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Special Issue

Abstracts of the 4th COST COREMI Conference

"Improving current understanding and research for sustainable control of the poultry red mite *Dermanyssus gallinae*"

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SUMMARY

ORAL PRESENTATIONS

POSTERS

Dear COREMI Cluj Participants

It gives us great pleasure in welcoming you to this Special Issue in Scientia Parasitologica. This issue is related to the annual COREMI (Control of the Red Mite, FA1404) conference on the poultry red mite, *Dermanyssus gallinae* held in Cluj, Romania from October 31st to November 2nd, 2018. This issue will give you access to the latest state-of-the-art research activities, field work and poultry stakeholder collaborations from the worldwide membership of the Cost Action COREMI (www.coremi.eu), which started in 2014 and is now made of 28 collaborating countries in Europe and beyond representing over 330 members. The abstracts in this issue will demonstrate the advancement in monitoring, treating, preventing and controlling the poultry red mite (PRM) *Dermanyssus gallinae,* which is a worldwide arthropod pests attacking poultry birds and other animals including humans.

We hope you will enjoy the conference and reading these abstracts.

Associate Professor Cristian Magdaş
Chair of the COREMI Cluj Conference

Professor Olivier Sparagano
Chair of the COST FA1404

ORAL PRESENTATIONS

MOLECULAR CHARACTERIZATION OF DERMANYSSUS GALLINAE ISOLATES FROM GREECE AND COMPARISON WITH A EUROPEAN FRAMEWORK, USING THE COI GENE

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Acaricides are currently the most widely used method of population control for *Dermanyssus gallinae* (the poultry red mite), although resistance is widely recognized across the poultry industry. Therefore, there is need for alternative control methods including the development of a suitable and effective vaccine. An essential consideration for understanding of both acaricide resistance and vaccine development is genetic structure of *D. gallinae* populations, but no data is presently available regarding the genetic diversity of *D. gallinae* populations in Greece. The primary objective of this study was to begin molecular characterization of *D. gallinae* isolates from Greece, targeting the cytochrome c oxidase subunit I (COI) gene. For this purpose, mites were collected using cardboard traps from four farms each located in a different region. Traps were collected and transferred to the Laboratory of Parasitology and Parasitic Diseases in Aristotle University of Thessaloniki and examined for the presence of *D. gallinae.* Mites from traps were stored at -20°C in 70% ethanol for further molecular analysis. At the Royal Veterinary College, Department of Pathobiology and Population Sciences, all samples were processed for molecular analysis. Genomic DNA was extracted from one individual per sample using a Qiagen DNeasy Blood & Tissue kit, followed by PCR reaction using specific primers targeting a 737-bp fragment of the *D. gallinae* COI gene. Afterwards, the PCR products were purified using a Qiagen QIAquick PCR Purification Kit and sent for Sanger sequencing to Eurofins Genomics. According to the results of the analysis, two haplotypes differing from each other by five polymorphic sites (0.67%

differences) were found within the sampled sequences. Preliminary network analysis illustrated the distribution of haplotypes across all four sampled Greek farms. Overall, these results demonstrate low genetic diversity within the sampled farms and they will be more informative when they will be intergraded in a much wider European study and compared to other European populations in the future.

ATTRACTIVENESS OF SOME VOLATILE ORGANIC COMPOUNDS FOUND IN HENHOUSES ON *DERMANYSSUS GALLINAE*

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Increasing acaricide resistance and strengthening of environmental policies makes poultry red mite (PRM) control challenging and requires alternative control tools. Whilst the general life cycle and habits of PRM are well established, determinants of it's interaction with the environment remain poorly understood. The role of generalist host-related stimuli on the behavioral response of PRM, such as CO_2 and temperature, has been well investigated. However, the role of volatile organic compounds (VOCs) other than CO_2, found in the chemical landscape constituted by the henhouse environment, on PRM behaviour has received little attention. The aim of the study was to explore the attractiveness of some VOCs present in farm buildings. We evaluated the attractive response of starved PRM adult females to 7 volatile compounds commonly found in the farm context, either alone or associated in a blend. Experiments involved both lab choice test bioassays (using Y-tube olfactometers) and field trials (preliminary results). Our results showed that an individual VOC (ammonia) and one blend of five VOCs ((*E*)-non-2-enal, nonanoic acid, octanal, nonanal, oct-1-en-3-ol) were significantly attractive to PRM in our laboratory conditions. Interestingly the latter five VOCs only attracted PRM when combined together: their attractant power was lost when they were tested individually or when one of them was lacking. In

the field, consistent trends were recorded. Our work brings fundamental information on chemical interactions between PRM and its environment in farm buildings. This study demonstrates the potential of using volatile substances to manipulate the behavior of PRM, combined or not with different stimuli, to elaborate an attract-and-kill control strategy.

DRAFT GENOME ASSEMBLY AND ANNOTATION OF THE POULTRY RED MITE, *DERMANYSSUS GALLINAE*: A NEW TOOL FOR RESEARCHERS

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The search for alternative control targets for *Dermanyssus gallinae* has been hampered by the lack of publicly available and comprehensive genomic information. Here we report the first draft assembly and annotation of the *D. gallinae* genome.

High molecular weight gDNA was purified from freshly laid mite eggs and PacBio sequencing libraries generated and sequenced using 10 SMRT cells on a PacBio RSII. Sequences were assembled using Canu and the resulting assembly scaffolded with 6Gb low coverage Oxford Nanopore Technology minION reads using PBJelly 2, followed by 8 iterations of genome polishing with Arrow. The final assembly contained 7,171 contigs, with an N_{50} value of 278,630 bp and an L_{50} of 800 contigs, the largest scaffold being 3,781,415 bp. The genome assembly comprised 959Mb and 63.5Gb of PacBio sequencing data provided ~66x–fold coverage.

Gene prediction utilised the MAKER pipeline with SNAP *ab initio* gene prediction trained with *Metaseiulus occidentalis*, *Ixodes scapularis* and Uniprot/Swissprot protein databases and supplemented with full-length PacBio Iso-Seq transcripts obtained from total RNA from mixed stages of *D. gallinae* (13,612 high-quality and 53,082 low-quality isoforms). The pipeline identified 14,608 predicted protein-encoding genes, of which 13,840 had significant Blast hits against the NCBI nr database. Predicted protein sequences were annotated with Pfam information and Gene Ontology (GO), which resulted in assignment of GO terms for 11,624 genes with functional annotation of 10,914 genes.

This Whole Genome Shotgun project is deposited in GenBank (accession QVRM01000000) and the annotated genome will be hosted on OrcAE as a publically available resource.

CHARACTERIZATION OF PREDATION INTERACTIONS BETWEEN ARTHROPODS FOR THE BIOLOGICAL CONTROL OF THE POULTRY RED MITE

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CONTEXT: Because the poultry red mite (PRM) lives off-host in habitats potentially shared by many predators (arthropods mainly), biocontrol agents have the potential to regulate this pest in poultry buildings. As a result, PRM is an excellent target for biological control, either by inoculating exogenous populations of enemies (usual biological control), or by stimulating local populations (conservation biological control CBC). In order to progress towards CBC, it is necessary to disentangle predatory interactions which occur between arthropods naturally present in henhouses. This will allow identifying direct natural enemies of PRM as well as their potential enemies and thus evaluating

their respective suppressive potential on PRM (associated or not with other organisms).

OBJECTIVES: In order to identify the ecological interactions to be favored, a reconstruction of trophic networks and an evaluation of interactions between the different species naturally-occurring in farming conditions were initiated.

METHOD: The predatory potential and food preferences of several arthropod species were evaluated with *in vitro* behavioral trials associated with chi2 tests. Correlations between predator and PRM abundances were measured using data collected on 20 henhouses (barn) as follows: by applying a Bayesian hierarchical model to direct inventory data (manure mites) and by calculating Spearman rho values using environmental DNA data (whole arthropodofauna of the henhouse).

RESULTS: In lab conditions, five mite species, a pseudoscorpion and a bug were found to be capable of repeated predation on PRM. The experimental results are consistent with correlation measures on field data, at least with predatory mites. Furthermore, it appears that some predatory species feed on each another. The implications of this information are discussed with regard to CBC.

ARE *DERMANYSSUS GALLINAE* AND *SALMONELLA ENTERICA* SUBS. *ENTERICA* SER. *GALLINARUM* ASSOCIATED IN POULTRY FARMS? A FIELD STUDY

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The aim of the this study was to evaluate the interaction between *Dermanyssus gallinae* and *Salmonella enterica* subsp. *enterica* ser. Gallinarum

during an outbreak of fowl typhoid in a intensive laying hens farm farm. The presence of *S*. Gallinarum was assessed and quantified by a semi-nested polymerase chain reaction (PCR) and real-time PCR, respectively, in mites collected during two subsequent productive cycles and the sanitary break. The anti-group D *Salmonella* antibodies were quantified by an enzyme-linked immunosorbent assay.

During the outbreak and the sanitary break, *S*. Gallinarum was constantly present in mites. In the second cycle, scattered positivity was observed, although hens did not exhibit signs of fowl typhoid, as a result of the vaccination with BIO-VAC SGP695 (Fatro, Ozzano Emilia Bo, Italy). The data strongly suggest that *D. gallinae* acts as reservoir of *S*. Gallinarum, thus allowing the pathogen to persist in farms. Furthermore, the present study has highlighted the interactions among *D. gallinae*, *S*. Gallinarum and hens with respect to enhancing the mite-mediated circulation of *S*. Gallinarum in an infested poultry farm.

UPDATE ON POULTRY RED MITE RESEARCH ACTIVITIES IN MACEDONIA

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Poultry red mite (PRM) remains a serious concern for the poultry industry. The poultry industry in Macedonia is shifting towards introduction of enriched cages. Previously, using different methodologies for the collection of the data (farm visits, post-mortem examinations and direct communications with the farmers) we found high prevalence of 69% of PRM at the farm level in the country. In another study, by using the COREMI Questionnaire designed within Working Group 1 we found that out of 29 farms 27.6% reported the presence of PRM in their farms. Almost half of the positive farms believe that decrease in egg production is related to the presence of PRM with the average decrease being 20%. Macedonian farmers reported 25 different agents used for regular

treatment and prevention against PRM. During the timeframe of the COREMI action we strived to establish morphological and molecular methods for identification of PRM and associated mites. For this purpose we have collected feces from four farms from different geographical regions in the country and we used the corrugated card trap method for collection, enumeration and identification of PRM. Recently, we have implemented a PCR diagnostic method by amplifying the mtCO1 gene using LCO1490 (forward) and HC02198 (reverse) primers followed by Sanger sequencing. This will be useful for running molecular epidemiology studies on PRM. Hereby, we present the results of our studies. Through participation in the COREMI action we initiated above mentioned research activities on PRM which were previously non-existent in the country.

PRACTICAL ASPECTS OF FLURALANER TREATMENT AND FIELD EXPERIENCE AT COMMERCIAL LAYER FARMS IN POLAND

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Fluralaner is a novel izoxasoline, which is used for reducing infestation of *Dermanyssus gallinae* in commercial layer and broiler-breeder flocks. The relevant publications and research show that *Dermanyssus gallinae* infestation is still a very important problem in poultry production, because of avian diseases spreading like salmonellosis, mycoplasmosis, Newcastle disease or avian influenza (1,2). Red mite infestation can induce stress in the birds (3), cannibalistic behavior, increased feed and water intake and decreased animal health. At commercial layer farms, high levels of red mite infestation lead to a drop in egg production, higher mortality and increasing morbidity of diseases (4). For the last one-year, field observations and production results after fluralaner treatment at commercial layer farms in Poland were collected. The level of red mite infestation after fluralaner treatment was monitored using red mites traps. The level of red mite infestation before fluralaner application was very high or medium. The parasites were visible on eggs and on the layer

cages as colonies. Usually the mortality rate was increasing at intervals of a few weeks as a result of virus or bacterial infections like *Escherichia coli*. According to the data collected, mortality decreased and bird health improved after fluralaner treatment. Due to red mite repopulation that was observed in some cases, all factors which can have an impact on that were analysed. In most of these cases, after economic analysis, eggs producers decided to use another round of treatment with fluralaner.

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INSIGHTS INTO *DERMANYSSUS GALLINAE*: THE ITALIAN CONTRIBUTION FROM BIRDS TO HUMANS

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The avian mite *Dermanyssus gallinae* (PRM) is a menacing blood-feeding arthropod of birds and mammals worldwide, including Italy. In the

last 20 years or so, Italian researchers have contributed to improving the knowledge of this pest, carrying out research in their own country and also collaborating with colleagues from several European and non-European countries. Over 80 contributions have been published, including refereed articles and book chapters, together with work presented at national and international conferences, academic lectures, seminars, technical meetings. Italy's PRM research has adopted a One-Health approach and examined several topics, including PRM morphology, its zoonotic role, traditional and molecular epidemiology, its vectorial role, acaricide-resistance and related issues, as well as alternative control challenges. In particular, besides providing practical tools for correct PRM identification, and highlighting the genetic diversity of mite isolates present inside and outside Italy, the Italian research group has improved understanding of PRM diffusion by pinpointing a prevalence of 20% in rural farms and up to 92% in industrial farms, as well as – interestingly – showing that farmers are generally unaware of PRM and unable to control infestations adequately. These socio-cultural limitations provided the impulse for further research on acaricide-related consequences. In fact, not only were field PRM populations collected from industrial farms found to be tolerant to carbamate (86%) and permethrin (42%) (research on target coding genes and the possible polymorphisms involved in this resistance is currently in its infancy), but the more worrying finding was that carbaryl residues were found to be present in 40% of poultry organ and meat samples, and permethrin residues in 1.7%. These disturbing results, coupled with the evidence that PRM transmit *Salmonella gallinarum* to poultry, and the presence of *Chlamidia psittaci* DNA in other birds, led the research group to focus its attention on biological control. Lab trials showed that entomopathogenic fungus *Beauveria bassiana* used with *Eucalyptus globulus* essential oil at 0.2% has a significant acaricidal effect. In addition, a field trial demonstrated the strong (up to 99.8%) and long lasting (over 2 months) bioactivity of a patented neem-based formulation used in a heavily infested industrial farm. The group has made an important contribution by reporting human cases in both rural and urban contexts. It has documented a large number of PRM attacks involving farmers (19%), so that PRM's inclusion as an occupational hazard has been strongly recommended. In the meantime, 26 urban outbreaks of pruritic dermatitis have been described over a period of 16 years (2001-2017), involving a total of 66 subjects in private or public buildings (offices, homes, hospitals); these were mostly caused by *D. gallinae*

(20/26) and thus provide the largest series of human dermanyssosis cases in Europe. Reports of PRM in humans have been enriched with detailed anamnestic and clinical documentation, source identification (abandoned pigeon and sparrow nests, pet canaries), together with data on the potential role of urban PRM in transmitting *Coxiella burnetii* and *B. burgdorferi sensu lato/Borrelia afzelii.* The group strongly supports the urgent need to reduce under-reporting by creating a shared diagnostic protocol to improve recognition of *D. gallinae* infestations in humans. All these completed or ongoing research initiatives have been supported by national or international grants and STSM opportunities, initially within the framework of an unofficial group of European scientists interested in this topic (2001-2013), and subsequently (2014-2018) as part of the European Cooperation in Science and Technology (COST Action FA1404 - COREMI), admirably led by the Chair O. Sparagano, and which we wish to thank.

MONITORING AND EPIDEMIOLOGY OF *DERMANYSSUS GALLINAE* (PRM) BASED ON DATA FROM THE NORWEGIAN PRM MONITORING PROGRAM

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Monitoring of poultry red mite (PRM) is mandatory for all Norwegian egg producers selling eggs to commercial egg packing plants. Animalia AS has since 2006 been running a PRM monitoring program for Norwegian egg producers. This monitoring is based on the use of cardboard traps that are sent to Animalia, where any PRM found are genetically identified and haplotyped.

Information is gathered and stored in an online database and PRM findings are shared with the producers, the egg packing plant and the pullet grower for each flock. Also the farm veterinarian can access the database and the PRM status.

We are currently receiving traps from approximately 50% of layer flocks annually. Data from 2011 showed that approximately 11% of the layer houses in the PRM monitoring program were positive for PRM. From 2011 to 2014 the number of new houses that became infested each year with PRM was low. However in 2015 and also in 2018 there was a peak in new PRM infestations bringing the numbers of PRM infested houses to approximately 20% (2016), 22% (2017) and 25% (1st October 2018). From 2009 -2014 we registered 2-8 houses each year infested by PRM.

When there is an increase in PRM infestation actions are taken. In 2015 PRM spread was successfully eliminated from a probable pullet grower source using a combination of acaricide and heat applied to empty houses. We emphasize that no PRM was found in the pullet grower houses.

Challenges regarding this national PRM monitoring program will be discussed.

UTILISING THE GENOME ANALYSIS TOOKLIT TO IDENTIFY SINGLE NUCLEOTIDE POLYMORPHISMS FOR USE AS GENETIC MARKERS

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The poultry red mite (*Dermanyssus gallinae*), an obligatory blood feeding ectoparasite, is primarily associated with poultry where it is predicted to incur losses of ~€130 million per annum from European farmers. Moderate to high infestation levels result in negative impacts on hen welfare, including an increase in cannibalism, irritation, feather pecking, restlessness, hen mortality and anaemia. Current control strategies, including the use of acaricides and desiccant dusts, are often ineffective and widespread resistance to acaricides has been demonstrated.

Alternative methods of control are urgently required for *D. gallinae* and methods under investigation include the development of a suitable vaccine. One major consideration for the development of a vaccine to protect chickens against *D. gallinae* is the extent and rate of occurrence of genetic diversity within mite populations. Understanding genome-wide diversity requires a panel of appropriate genetic markers, however resources available for *D. gallinae* are limited, with little or no available data on markers such as single nucleotide polymorphisms (SNPs). Here, the genome analysis toolkit (GATK) best practices pipeline for germline short variant discovery (SNPS + Indels) has been utilised via the UseGalaxy platform to identify SNPs across the *D. gallinae* genome. RNAseq 454 transcriptomic datasets produced at the University of Hannover and Moredun Research Institute have been compared with Illumina data produced at the Royal Veterinary College. Preliminary comparative mapping using BWA-MEM has detected in excess of 60,000 SNPs within each data set tested. High quality SNPs found to segregate between populations will be developed for use in future analyses of genome-wide genetic diversity.

STSM EXPERIENCE: CHARACTERIZATION AND CLONING OF THE POULTRY RED MITE (*DERMANYSSUS GALLINAE*) SUBOLESIN

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Tick subolesin has previously been tested as vaccine candidate against ticks, showing effects on the blood digestion and on development and reproduction. In addition, recombinant mosquito subolesin has been used in a trial to vaccinate hens against Poultry Red Mite (PRM) and was shown to increase mite mortality in an *in vitro* feeding assay. However, the *Dermanyssus gallinae* subolesin orthologue has not been yet described. Searching in the

PRM genome built by the COREMI Working Group I, a candidate *D. gallinae* subolesin (Dg-SUB-1) was identified. Dg-SUB-1 was PCR amplified from whole *D. gallinae* cDNA and cloned into *E. coli* expression vector pET-SUMO. Recombinant Dg-SUB-1 (rDg-SUB-1) was expressed in *E. coli* as an insoluble protein, and purified by nickel-affinity chromatography. Using Western blots, we demonstrate that laying hens immunized with tick-subolesin generate IgY antibodies that cross react with purified rDg-SUB-1. The characterization of Dg-SUB-1 will allow evaluation for use as a recombinant subunit vaccine.

CURRENT CONTROL STRATEGIES AGAINST THE POULTRY RED MITE *DERMANYSSUS GALLINAE* IN SWITZERLAND

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A questionnaire survey on current control strategies against the poultry red mite *Dermanyssus gallinae* has been carried out in Switzerland in collaboration with the egg producers association Gallo Suisse and supported by the organic producers within IG Bioei Suisse. 67 questionnaires were returned in January/February 2017. Responders covered an average flock size of 5'517 hens (440 – 18'300) aged between 10 and 105 weeks (average 43). 22% of responders registered as being organic producers, which corresponds well to the proportion of organic eggs produced and consumed in CH, 56% as free-range and 22% as indoor producers. Overall, 34% of the farmers reported having an infestation of *D. gallinae* in their layer houses at the time of responding, but almost 50% indicated to have noticed an infestation in the current flock in the past. Empty layer houses are usually cleaned and disinfected, followed in almost 40% of the cases by treatments directed to red mite control mainly using silicas, less frequently plant extracts (Pyrethrum) or chemical treatment (CBM8). When hens are in the house, about 1/3 of the farmers start mite

control as soon as mites are first detected, 1/3 wait until infestation becomes apparent and 1/3 start before mites are detected. On average, farmers spent 2h and 694€ (0 – 4'400€) for mite control in the current flock. In summary, the survey confirmed that *D. gallinae* are common in Swiss layer houses. Producers are aware of the problem and usually start mite control in empty houses between two flocks of layers.

EFFECTS OF ON-FARM APPLICATION OF IPM FOR THE POULTRY RED MITE *DERMANYSSUS GALLINAE;* PRELIMINARY FINDINGS

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Dermanyssus gallinae (Poultry Red Mite, PRM) is a blood-sucking mite with an almost worldwide distribution and a negative impact on animal health, animal welfare and production parameters. This pest in birds and poultry is difficult to control due to its reclusive behavior, fast reproduction cycle and ability to quickly develop resistance to pesticides (chemical, synthetic products against pests). Integrated Pest Management (IPM) is a sustainable method, successfully applied in horticulture to prevent and control outbreaks. IPM applies pesticides only if other measures are generating limited results and thus reduces problems regarding pesticide residues and development of drug resistance (Barzman et al., 2015). Twenty laying hen farmers (organic, aviary, outdoor and enriched cages) apply the method of IPM for PRM and apply suggested measures as described by Mul et al. (2017) for more than one year. All farmers are supported by their self-chosen farm advisors and will receive up-to-date information on PRM, monitoring and control methods via meetings, training sessions, discussions and WhatsApp chat. A control group consisting of 10 laying hen farmers will not receive that information. This trial will identify the effect of support on control of PRM via the IPM method on 1) the PRM population in the hen house, 2) the egg production,

3) the number of measurements to control PRM and 4) the costs of PRM. The results of the first 5 months of application of IPM on the number of taken measures and the experiences of the farmers applying the IPM method will be discussed.

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OPTIMISATION OF AN ON-HEN FEEDING DEVICE FOR ALL HEMATOPHAGOUS LIFE STAGES OF POULTRY RED MITE: A TOOL FOR MITE CONTROL EVALUATION

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Poultry red mites (PRM) are difficult to contain in an experimental environment that allows for natural feeding. *In vitro* feeding techniques were developed but demonstrate high mite mortality and variable feeding (Bartley *et al*., 2017).

A prototype *in vivo* on-hen mite feeding device for adult mites was further developed for hematophagous stages of PRM. Three nylon and three polyester meshes and two device designs were evaluated. The best device and mesh sizes (105 µm and 120 µm polyester) were further evaluated for feeding and survival rates.

No significant difference in feeding was observed between the two mesh sizes for adults and deutonymphs (p = 0.20, 0.65 respectively). Protonymphs showed a significant reduction in feeding with the larger mesh size (p = 0.02).

A mite conditioning study to maximise feeding and evaluate baseline mortality using 105 µm mesh was performed. Mites were starved for one week at room temperature (conditioning period 1, CP1) and then three additional weeks at 4°C (CP2-4). Adult feeding peaked at 57% (CP4), deutonymph at 51% (CP2) and protonymph at 18% (CP4).

Mortality of adults post feeding was significantly higher at CP1 than at CP3 or CP4 (p= 0.0006 and

p <0.0001 respectively). No statistically significant differences were shown between conditioning periods for deutonymphs and no protonymph mortality was demonstrated.

This device represents a high hen-welfare, *in vivo* method of allowing mites to feed on the host and has great potential as a tool to allow feeding of hematophagous life stages to evaluate systemic PRM controls.

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GENETIC INVESTIGATIONS OF *DERMANYSSUS GALLINAE* IN NORWAY

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Poultry red mite (PRM) *Dermanyssus gallinae* is a problematic pest for egg producers in many corners of the world and is impacting the economics of egg production, negatively affecting animal health, and

welfare. We have collected PRM from Norwegian layer farms and used molecular tools for identification and epidemiology of mites collected in a national surveillance programme over several years. A database of DNA-sequences from a region of the mitochondrial gene cytochrome oxidase C subunit 1 (CO1) have been constructed from isolates of PRM, sampled throughout the last decade. The molecular data from this study have been used to investigate possible dissemination routes of PRM. In the analysed material several different CO1-haplotypes have been identified. Attempts have been made to combine epidemiological data with haplotype information in the dataset, to help identify possible dissemination route(s) of the mite. Additional genetic investigations and future activities will be presented.

EVALUATION OF THE USE OF FLURALANER IN THE GREEK EGG LAYING INDUSTRY AGAINST THE POULTRY RED MITE (*DERMANYSSUS GALLINAE*): FIRST RESULTS

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Poultry red mite (PRM) infestation poses a costly threat for the global egg industry, as even low mite populations can negatively affect hens' welfare and operation productivity. Recently, fluralaner (Exzolt™, MSD) was registered in Greece as an innovative treatment for PRM. The aim of the study was to investigate the prevalence of this ectoparasite and to evaluate the efficacy of fluralaner administration to egg laying hens in industrial farms located in different parts of the country. Heretofore, a total of 15 farms were visited to investigate the presence of the PRM using the AviVet™ Red Mite Traps, which were evenly distributed and left for 48 hours. After collection, the traps were examined for mite presence, identification and counting. Each farm was characterized as negative or

with low, medium or high infestation according to the mean mite numbers trapped. 13 out of 15 (86.7%) farms were positive to PRM trapping, 2 of them with low, 4 with medium and 7 with high infestation rates. The evaluation of the effectiveness of fluralaner was carried out up to 3 months post administration by placing traps. In all cases no mites were detected, confirming a highly successful effect of fluralaner against PRM. Additionally, all farmers reported a fast clinical improvement and an increase in egg production and shell quality. It was shown that fluralaner was highly successful to maintain reduced mite population over 99% for at least 3 months, improving hens welfare and productivity. The study is continued to more layer farms in Greece.

PROGRAM CONTROL OF POULTRY RED MITE *DERMANYSSUS GALLINAE*, TODAY

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The control of *D. gallinae* has thus far been predominately based on acaricides (insecticides), the efficacy of which has decreased over time due to the development of resistance. *D. gallinae* is a species which has developed resistance to all acaricides used so far. From this point of view, it is possible to assume that the new acaricide fluralaner will, even with evident efficacy but also deficiencies, lead only to a short-term improvement in the overall control of *D. gallinae.*

The control of poultry red mite population in poultry production has had an unfavorable tendency across several decades, so in order to put an end to this trend and turn to another direction, certain measures must be taken in terms of the approach of controlling this disease. It is necessary to: eliminate toxicological risks; define short-term goals (suppression efficacy) and long-term goals (eradication); introduce principles of

biosafety, prevention and rational control; ensure an expert application of formulation and increase the quality of monitoring. Put together, these elements compose the program of *D. gallinae* control. The current program which is used on farms, in our view, has perspective; it is based on physical control and an active impact on technological processes. It is possible to combine this type of control with other efficient methods of suppressing mites on farms.

INTERDISCIPLINARY ONE HEALTH MODEL FOR POULTRY RED MITE – COREMI WG2 OUTPUT

Participants from the Workshop "One health overview of PRM infestation and treatment – interdisciplinary approach" in Skopje within the COREMI Working Group 2

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One Health (OH) represents an interdisciplinary approach for maintaining health in a much broader sense. The OH concept means "preventing, detecting, containing, eliminating, and responding" holistically to the constraints for human, animal and environmental health. Poultry red mites (PRM) in intensive poultry farms pose a threat for avian and other species. From the OH perspective, there are three main aspects of PRM impact on human, animal and environmental health: Infestation; Treatment and Prevention. PRM Infestation affects the health and welfare of avian and

non-avian species, there are a few studies which show PRM impact on human health, but the understanding for the environmental effects of PRM presence is very limited. PRM Treatment and Prevention includes a variety of methods, agents and techniques that might have impact on animals, humans, food safety and the environment. The members of the Cost Action COREMI Working Group 2, in Skopje, March, 2018, worked on developing an interdisciplinary OH model to identify the potential threats and factors affecting the health. The published studies and scientific literature relevant to the PRM and Health were summarized and categorized in a database. The OH model should provide information for the broader impact of PRM and identify the existing links between PRM and OH. The process of collecting the existing knowledge and embedding it into the model is still ongoing and the work and networking needs to continue. The developed model could be likely used as a methodology to propose the OH perspective of other vectors and pests.

THE EFFECTS OF TREATMENT WITH FLURALANER ON POULTRY RED MITE INFESTATION AND ON PRODUCTION OF LAYING HENS HOUSED IN ENRICHED CAGES AND AVIARIES

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Dermanyssus gallinae, is an important cause of welfare and health problems in laying hens, yet control remains a challenge. Recently, a new systemic acaricide was approved: fluralaner (Exzolt®, MSD Animal Health). A field study was performed to evaluate the effects of treatment with fluralaner on poultry red mite (PRM) infestation level and on egg production of laying hens kept in different housing systems.

31000 hens were housed under controlled conditions in 12 independent compartments equipped with enriched cages and aviaries. At the age of 51 weeks, fluralaner was administered twice 7 days apart at a dose rate of 0.5 mg/kg BW through the drinking water. PRM were monitored weekly. Production data were recorded daily from 4 weeks before until 6 weeks after the first treatment. For evaluation, obtained data were compared to the production standard of the breeds.

Before treatment, the infestation levels varied from light to very high. After the treatment, no mites were detected in 10 of the 12 compartments. In the remaining compartment, the percentage of mite reduction was more than 99.99%. In the 4-week period post-treatment, the laying percentage, egg mass, first choice eggs and feed intake increased.

In conclusion, fluralaner administered through drinking water was found effective in reducing the number of PRM with at least 99.99%, irrespective of breed and housing system. This was accompanied with an increase in egg production. Treatment with fluralaner can therefore mean an important aid to welfare of laying hens and profitability of laying farms.

ROLE AND INFLUENCE OF THE COREMI COST ACTION (FA1404) NETWORK ON THE EUROPEAN POULTRY SECTOR

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In 2014 the COREMI Cost Action (FA1404) was launched with 10 founding countries, which reached 18 and 28 (26 COST Countries + 1 IP country and 1 NNC country) by the end of 2014 and 2015, respectively. By the end of 2018 COREMI had **255** members from over **32** Countries covering 4 continents with more than **380** participants attending our events in the last four years

Since the creation of COREMI it is clear that members collaborated further together showing far more COST countries involved per COST publication. For instance the co-authorship index is 4.9 (compared to 4.1 for

non COREMI papers) with a field-weighted citation impact (FWCI) score of 0.855 (compared to 0.751). Moreover, 60% of COREMI papers have authors from least 2 countries (compared to 7.7% for non-COREMI papers), demonstrating that the COREMI Cost Action has created and supported European collaborations.

It is also reassuring to see other non-COREMI countries publishing on *Dermanyssus gallinae* (the poultry red mite, PRM), such as Iran, Korea, Japan and Brazil, showing how global the problem is.

Finally, so far **237** staff members all over the world at MSD Animal Health have taken the *Dermanyssus* online training organized by us.

EFFECT OF FLURALANER ON BEHAVIOURAL AND STRESS INDICATORS IN LAYING HENS INFESTED WITH *DERMANYSSUS GALLINAE*

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Poultry red mite (PRM, *Dermanyssus gallinae*) infestation is widely recognized as a major welfare threat in layers. The effect of a new drinking water PRM treatment (fluralaner, Exzolt®, MSD Animal Health) on behavioural and stress markers was investigated.

This study involved a commercial layer house with enriched cages, containing 12,700 29 week-old hens infested with PRM. The infestation was quantified weekly using mite traps, over 13 weeks. Seven weeks after the study start, the hens were administered Exzolt®, twice seven days apart, at the recommended dose.

Behavioural parameters were assessed weekly through scan (activity) and focal sampling (other behaviours). One week before treatment (baseline), and one and five weeks after, blood corticosterone was measured from 50 hens.

The pre-treatment PRM infestation level was high (average ca. 1500-2200 mites per trap) and dramatically decreased immediately after treatment until the end of the study (< 2 mites per trap).

For the whole post-treatment period, hens showed significantly lower head scratching, head shaking and auto-preening, both during the day and at night. After treatment, severe feather pecking decreased during the day($p=0.004$). During the night, the percentage of active hens decreased from 34% before to 11% after treatment ($p<0.0001$).

Blood corticosterone decreased from 4.0 ng/mL (baseline) to 2.7 ng/mL ($p=0.02$) and 1.7 ng/mL ($p=0.003$) respectively the week of the second treatment administration and five weeks later.

It is concluded that effective treatment of PRM infestation improves welfare of laying hens.

EFFECTIVITY OF THERMOKILL TREATMENT

Peter van de Laar

Van Eck Bedrijfshygiene BV (The Netherlands)

The Poultry Red-Mite (PRM) is one of the biggest problems that egg-producing farms struggle with these days. Controlling an infection of PRM, and also trying to get rid of the (PRM) in the period between the old and new flock is not easy. Several years ago Van Eck Bedrijfshygiene developed and introduced the Thermokill treatment. Thermokill is a treatment method for red mites in the poultry house where red mites and eggs die/dry out through heating. The poultry house is heated up to the desired floor temperature under control. On all locations with the desired temperature the mites and the eggs will dry out. The process lasts multiple days so that the equipment heats up and cools down slowly. To prove the effectivity of this treatment Van Eck Bedrijfshygiene started research in cooperation with Wageningen University Livestock Research. The first experiments were done at 2 poultry houses and the first results of this research at 2 laying hen farms are looking very promising.

POSTERS

INFESTATION STATUS OF THE POULTRY RED MITE IN ISRAEL

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In the last 30 years, mite infestations in commercial poultry houses in Israel were mostly restricted to the Northern Fowl Mite (NFM), *Ornithonyssus sylviarum*. In the last 3-5 years, however, farmers are increasingly reporting infestations of the Poultry Red Mite (PRM) *Dermanyssus gallinae*. The number of reports are currently the same as for the NFM. A survey performed during 2016-17 in 80 poultry houses across Israel showed above 80% PRM infestation in free rage settings, while enriched and battery cage settings showed between 40-50% infestation rates.

Since farmers are unable to adequately distinguish between the two mite species, and because treatment management regimes (mostly based on Permethrin and Amitraz) target NFM, the current mite treatments in Israeli poultry houses do not provide adequate control of PRM. In addition, the lack of newly registered acaricides and the observed mite resistance, are causing the use of unregistered and sometimes forbidden pesticides.

Since 2016, the Agricultural Extension Service of Israel has organized several mite identification and treatment workshops for farmers, and performed preliminary trails with natural mite repellents which showed 70-80% infestation reduction. Furthermore, application of Fluralaner is also under examination.

We anticipate that better-informed farmers, together with the recommendation to use repellents under moderate infestation, and registered acaricides only under high infestation, will improve both PRM control and food safety.

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THE RESIDUE LEVELS OF ABAMECTIN AND SPINOSAD IN EDIBLE TISSUES OF LAYING HENS FOLLOWING APPLICATION USING A SPRAYER

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Dermanyssus gallinae is the most economically important ectoparasite in layers throughout the world. One of the widely used control strategies is the use of acaricides including spinosad (SPN) and abamectin (ABM). However, there is no information on the residues of these products in edible tissues of layer hens following application. The aim of this study was to determine the residue levels of ABM and SPN in edible tissues (liver, fat, muscle and skin) of layers.

A total of 36 laying hens, 11-12 months old and weighing 1674±155 g were used in this study. The animals were divided into 4 groups of 9 animals each and they were kept in individual cages. Two different doses of SPN and ABM (SPN: 2 and 4 g/l, ABM: 0.025 and 0.033 g/l) were applied in empty and stocked cages, respectively, to the floor, side and ceiling wires, to all folds and connection points and to the egg canals of the cages by using a mechanical sprayer. In the ABM group, the animals were placed in cages 30 minutes after the application. All animals were sacrificed at day 30 after spraying and, plasma and tissue samples (liver, breast muscle, fat and skin) were collected. The samples were kept at -20 ºC until analysis. Residue levels of SPN and ABM were determined by High-Pressure Liquid Chromatography (HPLC).

ABM was not detectable in plasma samples after low and high dose applications. The residue levels of ABM in liver, fat and muscle were 0.24±0.06 ng/g, 0.26±0.06 ng/g and 0.26±0.05 ng/g, respectively, following the low dose application and, 0.27±0.07 ng/g, 0.48±0.20 ng/g and 0.42±0.16 ng/g, respectively, following high dose application. Relatively

much higher residue levels of ABM (at low dose: 11.12±4.32 ng/g and at high dose: 25.13±14.34 ng/g, respectively) were detected in skin samples. Subsequently, higher SPN (spinosyn A + spinosyn D) residues were detected in the skin (at low dose: 154.22±85.49 ng/g and at high dose: 446.64±211.73 ng/g) and fat tissue (at low dose: 129.07±65.59 ng/g and at high dose: 445.18±287.46 ng/g) compared with liver (at low dose: 15.97±5.77 ng/g and at high dose: 38.18±20.53 ng/g) and muscle samples (at low dose: not detectable and at high dose: 17.17±7.05 ng/g).

Maximum residue limits (MRLs) of ABM and SPN have been set as 0.01 mg/kg (10 ng/g) (EFSA, 2014) and 0.2 mg/kg (200 ng/g) (EFSA, 2011), respectively, for edible tissues [(meat, kidney, liver and fat (fat MRL: 1.0 mg/kg for SPN)] of chicken. The ABM residues in meat, liver and fat after both low and high dose applications were under the MRLs (EFSA, 2014). Although, the MRLs have not been established for ABM and SPN in the skin tissue of chicken, the residue levels of ABM and the high dose of SPN in skin samples detected in the present study exceeded the MRLs of both ABM and SPN in respect of the MRL values for other edible tissues in chicken. Therefore, much higher ABM and SPN residues in the skin than the other edible tissues indicate that also the skin should be considered as a further parameter in determining the "withdrawal period" of acaricides in layers.

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INVASION OF *DERMANYSSUS GALLINAE* IN POLAND – THE PRESENT SITUATION

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In Poland, up to the mid 1990s, *Dermanyssus gallinae* infection was a problem only in small farms. The situation changed when cages, often contaminated with colonies of *D. gallinae* imported from the European Union, started to be used. This fact and the import of hens to reproductive flocks from countries of western Europe became the beginning of the serious problem with *D. gallinae* infection in large industrial poultry farms in Poland (3).

Research on prevalence of *D. gallinae* was conducted in the years 1999-2017 by several scientific centers in Poland. Red mites were detected in poultry farms in 15 of 16 voivodships examined, with extensiveness of invasion in up to 70% of farms. The most reports come from the western and central parts of the country. There are no reports from the Podlasie voivodship, which is located on the north-eastern edge of Poland (1,2).

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RED MITES *(DERMANYSSUS GALLINAE)* – A BRIEF OVERVIEW OF THE SITUATION AND THE ACTIVITIES IN SLOVENIA

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Slovenia is a relatively small producer of poultry meat and eggs among EU countries. The number of breeders in our country does not exceed 400,000 birds per year and all breeder flocks are owned by three integrated poultry companies. In Slovenia we have around 1.5 million commercial layers per year. More than half of these are reared in furnished cages (60.7%) and around 36% of hens are placed in litter system. We have also some flocks - less than 5%, placed in organic and free range systems.

Red poultry mite (*Dermanyssus gallinae*) is one of the most economically important parasites in poultry production. The presence of red mites among breeder flocks and layers in the territory of Slovenia is presented. The effectiveness of therapy with various preparations (such as foxim and fluralaner) which are permitted for use in poultry in our country is the subject of discussion. Red mites are a very common poultry nuisance appearing usually in the warmer summer months. They are blood feeding ectoparasites and eventually invade all poultry flocks. The biggest problems occur in commercial layers and breeder flocks after 20 weeks of age. Mite bites are mostly visible in the area of breast, wings, thighs and cloaca. For this reason, clinical sings in correlation with the age of birds, degree of anaemia (values of haemoglobin and haematocrit were measured) and the degree of infestation are shown. The impact of the rearing system (furnished cages, litter system, organic and free range) on the level of mite infestation was also studied.

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EFFECTS OF FEED RESTRICTION ON *DERMANYSSUS GALLINAE* INFESTATION IN GROWING CHICKS

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Environmental factors may influence the degree of damage done to the host by poultry red mite (PRM). We investigated the effects of feed restriction in chickens on damage induced by PRM and on growth performance of the host. A total of 360 one-day-old chicks of 3 genotypes were used. Four chicks per cage were kept in two identical rooms. The birds in one room were infested with red mites. Birds were fed ad libitum in the first week. From the second week onwards half of the birds in both groups received 20% less feed as compared to ad libitum fed chicks. Lighting was provided as 16L:8D. At 12 weeks of age, 36 birds were slaughtered to measure heart, liver, spleen, gizzard weights, as well as hemoglobin and hematocrit values. Body weight of control group was higher than that of infested group (P=0.0055). On the contrary, the feed intake of infected group was higher (P=0.0008). However, an interaction between groups and genotypes was observed (P=0.0007). A slightly lower hematocrit value was observed in infested birds (P≥0.0689). Hemoglobin values were 10.2 g/dl, 9.2 g/dl in control and infested groups, respectively (P=0.0084). Heart, liver and spleen proportions to carcass weight were higher in the infested group (P≤0.0451). Feed restriction had significant negative effects on all traits (P≤0.05), except hematocrit and hemoglobin (P>0.05). No interactions between groups and feed restrictions was observed for all traits. However, hemoglobin values of ad libitum-fed control birds seem to be higher than the other subgroups (P=0.0880). In conclusion 20% feed restriction does not seem to have any influence on the negative effect of parasite infestation of the host.

This study supported by Canakkale Onsekiz Mart University BAP Coordination Unit with Project Number FBA-2018-2502.

POULTRY RED MITE IN CROATIA – PRELIMINARY RESULTS FROM THE WG1 QUESTIONNAIRE

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The Republic of Croatia joined the European Union on July 1, 2013, which also led to the alignment of its legislation with the legal system of the EU. The Commission Directive 2002/4/EC on the registration of establishments keeping laying hens covered by Council Directive 1999/74/E was implemented in Croatian law since 2013. Due to that directive, the Ministry of Agriculture held an official list of establishments that are keeping laying hens and selling their eggs on the market (currently available list from September 3rd, 2018, contains 184 establishments).

Working Group (WG) 1 of the COST Action FA 1404 COREMI produced a questionnaire that was translated to the Croatian language. The establishments mentioned on the official list of the Ministry of Agriculture were used as a base for investigation of the current PRM situation in Croatia, based on the WG1 questionnaire. In 2016, the list contained 145 establishments, which kept from 30 to 259200 laying hens. The questionnaire was sent to the 56 establishments that kept more than 3000 laying hens via email. Out of these 40 kept laying hens in cages, 15 in barns and one as a free-range flock.

The questionnaire had 5 groups of questions, as follows: System background, Poultry Red Mite indicators, Hygiene and red mite treatments, Implementation of red mite control and Costs of red mite control, as well as extra remark and personal details field (to be filled optionally).

Out of 56 sent emails, the filled questionnaire was received from 5 of them (8.93%). Although only less than 10% of the establishments sent the filled questionnaire, there are still some conclusions that can be taken: the majority of the establishments used legal insecticide for the treatment of the full houses, as well as some monitoring methods to indicate the presence of PRM. On the other hand, the majority of them start with treatment when the

mites are clearly visible, or have an impact on production or caretakers start to complain.

Due to the significance that PRM has to the layer industry, there is a clear need to approach the farmers or establishments more personally, to be able to obtain more significant national data, comparable to other EU countries.

INSEPARABLE DUO: *DERMANYSSUS GALLINAE* AND ITS BIRDS

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This presentation aims to provide a summary of our recent research on the effects *Dermanyssus gallinae* infestation has on overall health, performance, reproduction and product quality in different poultry species. Results obtained from experimentally induced infestations indicated that a slight infestation influences blood parameters and behavior, but not the growth of the host animal, whereas, a high-infestation clearly leads to anemia, and in quails to a higher mortality. The relative difference in growth performance between infested and control birds in five chicken genotypes (1 broiler, 4 layers) was nearly identical. A poultry red mite infestation in early age leads to delayed sexual maturity in layer chickens. Significant differences were found between laying performance and egg weight in favor of control birds to infested birds, and the shell quality of infested chickens was also worse. Also, a bad quality was noted of chicks hatched from infested chicken eggs. The findings of carcass characteristics show potential damages of mite infestation on quail and broiler meat quality and quantity. As a conclusion poultry red mite has similar effects on studied birds with regard to several parameters. The selection of one or more parameters could be assisted to develop

alternative control approaches not only parasite focused but also underlying host and environment. If the economic loss threshold can be estimated, the mite infestation may be managed without chemical treatments.

NATIONAL QUESTIONNAIRE ON *DERMANYSSUS GALLINAE* PREVALENCE AND CONTROL METHODS AND THE ON-FARM EFFECT OF THE FIPRONIL CRISIS IN THE NETHERLANDS

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Dermanyssus gallinae is an ectoparasitic pest with negative effects on animal health, animal welfare and egg production, costing the egg producers between € 0.5 – 1.0 per hen per year. In the Netherlands, the last prevalence study on *D. gallinae* was carried out in 2011 (Friesen et al., 2011). To obtain more insight into current *D. gallinae* prevalence, risk factors and control methods in laying hen farms in Europe, the COST-action FA 1404 initiated a European wide questionnaire related to *D. gallinae*. In the Netherlands this questionnaire was translated to Dutch and digitalised enabling the egg producers to complete the questionnaire online. Egg producers were asked via newsletters and email by the egg producers associations to complete the questionnaire during the period from December 2016 until March 2017. Five percent (44 respondents) of the Dutch egg producers completed the questionnaire through which only qualitative conclusions could be drawn. The obtained answers revealed a wide variety of products used on-farm to control *D. gallinae*, both registered and non-registered. To date, after the Fipronil crisis, Dutch egg producers are more aware about the risk of non-registered products and put effort into the identification of legal products against the poultry red mite which comprise two veterinary medicines, five biocides (including four products with diatomaceous earth) and predatory mites. With

such a limited number of effective products resistance of *D. gallinae* against non-mechanical products may quickly develop. Egg producers are asking for legalisation of non-patentable safe products such as green soap to treat against *D. gallinae*.

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EFFECT OF A CRISIS; HOW THE DUTCH POULTRY FARMERS AIM TO COMBAT THE POULTRY RED MITE IN THE FUTURE

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Dermanyssus gallinae (Poultry Red Mite, PRM) is a micro predator of birds with an almost worldwide distribution. In a laying hen house with hens this mite can cause reduced animal health and welfare and lower production parameters. The estimated cost of this pest is €0.50 – 1.0 per hen per year, which includes costs for control, which amounts to 15% of the balance per laying hen. Integrated Pest Management (IPM) is a suggested method to control PRM (Harrington et al., 2011) that is successfully applied in horticulture to control pests and diseases. IPM consists of eight steps including several preventive measures and monitoring of the pest population (Barzman et al., 2015).

In 2017 and 2018, the unintended application of Fipronil in farms in The Netherlands resulted in financial damage for the whole egg production column. Nowadays it is estimated to be a multiple of the 70 million euro, an estimation from Oktober 2017 (Van Horne et al., 2017). To

avoid such excesses in the future, government bodies, municipalities and farmer associations together aim to develop a sustainable solution to control PRM by funding a project that includes implementation of IPM for PRM in laying hen farms, development of a monitoring plan for PRM and knowledge transfer. Moreover, this project focuses on a plan to increase the responsible use of products to control PRM. Results of this two year project are expected to be: knowledge on the effects and costs of implementation of IPM, knowledge on measures to control PRM, a course and e-learning tool on using IPM for PRM infestation, an automated monitoring tool including an advice algorithm on treatment, a monitoring plan for PRM and a list with registered products against PRM.

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IN VITRO USE OF AG NANOPARTICLES AGAINST THE POULTRY RED MITE (*DERMANYSSUS GALLINAE*)

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Silver nanoparticles (Ag NPs) combine unique physiochemical characteristics with a growth inhibitory capacity against pathogens. Tailoring the shape, morphology and surface of these particles can manipulate their potent antimicrobial efficacy for desired applications. As

bacteria are becoming increasingly resistant to conventional biocides, Ag NPs constitute a promising alternative as a new generation of flora controlling agents for veterinary applications.

The aim of this pilot study was to evaluate the potential effect of Ag NPs against the poultry red mite (*Dermanyssus gallinae*). Towards this end, we collected live mites from a laying hen farm, where no acaricides were recently applied, using traps made of corrugated cardboard paper and left overnight in the farm. Mites were divided into groups of 10 mites each and placed into petri dishes. From the same farm feathers were also collected from the hens, which were used as a vehicle to apply the Ag NPs. Feathers were sprayed with 15, 20, 25 and 50 ppm of the Ag NPs solution, while some were sprayed with deionized water to serve as controls, and they were placed into the petri dishes with the mites. They were incubated at 25oC for 24 hours. All bioassays were run in triplicate. The mean efficacy (mite mortality) for the different concentrations 0, 15, 20, 25 and 50ppm Ag NPs were 13.3, 53.3, 70.0, 93.3 and 100%, respectively. It was concluded that there is a strong indication for the use of Ag NPs as a potential effective tool to control the poultry red mite.

DETECTION OF *DERMANYSSUS GALLINAE* IN NESTING BOXES OF THE LESSER KESTRELS (*FALCO NAUMANNI*) IN GREECE

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The lesser kestrel (*Falco naumanni*) is a small migrating falcon with a relatively wide Palaearctic distribution ranging from southwest Europe, through the Middle East to central and western Asia. Its population suffers from considerable declines and it is of conservation concern. The Greek population of this falcon species, which comprises c.15% of the European total, has been declining and is mostly concentrated in central Greece, in 98 colonies counting 2900 pairs. They live in association with human activities

and their food mostly comprises grasshoppers and locusts, but occasionally small mammals are also provided for the young. Nests may be found in old houses, buildings and often in close contact with chicken and pigeons. Within the framework of the conservation of this falcon and its habitats in Greece, we examined nesting material obtained from the boxes used for their reproduction to detect the presence of any ectoparasites. Towards this end, material (mostly sand) from a total of 52 nesting boxes was collected into plastic containers. The study area was in the eastern part of the Larissa plain, Thessaly, central Greece (39 ◦ 29'07'' N, 22 ◦ 41'39'' E) and included the lesser kestrel colony of c.120 pairs. The material was examined with salt flotation and any arthropod was collected. Five (9.6%) samples contained *Dermanyssus* spp. mites. The identification was made morphologically using standard identification keys. The presence of *Dermanyssus* spp. into the nesting boxes highlights the potential implication of this parasite in the health and successful reproduction of this falcon species and deserves further investigation.

ESSENTIAL OILS AS NATURAL ACARICIDES AGAINST *DERMANYSSUS GALLINAE*

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Dermanyssus gallinae is one of the most dangerous external parasites in domestic and wild birds. The result of infestation includes decreased laying, invasive diseases, immunosuppression, anemia and more frequent falls of birds. Increasing evidence suggests that *D. gallinae* populations have significant genetic resistance to commonly used drug classes, including many synthetic pyrethroids. Therefore, it is necessary to look for alternative methods of controlling and limiting these ectoparasites. Many plant species produce toxic secondary metabolites that limit the attacks of insects and can thus limit the spread of insects on new

hosts. The aim of the study was to test the effectiveness of essential oils as natural acaricides against *D. gallinae* and compare their actions with the spinosad active substance and mineral oil. The method of Zdybel et al. (2011) with our own modification, was used for the experiment. Mites were collected from three farms in Poland and placed on the disc (minimum 100 mites). The mortality of the exposed mites was measured after 24 and 48 h.

Essential oils with the highest acaricidial activity were used for the experiment: rosemary, cinnamon, thyme and clove oil in three concentrations (50%, 80% 100%) at a dose of 0.28 mg/cm^2. All the oils reduced *D. gallinae* survival. The best results were observed for 100% thyme oil (more than 90% mortality), 100% cinnamon (84% mortality), 100% clove oil (76% mortality). The active substance spinosad limited the survival of *D.gallinae* by over 95%. Mites after mineral oil treatment behaved in a similar way to the control treatment. Presented oils deserve further research as natural acaricides reducing *D.gallinae* survival.

MITES AND THEIR INFECTIONS IN SLOVAKIA

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The mite superfamily Dermanyssoidea is the most ecologically diverse group of Mesostigmata. Parasitic and non-parasitic species can be found in large numbers in the nests and burrows made by their vertebrate hosts. Some species of mites occasionally infest man, and some transmit diseases to humans. Mites have been found to transmit rickettsial agents such as *Rickettsia akari, Orientia tsutsugamushi* and they can contain *Anaplasma* spp., *Bartonella* sp., *Spiroplasma* sp., *Wolbachia* sp., and other unclassified Rickettsiales. *Dermanyssus gallinae* is of the greatest economic and veterinary importance among the studied species because it occurs worldwide and is closely associated with domesticated birds. The most frequent infestation was

found in the nests of *Columba livia* f. *domestica*, *Passer montanus* and *Gallus gallus* var. *domesticus*. Among wild birds, *D. gallinae* was more frequently found in the nests of birds that are common in synanthropic and urban habitats. An unusual finding of *D. gallinae* was observed in a dung beetle, *Geotrupes stercorarius* (Mašán et al. 2014).

Briefly, 886 mites were collected from small mammals (*Apodemus flavicollis, A. agrarius, Myodes glareolus, Microtus arvalis* and *Rattus norvegicus*) from five localities of Eastern Slovakia. The Eudominant species was *Laelaps agilis* and the dominant were *Laelaps jettmari* and *Haemogamasus nidi.* In addition, *Myonyssus gigas, Eulaelaps stabularis, Euryparasitus emarginatus, Eviphis ostrinus, Haemogamasus hirsutosimilis, Hyperlaelaps microti,* and *Laelaps hilaris* were species also parasitizing mammals. Rickettsial DNA was found in 9.4% of mites and another analysis showed the presence of *Rickettsia helvetica, R. slovaca, R. raoultii* and other unidentified *Rickettsia*.

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LOW COST LIGHT INDUCED THERAPY OF RICKETTSIAL INFECTION

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Photodynamic therapy (PDT), was discovered in 1900, when the toxic effect of acridine orange on microorganisms in the presence of light was observed. However, the first origins of PDT started during ancient times in Egypt, Greece and India [1].

PDT is a non-invasive method found in many bioapplications. It is based on light, oxygen and a photosensitiser [2]. PDT can cause specific

biological response at the cellular or sub-cellular levels, e.g. apoptosis or necrosis [3].

Rickettsiae are Gram-negative obligate intracellular bacteria growing within the cytoplasm of their eukaryotic host cells. They are associated with arthropod vectors (ticks, flees, lice and mites) and are responsible for mild to severe diseases in humans. In 1997, *R. slovaca* was described as a human pathogen and the etiological agent of the TIBOLA /DEBONEL human disease.

We evaluated *in vitro* effect of PDT on rickettsial infection. The interaction studies showed a statistically significant reduction number (by 96%) of alive rickettsial microorganisms in cells after using the photosensitizer and the light.

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DESCRIPTION OF *ORNITHONYSSUS BURSA* IN POULTRY IN THE MADEIRA ISLAND, PORTUGAL

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Dermanyssus gallinae, *Ornithonyssus sylviarum* and *Ornithonyssus bursa* are blood-feeding ectoparasites of birds. Although all three mites are considered a serious pest in poultry and capable of causing human dermatitis, *O. sylviarum* and *D. gallinae* are by far the most reported in literature. Both are adapted to temperate climates, but for yet unclear reasons, *O. sylviarum* is predominantly encountered in North America and *D. gallinae* in Europe. *O. bursa* is mainly a tropical and subtropical mite and its presence has been recorded only twice in Europe, in migratory birds in Slovakia and one case of human dermatitis in Sicily.

We report the occurrence of *O. bursa* in poultry in the Madeira Island, Portugal. The specimens were found in a backyard laying flock and collected from the plumage of hens. The owner reported frequent infestations of the flock and complained of mites crawling on the skin. *O. bursa* mites were identified microscopically. They differentiated from *O. sylviarum* and *O. bacoti*, the tropical rat mite, based on the arrangement of setae on the sternal shield, morphology of epigynal and dorsal shields and length of dorsal setae. This is the first report of *O. bursa* in Portugal and one of the few in hens in Europe. No information on dermanyssoid mites parasitizing poultry in the Madeira Island is available. Therefore, more investigation on *O. bursa* is needed to assess the extent of colonization of poultry and the possible epidemiological consequences on this island.

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CO-OCCURRENCE OF *CHEYLETUS* SPP. AND *DERMANYSSUS GALLINAE* IN COMMERCIAL LAYING FARMS IN PORTUGAL

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The genus *Cheyletus* consists of predacious species that are mostly associated with bird nests and a common component of the house dust acarofauna. These mites play an important role in biocontrol programs of agricultural pests and recent evidence suggests that some species may be natural enemies of *Dermanyssus gallinae*. This study assesses the co-occurrence of *D. gallinae* and *Cheyletus* spp. in commercial laying farms in Portugal. Mites were collected with cardboard traps in 24 laying farms located in 21 civil parishes. Traps were fixed on cages at a ratio of 1:1000 birds for flocks up to 20000 birds, plus an extra trap for each additional 5000 birds. A total of 562 traps were recovered, of which 86.1% contained *D. gallinae* and 31.9% *Cheyletus* spp. mites. The prevalence of *D. gallinae* and *Cheyletus* spp. among farms was 95.8% (95% CI: 79.8-99.3%) and 83.3% (95% CI: 64.2-93.3%), respectively. The average number of trapped *D. gallinae* mites in each farm was 5200.4 ± 16522.7, with a median of 358.6 mites per trap (IQR = 45.7-3135.2) while that of *Cheyletus* spp. was 4 ± 8, with a median of 1.1 mites per trap (IQR=3.8). The mean number of trapped *Cheyletus* spp. per farm was positively correlated with the mean number of *D. gallinae* (ρ=0.64; P<0.001). Further in-depth studies in commercial poultry holdings are needed in order to investigate the population dynamics of both species and evaluate the use of *Cheyletus* spp. in the biological control of *D. gallinae*.

ESTABLISHMENT OF AN REARING SYSTEM FOR THE POULTRY RED MITE, *DERMANYSSUS GALLINAE* AND THE EFFECTS OF DARKNESS ON THE REPRODUCTION RATE OF POULTRY RED MITE

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The poultry red mite (PRM), *Dermanyssus gallinae*, is one of the most economically deleterious ectoparasites affecting egg-laying hens worldwide. New approaches for control are needed and often require the maintenance of PRM under laboratory conditions. A rearing system for PRM, consisting of a metal cage, a plastic storage box, novel traps and a tray filled with water was established, which proved very effective in maintaining and reproducing colonies of PRM. PRMs feed on the hens usually at night not in daytime, indicating it may be possible to control PRM by manipulating lighting regimes. The effect of darkness on the reproduction of *D. gallinae* was investigated on Chinese strains of PRM. Using molecular tools we confirmed these mites to be *D. gallinae,* and we performed an assessment on the phylogenetic position of these mite strains identified by phylogenetic assessment of mtCO1. It was found that the reduction of light positively increased the population of PRM in culture by improving the fertility of females via increasing engorgement levels of females and feeding success of mite.

IN VITRO EVALUATION OF THE EFFECTIVENESS OF ACARICIDES USED IN POLAND AGAINST RED MITES (*DERMANYSSUS GALLINAE*)

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The control of poultry red mites, *Dermanyssus gallinae* (De Geer, 1778), is very difficult because of high resistance of these parasites to acaricides. In order to be highly effective in disinfestation, it is necessary to identify the susceptibility of the local red mite populations to the acaricide. In the years 2015-2017 an investigation was carried out in 34 battery cage farms of laying hens, localised in 13 Polish provinces. For the study, acaricides containing the active substances: avermectin, cypermethrin, permethrin, carboxylate, phenoxybenzyl, carbamate and silicon dioxide were used. The investigation was carried out by previously described methods (Cencek *et. al.,* 2011; Zdybel *et. al*., 2011). Acaricides containing bendiocarb FICAM 80 and also preparations containing silica BIOBECK (silicon dioxide) and RECIDAL SIL (diatomaceous earth) demonstrated the highest efficacy against the majority of the red mite populations. Their mean efficiencies were 96.5%, 90.0% and 84.7%, respectively.

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