7%) were significantly more prevalent than males (13.3%). Similar trend was found out for *I. ricinus* collected from red foxes - 66.7% of the ticks were female and 33.3% male. Infectious agents were found in 31.7% of the investigated ticks. *Ehrlichia* spp. was established in 79% and *Rickettsia* spp. in 21% of the infected ticks. *Ehrlichia* spp. was found only in ticks collected from dogs. The majority of the ticks infected with *Ehrlichia* spp. were *Rh. sanguineus* (93.3%) and only one tick was *I. ricinus* (6.7%). Four ticks were positive for *Rickettsia* spp., two were *Rh. sanguineus* and two - *I. ricinus*, one of the latter was found on a fox. This is the first report about detection of *Ehrlichia* spp. in *Rh. sanguineus* ticks from Bulgaria as well as *Rickettsia* spp. in *I. ricinus* ticks collected from red foxes from this country.

Key words: Bulgaria, dog, *Ehrlichia canis*, *Ixodes ricinus*, red fox, *Rhipicephalus sanguineus*, *Rickettsia* spp.

**Carnivores and Ixodid Ticks as Important Factors in the Emergence,
Circulation and Distribution of Dangerous Infections**

Delka Salkova, Mariana Panayotova-Pencheva, Vassilena Dakova, Zuzana Hurnikova, Martina Miterpakova, Bronislava Víchová, Viktória Čabanová

Abstract

Climate changes in the recent years led to a sharp rise in the tick population and an increase in the number of animals and people with tick-borne infections. The domestic and wild carnivores, especially the dogs, have a huge role for the distribution of ticks in certain areas. It is necessary to carry out a complex fight against the ticks and diseases transmitted by them, which includes systematic control of the tick population in a given area, as well as in-depth studies on their contamination with particular pathogens, especially those causing zoonoses. This work presents a short review on the recent research on the role of carnivores and ticks in the emergence, circulation and distribution of some dangerous viral, bacterial and parasitic infections.

Key words: carnivores, ixodid ticks, vector-borne diseases

Feeding of the Bee Families with an Addition of CoSO_4

R. Balkanska and D. Salkova

Abstract

For their normal living, honey bees (*Apis mellifera*) require proteins, carbohydrates, lipids, vitamins and minerals. It is well known that Co and vitamin B₁₂ are two nutrients that have been reported in pollen and plants that have had positive results in bees feeding. In this respect, the aim of the study is to determine the influence of feeding of the bee families with CoSO_4 as a supplement on the chemical composition of non-flying worker bees' bodies and some bee products components. The study was conducted during May-August 2015 in the experimental apiary of the Institute of Animal Science, Kostinbrod. A total of 6 bee families were observed-3 experimental (fed with sugar syrup, sugar: water 1:1 and 4 mg/L CoSO_4 as a supplement) and 3 control (fed only with sugar syrup). The results show that the addition of 4 mg/L CoSO_4 in the sugar syrup of the bee families do not have influence on the average weight and chemical composition of non-flying worker bees. Statistically significant differences ($P < 0.05$) in the diastase activity of the honey in the experimental group bee families were found.

Key words: bee haemolymph, bee products, CoSO_4 , lysozyme, total protein.

Genetic Diversity and Population Structure among Arabian Horse Genealogical Lineages in Bulgaria

Peter Hristov, Georgi Radoslavov, Ivan Mehandjyiski, Delka Salkova and Georgi Yordanov

Abstract

The present research aimed to characterize the genetic diversity and relationships among extant Arabian horse sire lines in Bulgaria, using 15 equine microsatellite markers. The evaluation included 537 Arabian horses representing nine sire lines (SAKLAWI I, LATIF, SEANDERICH, IBRAHIM, SHABAB, DJEBEL MOUSA, KUHAILAN AFAS, BAIRACTAR, and SARHAN). The obtained results indicated that within these lines, the mean number of alleles ranged from 4.15 in SARHAN to 5.54 in SAKLAWI I and LATIF. The mean expected heterozygosity (H_e) ranged from 0.54 in the SEANDERICH line to 0.67 in SAKLAWI I. The inbreeding coefficient for the entire Arabian populations was rather low: $FIS = -0.109$, fluctuating from -0.204 in SHABAB to -0.041 in SAKLAWI I. The mean genetic differentiation, FST , was 0.096, demonstrating that nearly 90% of the total genetic variation was due to genetic differentiation within each population. STRUCTURE analysis indicated a genetic similarity between SHABAB and LATIF, between IBRAHIM and KUHAILAN AFAS, as well as between SAKLAWI I, SEANDERICH, and BAIRACTAR. This study of the genetic diversity of Arabian sire lines in Bulgaria can assist in developing a national strategy for the exclusion of non-purebred animals from breeding programs in order to preserve the genetic profile of the original Arabian lines.

Keywords: Arabian sire lines; microsatellites; genetic variability; genetic differentiation; conservation

HONEY BEE DISEASES AND THEIR CONTROL IN BULGARIA AND ESTONIA: A REVIEW

Delka Salkova, Mariana Panayotova-Pencheva, Sigmar Naudi

Abstract

The Western honey bee (*Apis mellifera*) is a species of crucial economic, agricultural and environmental importance. Honey bee colonies suffer from numerous pathogens. These include various bacteria, viruses, fungi and parasites. The aim of the present work is to review and compare information on the most important bee diseases and their control in Bulgaria and Estonia. Based on the data about honeybee diseases from available research and those supplied by the National Reference Laboratories of Bulgaria and Estonia it could be said that in both countries the main problem for beekeeping are the diseases varroosis, nosemosis, American foulbrood, and European foulbrood. Estonia seems to be one of the few countries in the world where *N. apis* (43%) is still individually prevalent, while in Bulgaria nosemosis caused by *N. ceranae* (98%) predominates. Principles of prevention and treatment of bee diseases are similar in both countries and comply with European recommendations.

Key words: *Apis mellifera*, bee diseases, Bulgaria, Estonia, control

Genetic Differentiation and Population Structure of Two Bulgarian Local Goat Breeds Using Microsatellite Markers

Georgi Yordanov, Georgi Kalaydzhiev, Nadezhda Palova, Delka Salkova, Lyudmila Lozanova, Heliana Dundarova, Tsonka Odjakova, Pavel Todorov, Georgi Radoslavov and Peter Hristov

Abstract

Establishing genetic diversity in the population is an essential first step for the sustainable conservation of valuable genetic resources under threat of extinction. Two local goat breeds, Kalofer long-haired (KLH) and Bulgarian screw-horned long-haired (BSHL), were genotyped using a panel of 14 microsatellite markers to determine their population structure and differentiation. The overall mean number of alleles was 8.82, higher in BSHL (9.71) as compared with KLH (7.92). The H_o and H_e values were almost equal for BSHL (0.75; 0.76, respectively) and KLH goats (0.74; 0.76, respectively). The value of the coefficient of population differentiation (F_{ST}) was very low (0.024), indicating only 2% genetic differentiation between the two goat breeds. The structure and PCoA analyses revealed considerable overlap between the two breeds, although some flocks of each breed differentiated from the common genetic pool. The results obtained in the present study can serve to differentiate purebred animals that are the basis of a selection process and a breeding strategy with a view to the conservation of these two local goat breeds.

Keywords: genetic diversity; microsatellites; population structure; native goat breeds

A study on the genetic diversity and subpopulation structure of three Bulgarian mountainous sheep breeds, based on genotyping of microsatellite markers

Tsonka Odjakova, Pavel Todorov, Georgi Kalaydzhiev, Delka Salkova, Heliana Dundarova, Georgi Radoslavov, Peter Hristov

Abstract

Traditional domestic breeds of domestic animals are part of the “living” wealth of the world, part of the disappearing biodiversity and a valuable local gene pool. Nowadays, they are increasingly included in the category of “rare and disappearing” breeds. Therefore, the purpose of this study was to reveal the genetic diversity and population structure of three Bulgarian mountainous sheep breeds (Rhodopean Tsigai, RT; Middle Rhodopean sheep, MRS; and Karakachan sheep, KS). A total of 113 unrelated individuals (two flocks of each breed) from different regions of the country were investigated based on 11 STR (short tandem repeat) markers. The obtained results showed a total of 116 alleles in the three sheep breeds. The mean number of alleles, effective number of alleles, and polymorphism information content (PIC) values per loci were 8.21, 4.72, and 0.79. The largest number of alleles were found in the population of MRS (9.18 ± 0.76), followed by RT (8.27 ± 0.64) and KS (7.18 ± 0.47). The largest polymorphism was found in INRA005 and INRA06 loci with 11 and 10 alleles, respectively, and the smallest polymorphic loci were noted for the locus McM042 and INRA172 with only 6 alleles. The observed heterozygosity showed the highest value in KS (0.79 ± 0.04), followed by RT (0.76 ± 0.02) and MRS (0.73 ± 0.04). The range of expected heterozygosity was from 0.77 ± 0.02 in MRS to 0.75 ± 0.03 in KS. The fixation index F_{ST} , estimated by analysis of molecular variance (AMOVA), was 0.2090, indicating moderate levels of genetic differentiation among the populations. The F_{ST} between the investigated breeds indicated a moderate value of 0.216, and the analysis of variance (ANOVA) data showed only 1% variation among the populations, whereas 99% was due to variation within breeds. The results by the STRUCTURE analysis revealed that all breeds were heterogeneous and formed three distinct clusters. The results of the genetic diversity and structure analysis of the investigated breeds provide useful information for assessing the genetic

diversity of other Bulgarian autochthonous sheep breeds, serving as a cornerstone for designing effective conservation management strategies.

Keywords: Genetic diversity, Microsatellite DNA, Population structure, Sheep breeds

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INVASION OF BEE SAMPLES WITH VARROA DESTRUCTOR

Delka Salkova, Kalinka Gurgulova, Ilian Georgiev

Abstract

The aim of this work was to estimate the level of infestation of bee samples infested with *Varroa destructor*. It has been performed a laboratory assay of bee samples for the presence of the mite *Varroa destructor*. The investigation was for a period of two years – 2015 and 2016. The bee samples were collected from diseased and dead bee colonies owned by 149 beekeepers. The result showed that from 220 bee samples tested, 36% were positive for *Varroa* mite and negative samples were 64%. The level of infestation in positive samples was as follows: less than 5% were in 39.2% of samples, between 5 to 20% and more than 20% were found in 30.4% for each level, respectively. In conclusion more than a third of the bee samples were infested with *Varroa* mites. Most of the bee samples had a low degree of invasion (< 5%) and the average and the high level of invasion of bee samples were represented by the same values.

Key words: *Apis mellifera*, bee sample, *Varroa destructor*, laboratory assay.

Lysozyme levels in haemolymph of worker bees (*Apis mellifera* L.) from bee colonies with different degree of expression of hygienic behaviour

S. Lazarov, I. Zhelyazkova, D. Salkova, R. Shumkova, S. Takova

Abstract

A total of 24 bee colonies of apiaries with different business orientation were tested for the degree of expression of hygienic behaviour by modified method, different from the traditionally used for this purpose method. To outline the test field a square sized 5 x 5 cm was used, stuck onto a section of a honey comb with sealed worker brood (the area bounded by the stencil is equal to 100 worker bee cells). The brood in the outlined square is killed by a thin entomological needle by jabbing the sealed cells, without destroying their caps. Depending on the time and extent of cleaning bee colonies are divided into 3 groups: super hygienic - colonies which of the 24th hour after the jabbing have uncovered and cleaned over 95% of the outlined area; hygienic – colonies which on the 48th hour after the jabbing have uncovered and cleaned over 95% of the outlined area; non- hygienic – colonies which have cleaned less than 95% of the cells in the area on the 48th hour. From each bee colony samples of worker bees (200-250 pcs.) have been taken and haemolymph obtained. The amount of lysozyme has been defined at the Reference Laboratory “Honeybee health” at the National Diagnostic Scientific Research Veterinary Medical Institute – Sofia by the method of Motavkina et al. (1979), modified by Kostov et al. (1983). The results obtained show different values for the amount of lysozyme in haemolymph of worker bees, depending on the degree of expression of their hygienic behaviour – 10.49 ± 1.86 µg/ml for the group of super hygienic colonies; 9.11 ± 1.37 µg/ml for the group of hygienic ones; 15.22 ± 2.37 µg/ml for the group of non-hygienic bee colonies, respectively. The established values range from 4.59 µg/ml to 38.28 µg/ml, the greatest variation being in the group of non-hygienic colonies. The data suggests that in positive direction compared to the average for the model is the deviation of LS-means of bee colonies with low level of hygiene (non-hygienic). The reported LS-estimates suggest that in the nonhygienic bee colonies there is a tendency of increase the lysozyme content in the haemolymph.

Keywords: honeybees, bee colonies, hygienic behaviour, haemolymph, lysozyme level

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ROLE OF PROBIOTICS IN POULTRY COCCIDIOSIS

Delka Salkova, Mariana Panyotova-Pencheva, Ivelin Vladov, Milena Anissimova, Petar Dimitrov

Abstract

Chicken coccidiosis is the major parasite disease of poultry, with substantial economic burden estimated to cost the industry more than \$ 800 million in annual losses worldwide. Modern intensive poultry production is largely dependent upon chemoprophylaxis for the control of coccidiosis. Although there is a rising problem of drug resistant strains of *Eimeria*. In connection with that alternative solutions to the use of antibiotics have been sought and there are now available a number of probiotic products intended to help to maintain the balance of the intestinal micro flora in a range of food animal species.

Key words: coccidiosis, chickens, resistance, probiotics.

AMPLIFICATION OF DNA FRAGMENTS OF HELMINTHS

Ivelin Vladov, Delka Salkova, Svetlozara Petkova, Valentin Radev, Veselin Nanev, George Stoimenov, Dimitar Hrusanov, Valeria Dilcheva, Milena Anisimova

Abstract

Molecular-biological methods complement the fundamental microscopic, morphological and pathoanatomical methods. The aim of this study was after isolation and purification of DNA from helminths with different taxonomic affiliation to separate species-specific amplified fragments of DNA. The research were performed on the genus *Haemonchus* (Nematoda: Trichostrongylidae) and genus *Fasciola* (Trematoda: Fasciolidae). Obtained by this method DNA, was qualitatively and quantitatively analyzed by methods of spectrophotometers and gel electrophoresis, from each sample of helminths.

Key words: DNA amplification, helminths.

Furostanol Glycosides Group (Adaptogen) Used for Biological Control against Gall-forming *Meloidogyne* species

SVETLANA ZINOVIEVA, OLGA BAICHEVA , ANNA DAMIANOVA, ZHANNA UDALOVA,
IVELIN VLADOV, INNA VASILIEVA, DELKA SALKOVA

Abstract

The paper deals with the assessment of the adaptogenic effect of furostanol glycosides (adaptogen) extracted from *Dioscorea deltoidea* on the microelement content and development of invaded with *Meloidogyne arenaria* tomato plants. The experiments were carried out under laboratory conditions (20-22°C). The plants were cultivated on soil sterilized by heating. Tomato seeds were treated with 0.1 % solution of adaptogen by soaking for 1 h. The contents of Zn, Mn, Mg, Cu and Fe were analyzed using atomic absorption spectrometry method. A positive effect of adaptogen treatment on the gall formation and normalization of microelement content of tomato plants is observed.

Key words: microelement, parasite-host system, adaptogen, tomato plant.

**Investigations of radiation effect on the life cycle of *Meloidogyne arenaria*
(Neal, 1899) Chitwood, 1949**

A. DAMIANOVA, O. BAICHEVA, I. SIVRIEV, D. SALKOVA

Abstract

Root gall-forming nematodes (*Meloidogyne* spp.) are one of the most pathogenic groups of plant parasites of great economic importance. They cause tumor-like formations (galls) on the roots of the invaded plants and parasitize more than 2 000 plant species (Hussey and Jansen, 2002). The numerous species of the gall-forming nematodes and their different ecological and biological abilities to survive under unfavorable conditions make their control very difficult. The present work summarizes the results obtained after application of α - and γ -irradiation on the life cycle of *Meloidogyne arenaria*.

Key words: radiation effect, gall-forming nematodes, life cycle.

Effect of NH_4VO_3 on the tissues of germinating tomato plants

Y. MIZINSKA-BOEVSKA, O. BAICHEVA, D. SALKOVA, K. GEORGIEVA

Abstract

The goal of these investigations was to study the influence of NH_4VO_3 concentrations on the formation of young root tissues. Toxic concentrations, concentrations with no effect on the root tissues as well as a concentration which influences the root development positively have been found.

Key words: NH_4VO_3 , root tissue, cell wall, nucleus, chromatin.

Vanadium and its compounds as a possible method for control against plant-parasitic nematodes

O. BAICHEVA, D. S ALKOVA, A. DAMIANOVA, N. NICOLOVA, N. LIHAREVA

Abstract

Some of the biological activities of vanadium mainly connected with the plants are reviewed. Of special interest is approbation of alternative methods for plant nematode control in respect to the requirements of the contemporary ecological agriculture. On the basis of their own investigations for control against *Meloidogyne* the authors consider vanadium (its compounds respectively) to be perspective in the therapy of the invaded plants.

Key words: vanadium, biological role, plants, plant nematodes, *Meloidogyne*.

Growth and development of the invaded with *Meloidogyne arenaria* tomato plants under treatment by NH_4VO_3

O. BAICHEVA, D. SALKOVA, A. DAMIANOVA

Abstract

Under laboratory conditions the growth and the development of the experimentally invaded with *M. arenaria* tomato plants were investigated. The durations of the experiment were two months. Five measuring of the experimental plants were made during the vegetation- general condition of the plants, middle height and middle rate of the growth. In the variants “noninfected, treated plants” the lowest concentration - 0.01 mg NH_4VO_3 / 100 ml H_2O - influenced in the best way the experimental plants. In the variants “infected, treated plants” the concentration 0.13 mg /100 ml H_2O showed the best effect on the plants.

Key words: plants, growth, concentration, NH_4VO_3 , *Meloidogyne arenaria*.

[Experimental Pathology and Parasitology, 7/2, 2004, ISSN:1311-6851](#)

Life cycle of *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 under treatment of the host tomato plants with NH_4VO_3

DELKA SALKOVA, OLGA BAICHEVA, GALINA PALAZOVA, HARRY SAMALIEV

Abstract

The life cycle of *Meloidogyne arenaria* in the tomato plants treated with NH_4VO_3 was established. The experiments were realized under laboratory conditions. The penetration of J_2 , as well as the development and the formation of J_2 , J_4 , matured females and egg sacks were studied. The presence of males was given too.

[Experimental Pathology and Parasitology, 5/8, 2002](#)

Something more about zinc

R. ALEXANDROVA, G. RASHKOVA, D. SALKOVA, I. SAINOVA

Abstract

The first indication that zinc is required by a living organism appeared in 1869 when it was demonstrated that lack of zinc retarded the growth of *Aspergillus niger*. Eight years later came the observation that zinc was a constituent of plants, vertebrates and animals. Now we know that zinc is essential for all life forms and plays a vital role in human nutrition and biochemical functions. It is an essential co-factor in variety of cellular processes including DNA synthesis, behavioral responses, reproduction, bone transformation, growth, and wound healing. Zinc is the structural component of a wide range of proteins, neuropeptides, hormone receptors and polynucleotides. There is substantial evidence to support an important role of zinc in immune processes. This element is also present in the brain and contributes to its structure and function. Zinc deficiency can promote different disease states including cancer.

Key words: zinc, nutrition, zinc deficiency, hormones, enzymes, immunity, central nervous system.

**Detection of *Varroa destructor* Mite and *Nosema* spp. in Bee Samples from
Bulgaria**

Delka Salkova, Kalinka Gurgulova

Abstract

This study is focused on the investigation of honey bee samples for the presence of the two most common and widely distributed honey bee parasites. In a two-year period during 2020-2021, 185 bee samples were tested. All samples were examined by morphological and light microscopic methods. The obtained results showed that 32.43% of bee samples were infested with *Varroa destructor*. The degree of infection in the bees ranged from 0.5% to 60%. Spores of *Nosema* spp. were established in 25.40% of samples with a degree of invasion in the range from 3×10^5 to 26×10^6 per bee. Mixed infections of both parasites were observed in 32.43% of the samples. Negative samples were with the lowest value of 9.74%.

Keywords: Honey bee, Bee pathogens, Infestation rate, Bulgaria

Comparative Study of the Prevalence of Nosemosis in Honey Bees in Bulgaria and Estonia

Delka Salkova, Sigmar Naudi

Abstract

Nosemosis is a disease of honey bees, affecting bee colonies worldwide, including in Bulgaria and Estonia. The present study aimed to determine the prevalence of *Nosema* spp. (nosemosis) in Bulgaria and Estonia in 2017, the degree of invasion, as well as to compare the prevalence of nosemosis in countries with colder and temperate climates. For each sample, 60 forage bees were collected from the flying boards of hives. The samples were placed in plastic tubes, cooled immediately for transportation, and frozen at -20°C until laboratory analyses. Diagnostic methods used to proof spores and identification of *Nosema* spp. - *N. apis* and *N. ceranae*, including light microscopic examination (native and stained smears and counting spores in haemocytometer / flow cytometer) and multiplex PCR. During this period in Bulgaria 114 samples of bees from 82 apiaries located in different regions of the country were studied. The results showed 85 (74,6%) positive for nosemosis samples and 29 (25,4%) negative. Of the positive samples, 47.4% had an invasion rate of 2 to 10 million spores / bee, followed by those with up to 1 million spores / bee (17.5%) and the smallest number of samples showed an invasion rate of over 10 million / bee (9.6%). Among the 30 apiaries surveyed in Estonia the median number of spores per worker bee ranged from 1.6 to 14 million. *N. ceranae* has replaced *N. apis* in many countries. Estonia seems to be one of the few countries in the world where *N. apis* (43%) is still individually prevalent, while in Bulgaria in 98% of cases nosemosis caused by *N. ceranae* predominates.

Keywords: *Apis mellifera* L., *Nosema* spp., Bulgaria, Estonia, pathogens

Cold and Moderate Climate Beekeeping: Proceedings of the 5th International Scientific and Practical Conference. Moscow-Pskov. October 19-20. Pskov 2021

Investigation of bee samples from Bulgaria for *Nosema spp.* and *Varroa destructor* mite

Salkova, D., Gurgulova, K., Takova, S.

Abstract

This study is focused on investigation of two dangerous bee parasites which have been considered as the single most important causes of honey bee colony losses in different regions of the world, including Bulgaria. The aim of this study was investigated bee samples from different regions of Bulgaria for detection two bee pathogens: *Nosema spp.* and *Varroa destructor*. In a three-year project between NDRVMI and IEMPAM-BAS during 2015-2017, were examined 297 bee samples from 203 apiaries, located in 22 districts of the country. Samples were sent to the NRL "Honeybee health" mainly from colonies with different pathological problems such as abnormal behavior, depopulation of beehives, weakness and high mortality of colonies. Diagnostic methods used to detect *Varroa destructor* and proof spores and identification of *Nosema spp.* - *N. apis* and *N. ceranae*, included morphological identification, light microscopic examination and conventional PCR (OIE - 2008; 2013). Our results shown *Varroa destructor* was established in 11, 11% of samples. The degree of invasion in the bees was in the range from 0, 5% to 100%. Spores of *Nosema spp.* was demonstrated in 54, 89% bee samples with a degree of invasion in the range from 3,105 to 26,106 per bee. Mixed infections of both parasites were observed in 16, 84% of samples analyzed. Negative for both parasites were 17, 17%. *Nosema* invasion of the analyzed bee samples was more prevalent than *Varroa* infestation. From *Varroa* mite positive samples those with a low degree of invasion predominated, while most of samples positive for *Nosema spp.* showed degree of invasion between 1,105-1,106 per bee. In conclusion, we can say that our study shows prevailing higher percentage of positive bee samples for *Nosema spp.* than samples, infested with *Varroa destructor* mite in Bulgaria.

Keywords: honeybee (*Apis mellifera*), *Nosema spp.*, *Varroa destructor*, investigation

Resistance of *Varroa destructor* mite to some acaricides

Delka Salkova

Abstract

Varroa destructor is an ectoparasitic mite on the honey bee *Apis mellifera*, that is causing enormous damage to beekeeping worldwide. Synthetic varroacides have been used the longest time and most commonly by beekeepers worldwide. One of the most important reasons for the development of high resistance is the improper use of acaricide by the beekeepers themselves. The main mistakes that beekeepers most often make are applying lower or higher doses than necessary, frequent use of the same product or those of one synthetic group. This is the main reason of the low efficacy of the applied acaricides than expected efficacy according to the label. This involves risk of *Varroa destructor* mite developing acaricides resistance. Therefore it is necessary to monitor the effectiveness of acaricides treatments to ensure appropriate protection of the honeybee colonies. Recently in many honey bee apiaries, high infestation levels of *V. destructor* mite are being detected in colonies of *Apis mellifera* after treatment with some synthetic acaricide like as organophosphate coumaphos, formamidine amitraz, pyrethroids tau-fluvalinate, etc. The purpose of this work is to presents short review of recent studies to estimate the acaricide resistance of *Varroa destructor* mite populations to some common used varroacides in honey bee colonies worldwide.

Keywords: *Apis mellifera*, *Varroa destructor* mite, synthetic acaricides, resistance

Theory and Practice of Struggle Against Parasitic Diseases, 19, Federal State Budget Institution ASRI Parasitology Named After K.I. Skryabin, 2018, ISBN: 978-5-904798-57-4

The role of some natural compounds and ammonium vanadate in the regulation of relationship of *Meloidogyne arenaria* - tomatoes.

Baicheva, O., Samaliev, H., Zinovieva, S., Udalova, Z., Salkova, D.

Summary

Influence of adaptogene - furostanol glycosides, extracted from *Dioscorea deltoidea*, elicitor - chitosan derivative and NH_4VO_3 on the life cycle of *Meloidogyne arenaria* was investigated. The mineral content of Zn, Mn, Mg, Fe, Cu, as well as content of vanadium in the plant organs was fixed by means of atomic absorption spectrometry method. Three increasing concentration of NH_4VO_3 were used in the experiments (0.01 mg/100 ml H_2O ; 0.1 mg/100 ml H_2O and 0.13 mg/ml H_2O). Positive effect of the treatment (particularly positive effect of 0.13 mg/ml H_2O) was registered. The treatment with high concentrations made the mineral contents of invaded plants near to that of controls. Increasing of vanadium in the above ground plant organs and especially in fruits was not revealed. The usage of furostanol glycosides and chitosan derivative inhibited development of the parasite. The obtained results proved possibility of using natural composition for normal development of plants under root-knot nematode infection.

Review of Methods and Means for Control of Varroosis in Honey Bee *Apis mellifera*

Delka Salkova, Plamen Hristov, Kalinka Gurgulova, Tsvetan Tsvetanov, Rositsa Shumkova

Abstract

Varroosis is a parasitic disease in bees, which causes the greatest economic damage beekeeping worldwide. In addition to the direct damage to bees, the causative agent is also a vector of many infectious diseases, and its harmful impact on the bee family is increasing. It also proved that varroosis is key factor to manifestation of the collapse of bee colonies. The review presents the most frequently used means for *Varroa destructor* control. These include biotechnological methods, chemical and alternative preparations that are most commonly used in beekeeping. Biotechnological methods include: creation of new colonies, group incubation of comb-traps, use of building frames and uncapping of the drone brood. Chemical methods are based on authorized substances such as: amitraz, cumaphos, cymiazole and synthetic pyrethroids - fluvalinate, tau-fluvalinate, flumethrin and are suitable for use in conventional beekeeping. Alternative methods include: physical, mechanical and constructive methods, biogenic products - organic acids and essential oils, biological agents (bacteria, entomopathogenic fungi, etc.), selection methods, genetically modified bees and mites. The Integrated Pest Management (IPM) is a principle in which the treatment of bee families occurs when *Varroa destructor* populations are low before the mites reach bee damaging levels, where a pest species is kept at a harmless level by controlling it using a combination of methods each working in different ways and at different times of year. The IPM includes permanent monitoring of the infestation level, periodic control of mite resistance, periodic replacement of used means appropriate to the biology and the development cycle of the bee colonies, applying a combination of biotechnological, chemical and alternative methods according to the season, treatment of the region at the same time, maintenance of strong colonies and application of good beekeeping practices. The main advantages and disadvantages of the methods and means of combating varroosis are outlined.

The article highlights the advantages and disadvantages of the reviewed methods and means to control of varroosis.

Keywords: Honeybee (*Apis mellifera*), *Varroa destructor*, control.

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A SHORT REVIEW ON THE EXPERIENCE OF ANTHELMINTIC TREATMENT IN WILD BOARS

Vassilena Dakova, Mariana Panayotova-Pencheva, Delka Salkova

Abstract

A literary survey has been made about the use of anthelmintics in the European wild boar. The most frequently used anthelmintics have been from the Benzimidazole group (Albendazole, Mebendazole, Febantel, Fenbendazole, Flubendazole). They have been used against gastrointestinal and lung nematodes. Their effect has been strongest against gastrointestinal strongylids, it has been less effective against lungworms and *Capillaria* have been least affected species. Another popular group is that of tetrahydropyrimidine (Pyrantel, Morantel). They have been very efficient against intestinal nematodes. The efficiency against lung nematodes has been also good, with the egg output being stopped, although their adulticid effect has been smaller. Imidazotiazoles (Levamisol, Tetramisol) are a third anthelmintic group that is used in wild boars. The effectiveness of the Imidazotiazole group has been very good against gastrointestinal and lung nematodes, but the risk of drug overdose and intoxication has been higher than the other groups. Studies about the Ivermectin from the Avermectin group have shown that this drug is suitable for treatment of gastrointestinal and lung nematodes and mites, but the effect against *Capillaria* spp. and *Trichuris* spp. has been insufficient.

**ANTIPARASITE REMEDIES APPLIED IN MOUFLONS (*OVIS*
MUSIMON) AND OTHER WILD SHEEP**

M. Panayotova-Pencheva, V. Dakova, D. Salkova

Summary

The selection and use of antiparasitic remedies in wild sheep present one of the challenges to the wildlife managers and veterinarians. The present study represents a review of the literature on the experience accumulated in this respect. The summarized literature data show the following: The benzimidazoles most often have been applied for treatment of parasites in wild sheep. Their effectiveness varies according to the parasite species. It has been high against gastrointestinal nematodes and relatively lower against lungworms. Some drugs, for example oxfendazole has been 100% effective against *Moniezia* spp. It has been established that albendazole is readily accepted even by fastidious animals. Two-day treatment has been recommended during the treatment with mebendazole. For long-term control of protostrongylids free-choice availability of fenbendazole-medicated salt has been recommended as a potentially effective management tool. Ivermectin is other medicine tested for treatment of wild sheep. Applied parenterally it has been effective in control of the lung nematodes of *Protostrongylus* genus and mites *Psoroptes ovis*. The dosage of 600 µg/kg body weight is recommended for parenteral use. The results of orally administrated ivermectin are contradictory – in some cases the drug has been ineffective against lungworms, in others it has had good effect. Most probably this depends on the dosage and scheme of treatment. The parasites more difficult for cure, independently of kind of remedies, have been protostrongylids and *Trichuris* spp. Experiments have shown that repeated administration of some anthelmintics may contribute to the development of parasite resistance. Other ones have demonstrated positive effect of biological control on the gastrointestinal nematodes in animals in captivity.

Key words: mouflon, bighorn sheep, parasite treatment, benzimidazoles, ivermectin.

Труды Центра Паразитологии ИПЭЭ РАН, 2016, 49, ISSN: 0568-5524

***ANGIOSTRONGYLUS DASKALOVI (NEMATODA:
METASTRONGYLOIDEA) IN BADGERS FROM BULGARIA***

Panayotova-Pencheva M., Trifonova A., Dakova V.V, Salkova D.V, Movsesyan S.

Introduction. Angiostrongylids are nematodes of order Strongylida, superfamily Metastrongyloidea (Anderson et al., 2009). In most of the cases they parasitize in the pulmonary arteries and the heart of their final host which are insectivores, rodents, felids and canids. The man can also be infected by some of those parasites, although he is a nonspecific host. Intermediate hosts are different species of mollusks. The present work describes some cases of parasitizing of badgers (*Meles meles* L.) from Bulgaria with angiostrongylids.

Труды Центра Паразитологии ИПЭЭ РАН, 2016, 49, ISSN: 0568-5524

**PREVALENCE AND DISTRIBUTION OF NOSEMA SPP. AND VARROA
DESTRUCTOR IN HONEYBEE COLONIES IN BULGARIA**

*Salkova D.S., Georgieva T.E., Gurgulova K.I., Takova S.B., Panayotova-Pencheva M.S.,
Movsesyan S.O.*

Introduction. Nosemosis is a parasitic disease of adult honey bees (*Apis mellifera*) caused by two species of microsporidia, *Nosema apis* (Zander, 1909) and *Nosema ceranae* (Fries et al., 1996). The disease occurs throughout the world, including Europe and Bulgaria respectively. The parasitic mite *Varroa destructor* (Anderson and Trueman, 2000), is considered one of the most serious pests to beehives, causing great economic loss to the beekeeping industry (Baker and Peng, 1995). The purpose of this study was to compare the results of laboratory analysis for *Varroa destructor* and *Nosema* spp. of bee samples, obtained from professional and amateur beekeepers in period of two years (2014-2015).

**COMPARATIVE ANALYSIS BETWEEN FIELD AND LABORATORY
DIAGNOSIS OF NOSEMATOSIS AND VARROOSIS ON HONEYBEE**

D. Salkova, K. Gurgulova, S. Takova, M. Panayotova-Pencheva

Abstract

In 2015 and 2016 were conducted laboratory tests for parasites of 163 samples of bees of 105 apiaries from different regions of the country. Among 76 beekeepers were carried out survey for observed signs characteristic of these diseases. Results of laboratory tests were compared with the manifested symptoms of the field. The purpose of this study was to compare the clinical signs observed by beekeepers, and the results of laboratory analysis. Laboratory tests on samples of bees show a high degree of infestation with spores of *Nosema spp.* (52.8%) and the mite *Varroa destructor* (12,9%) alone and 18.4% in mixed invasion of both parasites. Negative are 15.9% of the samples. In many cases the anamnestic data is not confirmed by laboratory results. Comparing survey results on the field and laboratory studies conclude that nosemosis prevails while beekeepers indicate varroosis as a major problem. According to data from surveyed beekeepers on signs of parasitic diseases of the apiary at 39.6% it comes to varroosis, but only 2.6% - for nosemosis. Mixed invasion of the two parasites indicate 28.9 percent surveyed beekeepers and the same percentage (28.9%) of them have not observed signs of parasitic diseases. This study confirms the necessity of laboratory testing for accurate diagnosis.

Keywords: honeybees, nosemosis, varroosis, clinical signs, laboratory analyzes

**THE ROLE OF THE PARASITE *VARROA DESTRUCTOR* AS VECTOR OF
VIRUSES ON HONEY BEE *APIS MELLIFERA***

Delka Salkova Salkova

Abstract

Varroa destructor (Anderson and Truman, 2000) previously described as *Varroa jacobsoni* (Oudemans, 1904) (a closely related mite) is a parasitic mite of honey bees. According to results of scientific research projects, the main cause of honey bee colony loss is this mite, which can be found in almost every apiary in Europe. This mite is an external parasite that attaches to the body of *Apis* species, and breeds within the colony by laying its eggs within capped brood and feeding on *Apis* larvae. *Varroa* is present on all continents with the exception of Australia. Depending on climatic conditions, the damage caused by *V. destructor* appears from autumn to early spring during the overwintering phase, leading to general weakening and often complete losses of colonies. High level infestations can be a direct cause of colony loss, but the mite is also a vector of a number of viruses. The ectoparasitic mite *Varroa destructor* and honey bee pathogenic viruses have been implicated in the recent demise of honey bee colonies. Several studies have shown that the combination of *V. destructor* and deformed wing virus (DWV) poses an especially serious threat to honey bee health. Nowadays the bee viruses like as Sacbrood Virus (SBV), Acute Bee Paralysis Virus (ABPV), Chronic Paralysis Virus (CPV), Israeli Acute Paralysis Virus (IAPV), Kashmir bee virus – (KBV), Deformed Wing Virus (DWV), Cloudy wing virus (CWV), *Varroa destructor* virus-1 (VDV-1) are associated with mite. Although bee viruses usually persist as unapparent infections and cause no overt signs of disease, they can dramatically affect honey bee health and shorten the lives of infected bees under certain conditions. Even with proper management it is impossible to keep apiaries 100% free from *V. destructor* mites. This pathology causes commonly called varroasis (also known as varroatosis or varroosis).

Key words: *Varroa destructor*, bee viruses, vector

**Honey bees and their products as indicators of environmental pollution with
pesticides**

Salkova D. S., Panayotova-Pencheva M. S.

Abstract

In the present work a literature review of the experiments that explore the using of honey bee and their products as bio-indicator of environmental pollution with pesticides is presented. In all cases of pesticides implementation certain amounts of them have been always accumulated in the bees and their products. Bees in this respect, which are in a constant contact with the atmosphere, plants, waters and soils are the object of numerous ecological studies aimed at establishing their role in the detection of polluted areas. A great attention is also being paid to the honey bee products which also yield information about the state of the surroundings inhabited by the bees. Honey is most often tested for pollutants followed by the bee body, pollen, wax, propolis and faecal masses of the bees. The studies on the royal jelly, nectar honey and honeydew are in single numbers. According to the researchers the pollutants accumulate in the bees and their products to different extents. Pesticides have been established in the bodies of honey bees in larger quantities with relevance to the honey. Most of the authors show that bee honey is a suitable tool for monitoring the pollution with pesticides.

Key words: bee products, bio-indicators, honey bees, honey, pesticides, pollution

Cell growth inhibiting effect of thermolable biologically active substance isolated from *Fasciola hepatica*-infected rat spleens.

Tsocheva-Gaytandzhieva N.T., R. Toshkova, D. Salkova, M. Gabrashanska

Abstract

Thermolable biologically active substances (BASes) were isolated from healthy and *Fasciola hepatica* infected rat spleens. The effect of the newly isolated BASes was studied *in vitro*. A new property of mature *Fasciola hepatica* L. to inhibit experimental liver carcinogenesis was established in our previous investigations. This formed the basis for a hypothesis on the possible roles of some biologically active substances of parasite and host origin in the pathogenesis of this interaction. Thermolable and thermostable biologically active substances were isolated from the tissues of *F. hepatica* and from normal and *F. hepatica* infected host liver tissue, which were proved as inhibitors of cell proliferation.

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Antiparasite remedies applied to ruminant animals from the Cervidae family.

M. Panayotova-Pencheva, D. Salkova, A. Trifonova

Abstract

The present study represents a review of the literature on the experience accumulated in the antiparasite treatment of the ruminant animals of the Cervidae family distributed in Bulgaria as well as of species close to them. The summed-up literature data show that among the tested anthelmintics a good effect against the gastrointestinal parasites have displayed oxfendazole, albendazole, thiabendazole, mebendazole, fenbendazole, luxabendazole and tetramisolum. Febantel is also one of them having shown a relatively positive effect also against the dictyocaulus infection. No so good effect reaching up to unsatisfactory one against the lung helminths, especially protostrongylids, has been demonstrated by the preparations cambendazole, levamisole and rintal. Ivermectin has been most efficient in the struggle both against the gastrointestinal and lung nematodes. Its efficacy in the fight against the pulmonary helminthoses has been better when applied at higher doses such as 400µg/kg body weight and in its subcutaneous and pour on administration as compared to the per oral one. The attempts for controlling the extrapulmonary protostrongylidoses with levamisole and rintal have proven to be unsuccessful. Ivermectin has been in that respect the best of the rest of the anthelmintics but also at the high dosage (400µg/kg body weight) though even in these cases its effect has been incomplete. The treatment of trematode infections with albendazole has shown rating of efficiency between 20 and 80 per cent in per oral and almost 100 per cent in the intraruminal administration. Niclosamide has been effective in monesiosis. Ivermectin has been tested also as a remedy for fighting the external parasites in the deer. It has been highly effective in nasopharyngeal and subcutaneous myiasis, ticks, mange mites and lice.

Key words: parasite treatment; deer; Cervidae

Proceedings of Sixth Workshop “Experimental Models and Methods in Biomedical Research”, 2015, ISSN: 1314-9091

Preliminary studies on the spread of *Varroa destructor* and *Nosema* spp. in certain areas of Bulgaria.

D. Salkova, K. Gurgulova, S. Takova, M. Panayotova-Pencheva

Abstract

Biodiversity of ecosystems is largely provided by pollinator activity of honeybees. The parasitoses in bees caused by *Varroa destructor* and *Nosema* spp. play a very important role in colony losses, thus attracting the attention of many scientists. In the present work are made preliminary studies on the spread of varroosis and nosemosis in 66 samples from 11 regions of the country. Methods of OIE -MDTVTA are used for the identification of *Varroa destructor* and *Nosema* spp. and determining the degree of invasion in samples of bees from colonies died in the winter-spring period of 2015. It is found that the *Varroa destructor* is established in 16,6% of samples, and the degree of invasion in the bees is in the range 0.5% to 100%. Spores of *Nosema* spp. is demonstrated in 41%, the degree of invasion per bee is in the range of 3.105 / bee to 26.106 / bee. Mixed infections of both parasites are observed in 24,2% of samples analyzed. 18.2% of samples were negative for both parasites.

Studies on the project continued in other areas of the country, by including methods for identification and distinguish species *N. apis* and *N. ceranae*.

Key words: *Apis mellifera*, *Varroa destructor*, *Nosema* spp., spread, colony losses.

FASCIOLOSIS AND CARCINOGENESIS

N. T. Tsocheva-Gaytandzhieva, D. Salkova

Abstract

Some helminthoses are recognized as a significant factor in cancer development. However, the oncogenic potential of *Fasciola hepatica* is not certain. Data are reviewed about the combinations of fasciolosis and tumors in animals and humans. The interactions between fasciolosis and carcinogenesis are not well investigated and the received data are contradictory. In comparison with other helminthoses, in natural conditions fasciolosis is very rarely complicated with neoplastic degeneration in humans and animals. Chapman defines the chronic inflammation and the chronic alteration of the bile duct epithelium (which are observed under chronic infection with liver flukes) as a risk factor for the development of carcinogenesis of the bile ducts, but he considers that such a process is observed more rarely under *Fasciola hepatica* infection, in comparison with *Clonorchis sinensis* and *Opisthorchis viverrini* infections. The risk of the development of malignant diseases at the background of helminthoses is lower in children than in adults.

Key words: *F. hepatica*, carcinogenesis, treatment

OPISTHORCHOSIS, CLONORCHOSIS AND TUMOR GROWTH

N. T. Tsocheva-Gaytandzhieva, D. Salkova

Abstract

Some of the chronic helminthoses are recognized as a significant factor in cancer development. Data are reviewed about the combinations of trematodoses (opisthorchosis and clonorchosis) and tumors in animals and humans. A relation between the infections and development of the tumors is established.

**SCREENING STUDY FOR MICROFILAREMIA IN CANIDS
FROM DIFFERENT REGIONS OF BULGARIA**

Mariana Panayotova-Pencheva, Anetka Trifonova, Rossen Mirchev, Delka Salkova

Summary

Screening study for microfilaremia in canids from different regions of Bulgaria was performed. Three filariid species were found in the dogs, golden jackals and red foxes – *Dirofilaria immitis*, *D. repens* and *Acanthocheilonema reconditum*. In the most cases of the dogs with microfilaremia *D. repens* was found with the infection extensity (IE) 4.9 %, followed by *D. immitis* (4.2 % IE) and *A. reconditum* (3.5 % IE). In the golden jackals were established *D. immitis* (16.3 % IE) and *A. reconditum* (4 % IE). The most ranged species in the red foxes was also *D. immitis* (25% IE), in the less cases was established *A. reconditum* (15 % IE) and in the least – *D. repens* (5 % IE). Microfilariae by the three above mention species were found in the dogs from Veliko Tarnovo region and *D. repens* and *A. reconditum* were predominated. Only *D. immitis* was established in the gods originated from the South regions of the country (Plovdiv, Pazardjik and Smolyan). Microfilariae of *D. immitis* were found also in the golden jackals and red foxes from the regions of Plovdiv, Pazardjik and Burgas.

Key words: *Dirofilaria immitis*; *Dirofilaria repens*; *Acanthocheilonema reconditum*; *Canis familiaris*; *Canis aureus*; *Vulpes vulpes*.

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SOME ALTERNATIVE REMEDIES FOR CONTROL OF VARROOSIS

Delka Salkova, Mariana Panayotova-Pencheva, Rossen Mirchev

Summary

The ectoparasitic mite *Varroa destructor* Anderson and Trueman, 2000 is recognized as the most serious parasite of honey bee (*Apis mellifera*) worldwide, inflicting much greater damage and higher economic costs than all other known apicultural diseases. Besides its direct parasitic influence, the mite is appeared as a vector of many viral diseases in the bee colony and that's way its harmful impact on the bees is increased. The mite has developed resistance to the most of the acaricide drugs. In this connection recently the scientists have focused their efforts on finding and testing new spare agents for control of varroosis. The present work is a review on the performed in this field experiments as well as their effectiveness.

Key words: *Varroa destructor*, Varroosis, *Apis mellifera*, alternative treatment, essential oils, acaropathogenic fungus.

Honeybee pollen as bioindicator for environmental pollution

Delka Salkova Salkova, Mariana Panayotova-Pencheva

Abstract

Bee products possess therapeutic properties and are the source of many essential trace elements that is way they are regarded as valuable foods. Honeybee pollen is one of the most popular bee products used as a diet complement with immunostimulant effect. On the other hand, bee pollen as a product derived from the pollen of flowers around the hive, gives valuable information about the environmental situation in a given area. As the bees flit about collecting pollen and nectar from flowering plants, they also collect any pollutants that may have settled onto the plants from the air or been drawn up from the ground. The presence of pollutants in these products can lower their quality, which in consequence may endanger human health. In this work some investigations on the use and effectiveness of bee pollen as a bioindicator of environmental pollution with different trace elements, thoroughly heavy metals and pesticides, are presented.

Keywords: honeybee pollen, trace elements, toxic heavy metals, pesticides, environmental pollution

IN VITRO METHOD FOR SOME SPECIES EIMERIA IN RABBITS

I. Vladov, M. Gabrashanska, M. Anisimova, D. Salkova

Abstract

Eimeriosis is one of the most common parasitic diseases in domestic rabbit (*Oryctolagus cuniculus*) causing high mortality. *In vitro* culturing in cells culture of Eimeria allows more accurate studies in the absence of a complex immune response of the host to decode a complex host - parasite interactions. The present study describes a methodology for the preparation of merozoites by oocysts of species from the genus Eimeria in rabbits. The HeLa cell culture proved to be good for the development of the stage.

INFLUENCE OF PROPOLIS ON SOME PROTOZOAN PARASITES

Delka Salkova

Abstract

Although honey is perhaps the most famous bee product of interest to human beings, bees also make propolis, another substance that humans have used for thousands of years. Propolis (from the Greek "pro" + "polis" - "to the city", "protection of the City"), also known as clay is a mixture of resin, wax and pollen from flowers and buds of plants enriched with enzymes and subjected to lactic acid fermentation in the digestive system of bees. Propolis contains vitamins, essential oils, mineral salts, trace elements, hormones, and enzymes. Bees gather and bring to the hive and perform with him disinfection activities. It is their "chemical weapons" against infection. Bees coat the hive with propolis in much the same way we use paint and caulking on our homes. Propolis has been used as a popular remedy for several centuries for a wide array of ailments. Its antimicrobial properties, present in propolis from different origins, have been extensively studied. But, more recently, antiparasitic, antiviral/ immune stimulating, healing, anti-tumor, anti-inflammatory, antioxidant and analgesic activities of diverse types of propolis have been evaluated. The color of propolis is yellow, yellow-green, dark green or gray. Was dissolved in water slightly, most preferably dissolved in alcohol, ether, chloroform, acetone.

Key words: propolis, immunomodulatory activity, anti-protozoan properties

NOSEMOSIS – THE PESTS OF THE 21th CENTURY IN EUROPE

Delka Salkova Salkova

Abstract

In this review is presented an emerging disease caused by *Nosema ceranae*. Two microsporidian parasites are described from honey bees, *Nosema apis* and *Nosema ceranae*. *N. apis* was isolated in the European honey bee (*Apis mellifera*). *Nosema ceranae* is a microsporidian parasite described from the Asian honey bee, *Apis cerana*. Microsporidiosis caused by infection with *Nosema apis* or *Nosema ceranae* has become one of the most widespread diseases of honey bees and can cause important economic losses for beekeepers. Honey can be contaminated by spores of both species and it has been reported as a suitable matrix to study the field prevalence of other honey bee sporulated pathogens. Since 2006, beekeepers have reported increased losses of *Apis mellifera* colonies, and one factor that has been potentially implicated in these losses is the microsporidian *Nosema ceranae*. Within a few years after it was detected in Spain in 2005, the rest of European countries that had technical capacity to differentiate *Nosema apis* from *N. ceranae* reported its presence.

Keywords: *N. ceranae*, *N. apis*, *Apis mellifera*, nosemosis

**EFFECT OF BIOLOGICALLY ACTIVE SUBSTANCES ISOLATED
UNDER FASCIOLOSIS ON LYMPHOCYTE CELL CULTURES**

Tsocheva-Gaytandzhieva, N., E. Nikolova, D. Salkova

Abstract

Thermolabile biologically active substances (BASes) were isolated from the tissues of the helminth *Fasciola hepatica* and from healthy and *F. hepatica* infected rat liver. The effect of the newly isolated BASes was studied on nonactivated and mitogen activated lymphocyte cell cultures. An immunomodulating effect of the newly isolated BASes was demonstrated. Biologically active substances (BASes) inhibitors of cell proliferation from parasite and host origin were isolated and their growth inhibiting effect had been established *in vitro* on primary hepatocyte cell cultures. The newly isolated biologically active substances were two types: 1. from the tissues of the helminth *Fasciola hepatica* and 2. from *F. hepatica* infected rat liver. In the present work we aimed to study the effect of the newly isolated BASes on lymphocyte cell cultures, obtained from the spleens of healthy rats.

**EFFECT OF THERMOLABILE BIOLOGICALLY ACTIVE
SUBSTANCES UNDER FASCIOLOSIS ON TUMOR CELL CULTURE**

N. Tsocheva-Gaytandzhieva, R. Toshkova, A. Filchev, D. Salkova

Abstract

Thermolabile biologically active substances (BASes) were isolated from the tissues of the helminth *Fasciola hepatica* and from healthy and *F. hepatica* infected rat liver. The effect of the newly isolated BASes was studied on hepatoma MC29 tumor cell cultures. The strongest inhibiting effect on cell proliferation was by BAS isolated from *F. hepatica* tissues. The growth inhibiting effect of BAS isolated from *F. hepatica* infected rat liver was stronger than the effect of BAS isolated from normal liver tissue.

**REVIEW OF THE METHODS FOR CONTROL OF CHICKEN
COCCIDIOSIS**

D. Salkova

Abstract

Chicken coccidiosis is an intestinal infection caused by the intracellular protozoan parasite of the genus *Eimeria*. Seven species have been recognized to infect chickens: *E. tenella*, *E. necatrix*, *E. acervulina*, *E. maxima*, *E. brunette*, *E. mitis* and *E. praecox*. It is the major parasite disease of poultry, with substantial economic burden estimated to cost the industry more than \$ 800 million in annual losses worldwide (Williams, 1998). The life cycle of *Eimeria* comprises intracellular, extracellular, asexual and sexual stages, so it is not surprising that host immunity is also complex and involves many facets of nonspecific and specific immunity (cellular and humoral immune mechanism). The effective use of anticoccidial drugs over the past 50 years has played a major role in the growth of poultry industry and has allowed the increased availability of high quality, affordable poultry products to the consumer.

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Influence steroid glycosides on mineral composition of the plants infected by root-knot nematode, *Meloidogyne arenaria*.

Baicheva O., Damianova A., Zinovieva SV., Udalova Zh.V., Vladov I., Vasilieva I.S., Salkova D

Summary

In work data about influence furostanol glycosides on the maintenance of elements in tissues of tomatoes invasion by root-knot nematode are presented. It is shown, that processing of plants allows to stabilise the mineral composition broken by action of nematode.