COREMI

cost
EUROPEAN COOPERATION
IN SCIENCE AND TECHNOLOGY

3rd COST CONFERENCE

20, 21 September 2017

Oeiras, PORTUGAL

COREMI

ABSTRACT BOOK

PROGRAM

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National Institute for Agrarian and Veterinary Research

Instituto Nacional de Investigação Agrária e Veterinária, I.P.

COREMI

EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

3nd COST CONFERENCE

20-21 September, 2017

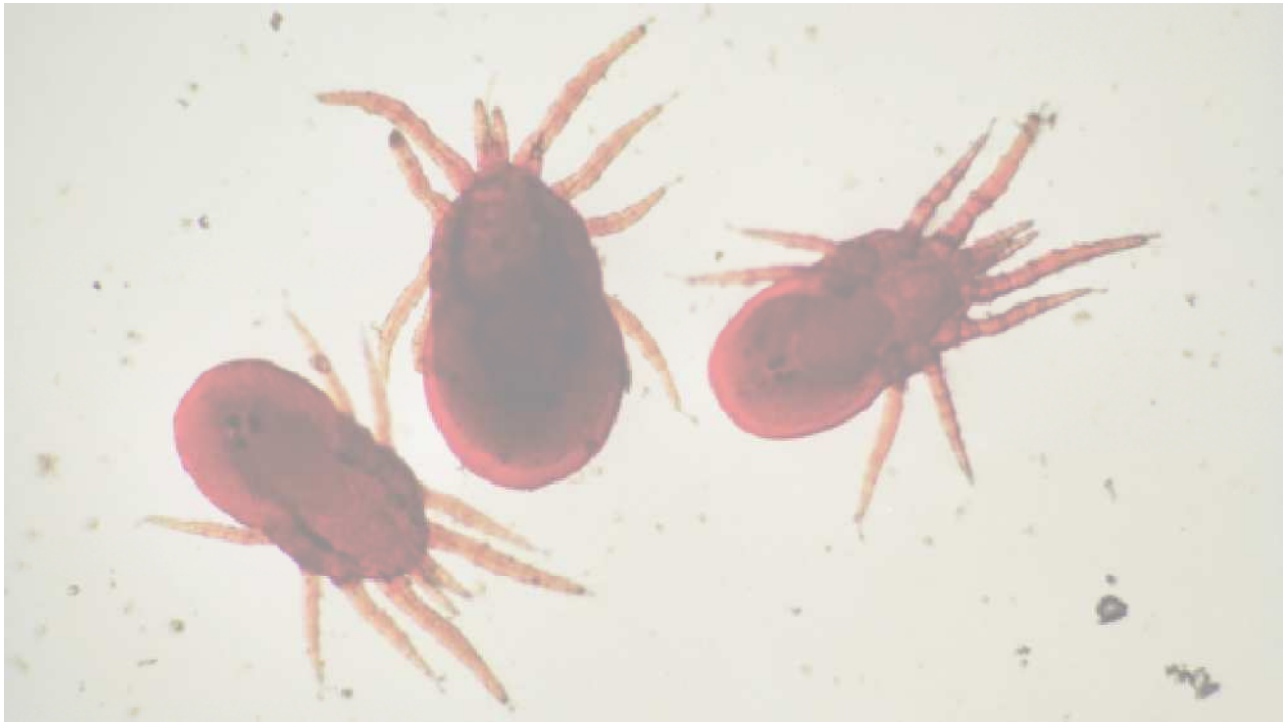
MANAGEMENT COMMITTEE (MC) MEETING

22 September, 2017

Oeiras, Portugal

COST ACTION FA1404

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



FINAL PROGRAM AND ABSTRACTS

SCIENTIFIC COMMITTEE AND CORE GROUP MEMBERS

Olivier Sparagano (Chair) UK
Fiona Tomley (Vice-Chair) UK
Robert Finn (WG1) UK
Mul Monique (WG2) NL
Lise Roy (WG3) FR
Danijela Horvatek Tomic (WG4) HR
Annunziata Giangaspero (STSM) IT
Julia Stew (GH) UK

ORGANIZING COMMITTEE

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WGs SUBJECTS

Working group/leader	Subject
WG 1 – Robert Finn	Developing alternative control measures
WG 2 - Monique Mul	End users (One Health) - interdisciplinary approach
WG 3 - Lise Roy	Genetic structure in a changing world
WG 4 – Danijela Horvatec Tomic	Epidemiology, pathology, geographical mapping and surveillance tools

WELCOME ADDRESS

Dear Colleagues,

The Committee members and of the COST Action FA1404 are pleased to welcome you in Oeiras at the 3rd COST CONFERENCE MANAGEMENT and COMMITTEE MEETING on Improving current understanding and research for sustainable control of the poultry red mite *Dermanyssus gallinae* (COREMI).

The overall aim of COREMI is to generate a synergic/holistic approach to improving the health, welfare and productivity of the 350 million laying hens, through more effective prevention and control of *Dermanyssus gallinae*, the Poultry Red Mite (PRM). This will be achieved by cooperation and multidisciplinary networking between scientists and other stakeholders from the different member states and from different disciplines, thus making the European poultry industry more competitive with other leading countries. COREMI plans to consolidate the existing expertise and knowledge in order to gain better understanding of PRM and its economic and social impacts, and to use this information to implement more efficient and sustainable control methods.

The Conference is open to all world scientists interested in this topic and will take place in the heart of the scientific Institution, the *National Institute for Agrarian and Veterinary Research (INIAV)-Oeiras, Portugal*.

We wish you a nice stay in Oeiras!

Olivier E. Sparagano
Coventry University, UK
COREMI Cost Action Chair

PROGRAMME

Tuesday September 19th, 2017

Location: **National Institute for Agrarian and Veterinary Research (INIAV)-Oeiras**

18:00 Welcome cocktail

Wednesday September 20th, 2017

Location: **National Institute for Agrarian and Veterinary Research (INIAV)-Oeiras**

8:00 – 9:00 Registration of participants

9:00 – 9:30 Opening ceremony:
Professor Doutor Nuno Canada (INIAV President)

Professor Fernando Bernardo (Directorate-General of Food and Veterinary Medicine [DGAV]– General Director)

Dr. Fernando Moreira (Portuguese Association of Avian Sciences [APCA] – President)

Professor Olivier Sparagano (COREMI Chair)

SESSION 1

Chair: Fiona Tomley and José Francisco Lima Barbero

9:30 -10:00 Key note lecture 1

An overview of the history, biology and control of bedbugs: what have we learned that could be applied to the poultry red mite?
Richard A. Naylor

10:00-11:00 Effective and perceived epidemiology of PRM

Oral presentations (15 min each plus discussion)

Internationalisation of research publications on *Dermanyssus*: myth or reality?
Olivier Sparagano, Fiona Tomley, Robert Finn, Monique Mul, Lise Roy, Danijela Horvatek Tomić, Annunziata Giangaspero

A survey on *Dermanyssus gallinae* in intensive poultry units in Portugal
Helga Waap, Telmo Nunes, Jacinto Gomes, Paulo Leite

Farmers' perceptions about poultry red mite in Macedonia – findings from the Coremi questionnaire
Miroslav Radeski, Aleksandar Dodovski

11:00-11:30 Coffee break

SESSION 2

Chair: Annunziata Giangaspero and Miroslav Radeski

11:30-12:00 Key note lecture 2

The 'other' poultry mite, *Ornithonyssus sylvarium*
Bradley A Mullens, Amy C Murillo, Alec C Gerry

12:00-13:20 Biology, ecology, physiology, mite-host relationship

Oral presentations (15 min each plus discussion)

General knowledge of *Dermanyssus gallinae* reproduction after feeding
Ivan Pavlovic, Aleksandar Pavlicevic, JongUng Yoon, Milica Dotlic

Development of a poultry red mite on-hen feeding device: a potential tool for mite control evaluation and vectorial studies
Kathryn Bartley, Frank Turnbull, Fran Nunn, Harry Wright, Alasdair Nisbet

Characterization of mite communities in wild bird nests from south-central Spain
José Francisco Lima-Barbero, Marta Sánchez, Monica Young, Maria L. Moraza, Shira Gal, Ursula Höfle, Eric Palevsky

An integrative pilot study to assess the feasibility of conservation biological control of the poultry red mite in barn layer farms
Lise Roy, Marine El Adouzi, Maria Lourdes Moraza, Geoffrey Chiron, Etienne Villeneuve de Janti, Guénolé Le Peutrec, Olivier Bonato

13:20-14:30 Lunch

SESSION 3

Chair: Elias Papadopoulos and Martina Lichovnikova

14:30-15:50 Available and future control methods - towards better treatments?

Oral presentations (15 min each plus discussion)

Efficacy of a novel neem oil formulation (RP03™) to control the poultry red mite *Dermanyssus gallinae*
Annunziata Giangaspero, Nicola Pugliese, Antonio Bevilacqua, Elena Circella, Marianna Marangi, Luigi Gradoni, David George, Olivier Sparagano, Antonio Camarda

Comparative in vitro susceptibility of *Dermanyssus gallinae* field isolates to fluralaner, phoxim, spinosad, deltamethrin and propoxur
Emmanuel Thomas, Hartmut Zoller, Gabriele Liebisch, Annie Flochlay-Sigognault

Field safety and efficacy of fluralaner in drinking water for the treatment of poultry red mite (*Dermanyssus gallinae*) infestations in commercial flocks in Europe
Emmanuel Thomas, Annie Flochlay-Sigognault

Introduction of Exzolt (fluralaner 10 mg/ml solution)—A new product for treatment of poultry red mite infestation in chickens
Roser Dolz

15:50-16:30 Poster session and extended coffee break

Biological control of the chicken red mite in layer farms in Europe: state of the art and perspectives
Damien Morel, Tom Groot

Control of red poultry mite (*Dermanyssus gallinae*) by mechanical effect: chosen current improvements of formulations, application and concept
Aleksandar Pavlicevic, JongUng Yoon, Ivan Pavlovic

An integrative approach to the molecular and morphological identification of mites associated with the red poultry mite
Shira Gal**,** Eric Palevsky, Eitan Recht, Yuval Gottlieb, Efrat Gavish, Lise Roy, María L. Moraza, Eddie Ueckermann Monica Young

Description of proteomes for different fed and unfed stages of the poultry red mite (*Dermanyssus gallinae*)
José Francisco Lima-Barbero, Olivier Sparagano, Robert D. Finn, Lourdes Mateos-Hernández , Mariana Boadella, Ursula Höfle, José de la Fuente, Margarita Villar

In vitro* effect of plant essential oils as acaricides against *Dermanyssus gallinae
Monika Roczeń-Karczmarz, Marta Demkowska-Kutrzepa, Jolanta Zdybel, Krzysztof Tomczuk, Maria Studzińska, Tomasz Cencek, Magdalena Włodarczyk-Ramus

***In vitro* evaluation of the effectiveness of commercially available acaricides against the populations of red mites (*Dermanyssus gallinae*) occurring in Poland**
Tomasz Cencek, Jolanta Zdybel, Magdalena Włodarczyk–Ramus, Jacek Karamon, Marta Dempkowska-Kutrzepa, Monika Roczeń-Karczmarz

***Dermanyssus gallinae* status in Iran: an emerging problem**
Shayan Rahimian, Olivier A. E. Sparagano

The residue depletions of spinosad and abamectin in eggs of laying hens
Veli Yilgor Cirak, Cengiz Gokbulut, Mehmet Ozuicli, Levent Aydin

Results of a pilot study regarding *Dermanyssus gallinae* in the greek laying hen industry
Konstantinos Arsenopoulos, Athanasios Angelou, Elias

16:30-18:00 WG2 individual session:

Chair: Monique Mul

WG2: End users (One Health) – an interdisciplinary approach

19:00- Social dinner "A PASTORINHA"

Location: **Avenida Marginal, Praia de Carcavelos**

Thursday September 21st, 2017

Location: **National Institute for Agrarian and Veterinary Research (INIAV)-Oeiras**

SESSION 4

Chair: Katherina Tiligada and Igor Stojanov

9:00 -9:30 Key note lecture 3

Fipronil in eggs, hens and in laying hen facilities
Guillaume Counotte and Ruth Bouwstra

9:30-10:50 Available and future control option: IPM strategies

Oral presentations (15 min each plus discussion)

A practical elaboration of integrated pest management for *Dermanyssus gallinae*; a farmer-science co-creation
Monique Mul, Alex Visch, Dirk Lagerweij, Linda van Lith

Mite monitoring can improve hen welfare
José Francisco Lima-Barbero, Ursula Höfle, Mariana Boadella, Xavier Manteca, Monique Mül, Deborah Temple

A model forecasting the *Dermanyssus gallinae* population in laying hen houses using monitoring data, housing temperature and dates of treatment.
Monique Mul, Johan van Riel, David George, Marcel Dicke, Bastiaan Meerburg, Johan Zoons, Lise Roy, Simon van Mourik, Peter Groot Koerkamp

The potential of an integrated PRM strategy in practice
Nathalie Sleeckx, Ine Kempen, Johan Zoons

10:50-11:20 Coffee break

SESSION 5

Chair: Robert Finn and Marta Demkowska-Kutrzepa

11:20-11:50 Key note lecture 4

Tropical and emerging diseases: new developments for tackling insecticide resistance
Mark Paine

11:50-12:30 Resistance of mites and associated pathogenic microorganisms

Oral presentations (15 min each plus discussion)

Preliminary results towards the molecular characterization of voltage gene sodium channel in *Dermanyssus gallinae* isolates
Marianna Marangi, Harry Wright, Alasdair Nisbet, Annunziata Giangaspero, Lise Roy, Kathryn Bartley

The bacterial flora of *Dermanyssus gallinae* and its antimicrobial resistance
Igor Stojanov[1], Danijela Horvatek Tomić, Ivan Pušić[1], Ivan Pavlović, Dalibor Todorović

12:30-13:40 Lunch

SESSION 6

Chair: Alasdair Nisbet and Marianna Marangi

13:40-14:40 Control: Protective antigens and immune responses to PRM

Oral presentations (15 min each plus discussion)

Understanding population genetic and antigenic diversity of the poultry red mite to improve prospects for successful vaccine development
Eleanor Karp-Tatham, Tatiana Küster, Alasdair Nisbet, Øivind Øines, Fiona Tomley, Damer Blake

Enhanced delivery of a prototype poultry red mite vaccine
Tatiana Küster, Dan Price, Alasdair Nisbet, Oivind Oines, Damer Blake, Fiona Tomley

Identification of gut antigens in *Dermanyssus gallinae*
James Pritchard, Tatiana Küster, Rob Noad, Dominic Kurian, Olivier Sparagano, Fiona Tomley

WG3 and WG4 individual sessions

14:40-15:50 WG3: Genetic structure in a changing world
Chair: Lise Roy and Øivind Øines

15:50-17:00 WG4: Epidemiology, pathology, geographical mapping and surveillance tools
Chair: Danijela Horvatek Tomić and Olivier Sparagano

17:00-17:15 Closing ceremony

17:15 - City tour

Friday September 22nd, 2017 *MC Members only*

Location: **National Institute for Agrarian and Veterinary Research (INIAV)-Oeiras**

9:00 -13:00	MC Members meeting (including a coffee break) Report from previous Training Schools (Israel and Greece) and London workshop Report from previous STSM activities Financial Report Membership expansion Plans for WBP4 (Year 4) Future dissemination activities
13:00-14:00	Lunch
14:00-15:00	Final remarks
15:00-	Farewell

ABSTRACTS

Lectures and Oral Presentations

AN OVERVIEW OF THE HISTORY, BIOLOGY AND CONTROL OF BEDBUGS: WHAT HAVE WE LEARNED THAT COULD BE APPLIED TO THE POULTRY RED MITE?

Richard A. Naylor

The Bed Bug Foundation, UK

At the turn of the century, and after more than 60 years of near absence, bedbug numbers began to increase in all major population centres of the World (Boase, 2001). This has primarily been attributed to pyrethroid resistance, although cheap foreign travel, better central heating, and lack of public awareness have all been implicated (Romero *et al*., 2007). The resurgence triggered a wave of new research into bedbug biology and control measures. I present an overview of bedbug feeding biology, with parallels to the Poultry Red Mite. I discuss my own research into bedbug ecology and dispersal, and its implications for control strategies. I then show the broad range of control measures now available for bedbug management and present data on current trends. Finally I discuss implications for the management of Poultry Red Mite.

References

Boase, C. 2001. Bedbugs - back from the brink. Pesticide Outlook, 12: 159–162.

Romero, A., Potter, M. and Haynes, K. 2007. Insecticide Resistance in the Bed Bug: A Factor in the Pest's Sudden Resurgence?, J. Med. Entomol. 44: 175–178.

INTERNATIONALISATION OF RESEARCH PUBLICATIONS ON DERMANYSSUS: MYTH OR REALITY?

Olivier Sparagano[1], Fiona Tomley[2], Robert Finn[3], Monique Mul[4], Lise Roy[5], Danijela Horvatek Tomić[6], Annunziata Giangaspero[7]

[1]Coventry University, UK. [2]The Royal Veterinary College, UK.[3]Northumbria University, UK. [4]Wageningen Livestock Research, The Netherlands.[5]UMR 5175 CEFE Montpellier France.[6]University of Zagreb, Croatia.[7]University of Foggia, Foggia, Italy

Since 1929 when Ainslie published the first paper on *Dermanyssus*, 406 papers have been recorded on Scopus (July 2017 census) with at least 47 countries publishing at least one paper.

Year	Publications	Co-authors	Countries	Top publishing countries (papers)
2016/2017*	24	109	17	Iran (6), UK (5), Germany (3)
2006/2007	29	76	12	France (7). Germany (5), UK (5)
1996/1997	14	35	8	Slovakia (4), Netherlands (2), USA (2)
1986/1987**	5	10	2	Czech republic (1), USA (1)
1976/1977***	2	3	2	Poland (1), USA (1)
1966/1967	4	4	1	USA (1)

*up to July 2017 ** no publications recorded in 1986 *** no publications recorded in 1977

In 2017 the top publishing scientists are all in Europe reflecting the importance of the *Dermanyssus* mite, with an average of 4.54 co-authors per paper. New countries have started to publish including Iran, India, Brazil, China and South Korea, showing how international this arthropod pest is considered by scientists, veterinarians and poultry producers. It is evident that the COREMI network (FA1404) has fostered collaborations between European Institutions that did not publish together before. Topics presented in recent publications show a predominance of research on control methods (chemical, biological, physical), mite physiology, proteomics, transcriptomics, vaccine development, prevalence and host species. It is also clear that much research is published in national journals that are not listed by Scopus or other databases.

There are still gaps in knowledge related to *Dermanyssus* in terms of individual and group behaviour, survival, reproduction and population genetic variation following natural conditions or treatment methods (Sparagano et al, 2014). However some recent publications describe key genomic/transcriptomics information, which would be valuable to identify new ways to control this important arthropod pest (Schicht et al, 2013, 2014; Hubert et al, 2017).

References

Ainslie CN (1929) Note on the occurrence of the mite, *Dermanyssus gallinae* in the nest of a house wren. The Canadian Entomologist 61, 39-40.

Hubert J *et al.* (2017). Comparison of microbiomes between red poultry mite populations (*Dermanyssus gallinae*): Predominance of *Bartonella*-like bacteria. Microb Ecology 1-14.

Schicht S *et al*. (2013). The predicted secretome and transmembranome of the poultry red mite *Dermanyssus gallinae*. Parasites & Vectors 6

Schicht S *et al.* (2014). Whole transcriptome analysis of the poultry red mite *Dermanyssus gallinae* (De Geer, 1778). Parasitology 141, 336-346

Sparagano OAE *et al* (2014). Biology, epidemiology, management and risk related to the poultry red mite, *Dermanyssus gallinae* (de Geer, 1778). Ann. Rev. Entomol. 59, 447-466.

A SURVEY ON *DERMANYSSUS GALLINAE* IN INTENSIVE POULTRY UNITS IN PORTUGAL

Helga Waap[1], Jacinto Gomes[1] , Telmo Nunes[2] and Paulo Leite[3]

[1]Instituto Nacional de Investigação Agrária e Veterinária, Portugal; Faculdade de Medicina Veterinária, Universidade de Lisboa[2]; [3]Zoetis, Portugal

Dermanyssus gallinae is endemic in many parts of the world, posing a worldwide economic problem. Despite the high prevalence rates reported in several European countries, epidemiological information on *D. gallinae* in Portugal is scarce (Pereira, 2011; Paulino, 2016). The aim of the present study was to obtain data on the prevalence, mite burdens and perceived importance of *D. gallinae* in industrial egg production systems in Portugal. A survey was performed between August 2016 and March 2017, which included 17 randomly selected laying farms in the NUTS 2 regions Centro and Norte. Traps made of corrugated cardboard were placed in cages at a ratio of 1:1000 birds for flocks up to 20000 birds plus an extra trap for each additional 5000 birds. The perceived importance of *D. gallinae* was assessed with a questionnaire. *D. gallinae* mites were found in 94% of the laying hen units sampled (95% CI: 73%-99%). A total of 440 traps were recovered, of which 91.6% contained mites. Considering the total sample, the mean number of mites per trap was 9093 ± 34208 and ranged between 1 and 81460 per farm. Linear regression analysis showed no significant relationship between the mean number of mites per trap and age of hens, flock density and room temperature. Eighty two per cent of the producers considered that *D. gallinae* was or had been a problem in their farms. The present data show that red mites are a problem in poultry farms in Portugal and underline the need for better control options to maintain poultry welfare and productivity.

References

Pereira D.M.C. 2011. *Dermanyssus gallinae* em galinhas poedeiras em bateria: carga parasitária, acção vectorial e ensaio de campo de um biopesticida. Master thesis. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária, Lisboa, 70 pp.

Paulino D. 2016. Detecção de *Dermanyssus gallinae* (De Geer, 1778) em aves exóticas de companhia no distrito de Setúbal, Portugal. Master thesis. Universidade Lusófona, Faculdade de Medicina Veterinária, Lisboa, 76 pp.

FARMER'S PERCEPTIONS ABOUT POULTRY RED MITE IN MACEDONIA – FINDINGS FROM THE COREMI QUESTIONNAIRE

Miroslav Radeski and Aleksandar Dodovski

Ss. Cyril and Methodius University in Skopje, Faculty of Veterinary Medicine in Skopje, Department of avian diseases and Department of animal hygiene and environmental protection, Macedonia

Poultry red mite (PRM) as the most important ectoparasite affecting laying hens is also one of the major challenges in Macedonian poultry industry. Yet, in Macedonia this problem was very little tackled in the past. The results from the preliminary investigation of the PRM presence in layer farms for the first time were presented at the second COREMI Conference in 2016. The presence of PRM, control practices and costs as well as farmer's awareness of the impact of this ectoparasite by using unified approach were not documented until now. This leads to absence of proper strategy and proposals of the best practices for control and eradication of this pest in laying hens. The aim of this study was to determine the farmer's attitudes and their perceptions considering PRM in laying farms in Macedonia. The National COREMI Questionnaire developed within the Working Group 1 of the Cost action FA1404 COREMI was used in the survey. The data collection and fulfilling of the questionnaire were performed by direct on-site communication with the farmers or managers from poultry farms with more than 1000 laying hens. In total, 29 poultry farms, out of 110 registered farms in the country, submitted their responses to the questionnaire. The farms included in this survey were not free-range, non-organic production systems with conventional battery cages for laying hens. The average number of laying hens per farm was 7460 (range, 600 – 29,000) and the average age of the oldest flock used as a representative of the farm was 61.57 weeks (range, 18 – 96 weeks). Regarding the presence of PRM in the farms, 25.00% and 27.59% of the farms responded that they currently have or had previously seen PRM in the farm, respectively. The presence of PRM in the farm is considered as a factor for drop in egg production by 48.28% of the farmers with the level of egg drop production being 20.31% (±15.78%). In total 25 different treatments against PRM were applied by the farmers. The most used chemical treatments in the poultry farms were Formaldehyde (35% solution) and Neopitroid® (*α-cypermethrin*) in 17.24% of the farms. The time of application of the control treatment practices varied according to the farm and the type of treatment, starting in empty building in some cases and up to 73 weeks old flocks. Regarding the time when farmers start with the treatment for PRM, the most frequent answers were "before mites are seen", "when mites are first seen" and "routine application regardless of the presence of PRM". The usage of PRM monitoring methods in the poultry farms is not a common practice: 86.21% of the farms don't use any kind of monitoring method. The average costs per flock for PRM control were 345 euros (range, 0 - 2000) and 20 labour hours (range, 4 – 80) were spent for PRM control of the oldest flock at the farm in the last month according to 41% of the farmers that responded to these questions in the survey. The number of responding farms and the involvement of the biggest poultry farms in the country in this study are the main reasons for considering the answers to the questionnaire as representative for the whole poultry sector in Macedonia. This type of survey was conducted for the first time in Macedonia and provides the baseline of the farmer's opinion about PRM presence, control and costs. However, further studies are needed in terms of monitoring, morphological and molecular identification, control and eradication of PRM and identification of best practices in order to manage this harmful silent problem in Macedonian poultry sector.

THE "OTHER" POULTRY MITE, *ORNITHONYSSUS SYLVIARUM*

Bradley A. Mullens, Amy C. Murillo, and Alec C. Gerry

University of California, Department of Entomology, Riverside, California USA 92521

The chicken red mite, *Dermanyssus gallinae*, has become a key pest of poultry in Europe due in part to changes in poultry housing triggered by welfare concerns. It is not a major pest in conventional cage housing in the USA, but that is likely to change (Mullens and Murillo, 2017). Before cages, *D. gallinae* was the most important USA ectoparasite in American poultry (Bishopp and Wood, 1917). Now *Ornithonyssus sylviarum*, the northern fowl mite (NFM) is the most damaging ectoparasite in American cage and non-cage systems. We will review some of our 20+ years of research on biology, economic impact and control of NFM in caged laying hen systems. It has not been well appreciated how similar red mite and NFM actually are. There are many aspects of NFM biology, impact and management that are quite relevant to red mite, and vice-versa. Both are blood-feeders and probably cause damage via the metabolic costs caused by the host immune response, causing reduced feed conversion efficiency and egg production. They have similar temperature preferences, life cycle lengths (at comparable temperature), broad host ranges, and survive well off-host. They respond similarly to pesticides, although those used against NFM must be applied to the host, rather than to the habitat, which entails a number of added concerns for host toxicity or pesticide residues.

The most intriguing questions related to the two mites are: 1) why is red mite not a worse problem in North America? 2) why has NFM not become a more severe pest in Europe (Roy *et al*., 2010)? The red mite, or closely related forms like the L1 lineage, probably will again become a key pest in North American non-cage systems which provide off-host habitat. The more difficult but interesting question relates to why NFM is not worse in European poultry. Here it is important to realize that the ancestral condition of NFM probably is as a nest parasite of wild birds, much like red mite. In 1917 NFM was not even listed as a pest of American poultry (Bishopp and Wood, 1917), but the revised 1931 review stated the new "feather mite" (NFM) had become a poultry pest "during the last few years". While historical evidence is scant and probably unattainable, it is notable that repeated efforts were made between 1850 and 1880, ultimately highly successful, to introduce *Sturnus vulgaris* and *Passer domesticus* from Europe into America. Repeated close proximity over about 50 years, especially between introduced *P. domesticus* and poultry, led us to hypothesize that a chicken-adapted form of NFM may have evolved that resides full-time on the host (Murillo and Mullens, 2017, submitted). We need surveys now to determine how widespread red mite or related parasites actually are in the full variety of American production systems, and molecular and host-parasite relationship studies to determine how amenable NFM are to host-switching.

References

Bishop F. C. and Wood H. P. (Ed.). 1917. Mites and lice on poultry. US Dept. Agric. Bull. 801: 1-26

Mullens B. A. and Murillo A. C. 2017. The Future of Poultry Pest Management. In Mench, J. (ed.). Advances in Poultry Welfare. Elsevier [in press]).

Murillo A. C. and Mullens B. A.. A review of the biology, ecology, and control of the northern fowl mite, *Ornithonyssus sylviarum* (Acari: Macronyssidae). Vet. Parasitol. (submitted).

Roy L., Chauve C. M. and Buronfosse T. 2010. Contrasted ecological repartition of the northern fowl mite *Ornithonyssus sylviarum* (Mesostigmata: Macronyssidae) and the chicken red mite *Dermanyssus gallinae* (Mesostigmata: Dermanyssidae). Acarologia 50: 207-219.

CONTRIBUTION TO THE GENERAL KNOWLEDGE OF *DERMANYSSUS GALLINAE* REPRODUCTION AFTER FEEDING

Ivan Pavlovic[1], Aleksandar Pavlicevic[2], JongUng Yoon[3] and Milica Dotlic[2]

[1]Scientific Veterinary Institute of Serbia, Belgrade, Serbia; [2]AVES MIT" LLC Cluster "Dermanyssus gallinae"Subotica -Bajmok,Serbia; [3]Biogenoci Co., Ltd., Seoul,South Korea

The reproductive potential of *Dermanyssus gallinae* determines the characteristics of the most important poultry ectoparasitosis - dermanyssosis, and it also conditions the effects of its control. We have tested the reproductive abilities of female adult *D. gallinae* after feeding. In lab conditions we separated fed female *D. gallinae* from insulation chambers where they had fed on a host, and placed them in petri dishes, at a density of 20 mites per petri dish. We repeated the test 42 times, thus performing it on 840 mites overall. We monitored the increase of reproductive elements and their development over the next 10 days at 23.6°C with 59.9% humidity. On average during the 10 days, 1.95 nymphs developed from each female mite. At this pace, *D. gallinae* infestation can triple its numbers in only 10 days. We suppose that reproductive potential is even greater in practice, because in the tests female mites were not able to take their next meal on time, nor were proto and deutonymphs, there was mortality and environmental conditions were not optimal for reproduction of red poultry mite. High reproductive potential (Tucci *et al.*, 2009), which is the result of egg production and short reproductive cycle (Chauve, 1998), especially emhasized in favourable environmental conditions (Maurer and Baumgartner, 1992; Nordenfors *et al*., 1999) necessitates highly efficient *D. gallinae* control. Otherwise there are consequences such as high intensity and extensity of flock infestation or frequent treatments of *D. gallinae* control.

References

Chauve C. 1998. The poultry red mite *Dermanyssus gallinae* (De Geer, 1778): current situation and future prospects for control. Vet. Parasitol., 79: 239–245.

Nordenfors H., Höglund J., Uggla A. 1999. Effects of temperature and humidity on oviposition, molting and longevity of *Dermanyssus gallinae* (Acari: Dermanyssidae*)*. J. Med. Entomol., 36: 68-72.

Maurer V., Baumgartner J. 1992. Temperature influence on life table statistics of the chicken mite *Dermanyssus gallinae* (Acari: Dermanyssidae). Exp. Appl. Acarol., 15: 27-40.

Tucci E.C., Prado A.P., Araújo R. 2009. Influência do jejum sobre a fecundidade de *Dermanyssus gallinae* (De Geer, 1778) (Acari: Dermanyssidae). Arq. Inst. Biol., 76: 23-26.

DEVELOPMENT OF A POULTRY RED MITE ON-HEN FEEDING DEVICE: A POTENTIAL TOOL FOR MITE CONTROL EVALUATION AND VECTORIAL STUDIES.

Kathryn Bartley, Frank Turnbull, Fran Nunn, Harry Wright and Alasdair Nisbet

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Poultry red mites (PRM) are small and highly mobile ectoparasites that usually reside 'off-host'. Consequently, it is difficult to contain PRMs in a controlled experimental environment, whilst permitting natural feeding on the host bird. Artificial or *in vitro* feeding techniques have been previously employed to overcome containment issues. These devices consist of a chamber containing the mites that is sealed at one end, with Parafilm or chicken skin stretched over the other end to serve as a feeding membrane through which mites can access a blood meal (e.g. McDevitt *et al*., 2006).
We refined the *in vitro* feeding technique for the preliminary screening of PRM vaccine candidates in small scale trials (Bartley *et al*., 2015). This strategy, while useful, has several major drawbacks: 1) the feeding membrane often fails resulting in drowned or escaped mites, 2) it requires invasive blood sampling of hens, 3) the blood meal is supplemented with anti-coagulants which may be toxic to the mites, 4) the devices lack natural feeding cues, thus high temperature and mite starvation are required to induce PRM feeding, and 5) a high variability in numbers of mites feeding and background mortality is observed. To overcome these issues a high level of technical and experimental replication is required. Our previous studies have shown that vaccine efficacy measured using the *in vitro* feeding device is not always translated into mite population reduction in field trials (Bartley *et al*., 2017). This is a major issue because it means that vaccines that appeared promising in the *in vitro* system when tested using hundreds of birds in a field trial, may fail to live up to their initial promise. To address these issues we have developed a prototype 'on-hen' *in vivo* mite feeding device to be used as an alternative to the *in vitro* feeding assays for adult mites for more accurate pre-screening of potential novel interventions before embarking on field studies.
The on-hen device consists of a sealed mesh pouch containing pre-starved, adult mites applied to the (plucked) skin of the hen's thigh and secured with medical tape and elasticated bandage. Adult mites can feed on the bird through a 250μm mesh and then recovered from the device, enumerated and maintained to monitor mortality and fecundity. Preliminary testing of the on-hen device resulted in a consistent feeding rate of 50% of adult mites following a 3 hour period where the device was applied to the hen. In addition, a low mite background mortality was observed. The device represents a high hen-welfare method of allowing mites to feed on the live host whilst maintaining PRM containment.
Further funding has been awarded from The National Centre for the Replacement, Refinement, Reduction of Animals in Research (NC3R^{s}) in order to further develop this prototype as a tool to allow feeding of nymph and adult life stages to evaluate systemic PRM controls (e.g. vaccines, systemic acaricides) and potentially for use in PRM vectored disease studies.

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CHARACTERIZATION OF MITE COMMUNITIES IN WILD BIRD NESTS FROM SOUTH-CENTRAL SPAIN

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Biological control is an increasingly used tool for pest containment. Because of the limitations in chemical control against the Poultry Red Mite (PRM, *Dermanyssus gallinae*), using predatory mites could be a promising alternative (Lesna *et al*., 2009). In order to identify indigenous predatory mites, we decided to characterize the communities of mites living in nests of different wild bird species including 12 white stork nests (*Ciconia ciconia*), 2 cinereous vulture nests (*Aegypius monachus*) and 2 northern-bald ibis nests (*Geronticus eremita*).

Mites were extracted from the bird nest material from Spain with Berlese funnels. For each nest, mites were sorted to isomorphic species groups by stereomicroscopic techniques. Five specimens per isomorph and per nest were imaged and placed one by one in a 96 well plate, designated for non-destructive DNA sequencing (Porco *et al*., 2010). DNA Barcode analysis was performed at the Canadian Centre for DNA Barcoding. Mite exoskeletons were returned for further morphological identification at University of Pamplona.

Higher amounts of mites were obtained from white stork and cinereous vulture nests, while northern-bald ibis nests provided very few mites. One possible cause may be that the humidity of the material in these nests was much lower. Also bald ibis nests were close to the coast (Mediterranean) while white stork and cinereous vulture nests were sampled in the central area of the Iberian peninsula under a more continental climate. Barcoding success rate was 38%. Mites which were not identified by molecular techniques were identified to genus or species by morphological characters. Finally, 25 mite species belonging to 8 taxonomic families were identified, including Macrochelidae, Digamasellidae, Parasitidae, Cheyletidae, Dermanyssidae, Tetranychidae, Trematuridae and Urodynichidae. The identified species included prey and predatory mites as well as parasitic species. Three Dermanyssidae species have been found in three different stork nests. Potential predatory mites for RPM identified in my STSM included species belonging to the families Cheyletidae, Parasitidae and Digamasellidae.

This study constitutes the first comprehensive taxonomic report on mite communities of white stork, cinereous vulture and northern bald ibis nests in Spain.

This work was a joint effort of several research groups collaborating within the framework of the COST Action “COREMI”.

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AN INTEGRATIVE PILOT STUDY TO ASSESS THE FEASIBILITY OF CONSERVATION BIOLOGICAL CONTROL OF THE POULTRY RED MITE IN BARN LAYER FARMS

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Conservation biological control (CBC) relies on naturally occurring ecological processes and aims to enhance these thanks to local adjustements that benefit natural populations of pest enemies. Because the hematophagous poultry red mite (PRM) lives off-host in habitats potentially shared by many micropredators, naturally occurring biocontrol agents have potential to regulate this pest in poultry buildings. Lesna *et al.* (2012) have shown in lab trials that at least one mesostigmatid mite species present in poultry farms was able to feed on PRM. Whole communities of predatory arthropods dwelling in poultry farms are poorly known. Extensive characterizations (based on systematic sampling) of poultry-associated mite communities were provided by Brady (1970) and by Horn *et al.*, (2016) in contexts different from current European layer farms. The present pilot study was conducted using a multidisciplinary approach to obtain a first insight into natural arthropod communities of barn layer farms in France and estimate how feasible CBC was according to regional and local factors. A morpho-molecular (mt 16S and nuclear 18S RNA) exploration resulted in a validated method for rapid biodiversity assessment of manure-dwelling mesostigmatid mite communities. Species composition of arthropod communities was determined from 20 different farm buildings. The feasibility of CBC was estimated by testing a series of hypotheses based on the 2-scale framework proposed by Begg *et al.* (2017): regional-scale factors were considered related to the source of natural pest enemies and local-scale factors to the ability of farming units to allow their local establishment. Additionally, the sensitivity of some dominant mite species to a common insecticide (deltamethrin) was measured via bioassays. The putative roles of recorded taxa within ecological guilds and their dispersal habits are discussed based on the results of multivariate and univariate analyses. Local and regional factors had significant effects on both high-level taxonomic arthropod groups and mesostigmatid morphospecies. In addition to documenting the occurrence and apparent establishment of highly mobile arthropods in hen houses, these results are considered promising for the development of CBC against PRM. Populations of two recurrent mesostigmatid morphospecies demonstrated an extreme sensitivity to the pyrethroid, suggesting that even very low levels of insecticide use are likely to have unnoticed deleterious effects on natural regulation processes in layer buildings.

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EFFICACY OF A NOVEL NEEM OIL FORMULATION (RP03™) TO CONTROL THE POULTRY RED MITE *DERMANYSSUS GALLINAE*

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The poultry red mite (PRM) *Dermanyssus gallinae* is of major concern for the poultry industry. Several chemicals are effective against PRM, but acaricide resistance, the limited number of active ingredients, and the risk of residues create a demand for alternative products, such as plant-derived acaricides.

We investigated the efficacy of neem oil against *D. gallinae* on a commercial laying egg farm with a high infestation level. The farm building was arranged in four blocks of cages, each consisting of two adjacent lines arranged over four tiers. A novel formulation of 20% neem oil dilution from a 2,400 ppm azadirachtin-concentrated stock (RP03™) was administered by nebulization three times, at three day intervals.

Using corrugated cardboard traps, mite density was monitored before, during and after treatment. Following trap removal, mites were frozen and their amount was estimated as total weight. The results were analyzed through multi-factorial ANOVA with trap position and time as criteria predictors. Mite populations in the treated block showed a 94.65%, 99.64% and 99.80% reduction after the first, second and third administration, respectively.

A reduction in mite population was observed also in buffer (59.93%, 75.68% and 83.68%) and control blocks (63.24%, 80.02% and 82.27%). Trap position was the most significant variable according to the analysis run, as well as the interactive term 'time/trap position'. Trap position showed a mean mite log-reduction of ca. 2.2-2.4 for the treated block, while in the control and buffer areas the mean reduction was 0.8 and 1.3, respectively.

The reduction rate of the mite population was significantly higher for treated block ($p<0.001$) compared to the buffer and control blocks. Nevertheless, it was also possible to observe a reduction in the population of the latter two blocks. It is postulated that forced ventilation may have spread the product, affecting mite density in the buffer and control block.

This result was independent from the effect of time and it suggests strong bioactivity of neem, and more specifically the patented neem-based RP03™, against *D. gallinae*. The treatment was most effective in the 10 days following the first application, and its effects persisted for over two months.

Further studies will aim to reduce the treatment schedule and neem concentration to overcome unwanted effects of treatment recorded on equipment and eggs, as related to the oily consistence and smell of the product.

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COMPARATIVE *IN VITRO* SUSCEPTIBILITY OF *DERMANYSSUS GALLINAE* FIELD ISOLATES TO FLURALANER, PHOXIM, SPINOSAD, DELTAMETHRIN AND PROPOXUR

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The poultry red mite (PRM), *Dermanyssus gallinae*, is the most damaging ectoparasite of laying hens and breeders, particularly in Europe, where it poses serious animal health, welfare and public health concerns. It also affects the productivity of the egg industry. The control of PRM infestations typically relies on the use of sprayed synthetic acaricides. However successful treatment is hampered by resistance development due to repeated use and misuse of existing acaricides and to uneven spraying especially inside crevices and cracks where mites hide most of the time.

Fluralaner is a new compound of the novel isoxazoline class, recently approved in the European Union as an oral solution for drinking water administration, for use in chickens (Exzolt™, MSD Animal Health). The present study was conducted to compare its activity against *D. gallinae* with that of other acaricides commonly used against mite infestations.

The susceptibility of *D. gallinae* to common acaricide compounds was evaluated on 12 recent field isolates collected in naturally infested commercial chicken houses in France, Germany and Spain, in 2014 and 2015. The 12 houses from layer, breeder or replacement farms had not received acaricidal treatments in the last 2 months preceding sampling. A MSD laboratory isolate originally collected from a chicken farm in 2001 was also tested. Susceptibility of mites to fluralaner, phoxim, spinosad, deltamethrin and propoxur was determined at the same laboratory using the larval package test (LPT), a contact test recommended by the FAO. Test papers were oily impregnated with the tested acaricide at different concentrations. Around 100 mites (nymphs and adults) were introduced in the impregnated and folded papers. Dead, moribund and living mites were counted under a stereomicroscope after 48 hours of exposure. The mortality rate was calculated using the Abbott's formula and the Lethal Concentration 90% (LC_{90}) was estimated.

Fluralaner demonstrated consistently a very high activity against *D. gallinae* (LC_{90} < 15.63 ppm for 9 field isolates, and ≤ 31.25 ppm for 2 field and the laboratory isolates, 1 isolate not tested). In contrast, LC_{90} values exceeded 1000 ppm for most of the isolates tested with the 4 other acaricides. LC_{90s} ranged from <1000 to >4000 ppm for phoxim (11 field isolates tested) and was < 500 ppm for the laboratory isolate. The values ranged from <1000 to >4000 ppm for spinosad (11 field isolates and the laboratory isolate). Deltamethrin exhibited LC_{90s} >250 ppm (1 field isolate), >1000 ppm (2 field isolates) and 500-1000 ppm for the laboratory isolate. Two field isolates showed a LC_{90} >1000 ppm for propoxur while it was < 125 ppm for the laboratory isolate.

For 6 isolates, the LC_{90} values of phoxim exceeded the recommended concentration (2000 ppm, Byemite®, Bayer), suggesting that these isolates had acquired resistance to this compound. One isolate showed an LC_{90} value of spinosad close to the highest recommended treatment dose (4114 ppm, Elector®, Elanco) suggesting that a part of the mites from these isolates would not be killed by a treatment with spinosad under field conditions. Resistance of *D. gallinae* to carbamates and pyrethroids has been previously reported in Europe and is further confirmed by the LC_{90} values obtained in this study.

This contact study demonstrated the very high and consistent *in vitro* activity of fluralaner against *D. gallinae* isolates collected under field conditions in Europe.

FIELD SAFETY AND EFFICACY OF FLURALANER IN DRINKING WATER FOR THE TREATMENT OF POULTRY RED MITE (*DERMANYSSUS GALLINAE*) INFESTATIONS IN COMMERCIAL FLOCKS IN EUROPE

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Welfare concerns, production losses caused by *Dermanyssus gallinae*, the poultry red mite (PRM), and widespread mite resistance to environmentally applied acaricides continue to drive an urgent need for new and effective control measures.

Fluralaner is a novel systemic acaricide developed to address that need. A series of field studies was initiated to investigate the safety and efficacy of Exzolt™ (10 mg/mL fluralaner solution, MSD Animal Health) when used in drinking water for the treatment of natural PRM infestations in chickens at a dose rate of 0.5 mg/kg administered twice, seven days apart.

Four negative-controlled and blinded studies were completed in Europe across eight layer, two breeder, and two replacement chicken farms. Chickens were housed in enriched cages (5 farms), aviaries / barns (5 farms) or in a free-range system. At each farm, two similar flocks were enrolled in similar PRM-infested units (either rooms within a building, or separate buildings) varying from 550 to 100,000 birds per unit. One unit at each farm was allocated to Exzolt™ treatment, administered in drinking water on Days 0 and 7. One unit remained untreated. Eight to 24 mite traps were placed throughout each unit on Days -1, 0 or 1, 3, 6, 9, and 13 or 14, then at weekly or two-weekly intervals, retrieved after 24 hours and processed for mite stage differentiation and counting at the same laboratory. Efficacy at each farm was assessed by mean PRM count reductions from traps in treated units compared with control units using the Henderson-Tilton formula. Production parameters and safety were also monitored.

Exzolt™ efficacy was 95.3 to 99.8% on Day 3 and 97.8 to 100% on Day 9. Thereafter efficacy reached 99 to 100% in all farms and remained above 90% for 56 to 238 days after treatment initiation. Post-treatment improvement in laying rate was greater by 0.9 to 14.6% in the treated group at 9 of the 10 layer or breeder farms. There were no treatment-related adverse events. Exzolt™ administered via drinking water at 0.5 mg/kg twice, 7 days apart, was well tolerated and highly effective against the PRM in naturally infested chickens representing a range of production types and management systems. The results indicate that this novel treatment has potential to be the cornerstone of an integrated approach to reducing or eliminating the welfare and productivity costs of this increasingly threatening pest.

INTRODUCTION OF EXZOLT (FLURALANER 10 MG/ML SOLUTION)—A NEW PRODUCT FOR TREATMENT OF POULTRY RED MITE INFESTATION IN CHICKENS

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Exzolt, 10 mg/ml fluralaner solution, is the first product with a European Veterinary Medicinal Product license approval for poultry red mites administered by drinking water, making it easy and safe to use by farmers. Fluralaner belongs to a new class of ectoparasiticide called isoxazoline. Since 2014, it has demonstrated a high degree of effectiveness in companion animals for flea and tick control. More recently, it has demonstrated a high level of efficacy for Poultry Red Mite control in chickens. Fluralaner has both acaricidal and insecticidal activity. It demonstrates a dual mode of action, acting as an antagonist of the GABA receptor and blocking L-glutamate-gated chloride channels. After oral administration of the product at 0.5 mg/kg bodyweight (2 times, 7 days apart), fluralaner is quickly absorbed and remains in the plasma for at least 15 days, which corresponds to 2 mite life cycles. This duration is sufficient to break the mite life cycle in infested buildings and reduce the mite population by over 99% after treatment. The treatment is very well tolerated in layer and breeder chickens. In addition, the product has a 0 day withdrawal for eggs which ensures safety for consumers. Following treatment, it is important to follow proper biosecurity measures to prevent reinfestation. Continuous monitoring of infestation levels after treatment is critical to control the risk of reintroduction of poultry red mites in laying farms. This new product has the opportunity to help fulfil an unmet need for the control of poultry red mites in chickens.

FIPRONIL IN EGGS, HENS AND IN LAYING HEN FACILITIES

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Fipronil is an insecticide for treatment of seed and young plants (Mundial, BASF). But fipronil is also a veterinary medicine for dogs and cats (Frontline, Merial). The lethal concentrations (LC50) of FIP, fipronil-desulfinyl and fipronil-sulfone for are respectively 83, 20 and 25 µg/L suggesting that FIP metabolites are much more toxic than the precursor. It interferes with the function of gamma-aminobutyric acid (GABA)-gated channels; fipronil disrupts normal neuronal influx (e.g., passage of chloride ions) by targeting the GABA-gated chloride channel and, at sufficient doses, causes excessive neural excitation, severe paralysis, and death.
Its use is not allowed in animals used for production of meat or animals that produce edible products (milk, eggs). Recently is was shown to be illegally used as treatment against poultry red mite in egg production facilities.
Fipronil is a very lipophilic compound. The pK-OW (logarithm of the octanol:water distribution of a substance) is 4: that means it is 10.000 times better soluble in fat than in water.
Fipronil is highly toxic to sea and freshwater fish and also highly toxic to sea and freshwater invertebrates. Two fipronil metabolites are even more toxic than fipronil itself. For humans, toxicity is moderate. The first signs of intoxication in humans are vomiting, agitation, seizures and normally has a favourable outcome with no signs of genotoxicity. The lowest published lethal dose is 1.43 mL/kg with signs of convulsions or effect on seizure threshold, coma and sweating. The ADI (acceptable daily intake) for humans is 0.0002 mg/kg BW / day. The ARfD (acute reference dose) is 0.009 mg/kg BW / day and the AOEL (acceptable operator exposure levels) is 0.0035 mg/kg BW / day. The highest dose in egg found in the Netherlands until now is about 1.1 mg/kg. Assuming an egg of 55 gram, this means that one egg contains 1.1 * 0,055 = 0.0605 mg fipronil. Assume a person of 60 kg, the ARfD is 0,540 mg per day. This means that at least 9 eggs must consumed per day before the first signs of intoxication will be visible. The MRL (maximum residue limit) in egg is 0,005 mg/kg. The median level of fipronil in eggs of poultry housed in treated stables, is about 0,20 mg/kg. At that level, one must consume 150 eggs per day before the first intoxication symptoms will be visible.
The degradation of fipronil and formation of metabolites in animals will be discussed. Halftime of fipronil in egg laying chickens (8 days) or non-egg laying chickens (40 days) is used in a kinetic model to calculate variation of fipronil in time.
Chemicals tested on the efficacy of the breakdown of fipronil and its metabolites in vitro and in walls of stables of hen facilities will also be discussed.

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A PRACTICAL ELABORATION OF INTEGRATED PEST MANAGEMENT FOR *DERMANYSSUS GALLINAE*; A FARMER-SCIENCE CO-CREATION

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Pests and diseases in the agricultural sector lead to a reduction of available food and feed throughout the world (FAO, 2001). In order to continue to meet the increasing demand for food and feed, losses must be reduced, preferably in a sustainable manner.
Integrated Pest Management (IPM) is a sustainable method of limiting economic losses due to pests and diseases (Kogan, 1998). It is based on eight steps: 1. Prevention and suppression of the population, 2. Monitoring, 3. Decisions to apply treatments based on monitoring and a threshold value, 4. Use of non-chemical methods, 5. Use of selective chemical agents specific for target agents, 6. Reduce use of chemical agents (e.g. reduced frequency, partial application) 7. Apply anti-resistance strategies, and 8. Evaluation. By carrying out these steps it is possible to prevent and control outbreaks where synthetic and wide-spectrum pesticides are used only if other means are generating limited results. This may reduce the number of problems regarding pesticide residues and the development of drug resistance (Barzman *et al*., 2015). At present, IPM is mainly applied successfully against pests and diseases in crop production. In the livestock industry, IPM is also applied, though less so than in crop production. Despite the fact that the benefits of IPM have been described for poultry diseases and the vectors of poultry diseases, this methodology is only rarely used on layers to control of pests and diseases (Harrington *et al*., 2011).
A pest in laying-hen farming that is difficult to control at present is *Dermanyssus gallinae* (Poultry Red Mite). This is a blood-sucking mite with an almost worldwide distribution and a negative impact on animal health, animal welfare and production parameters. The number of IPM measures currently applied to *D. gallinae* is limited to cleaning between rounds, a number of preventive measures and the use of chemical agents or products that physically affect the mites. Improved IPM programs for *D. gallinae* will lead to a better control of this pest in laying-hen farms.
Together, three egg producers and a research scientist specialised on *D. gallinae*, elaborated on the eight steps of IPM for *D. gallinae*. They discussed all steps for feasibility and practicality. The result is a document describing the possibilities to carry out IPM for *D. gallinae*, providing egg producers with a tool for improved control of *D. gallinae* and thus limiting the problems of mite resistance and pesticide residues. The document is published on henhub.eu.

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MITE MONITORING CAN IMPROVE HEN WELFARE

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The Poultry Red Mite (*Dermanyssus gallinae*) is the most relevant ectoparasite in layer farms, and highly prevalent in European farms. This situation is a serious challenge for the poultry industry as the biology of the Poultry Red Mite (PRM) and the lack of effective tools make its control complicated. Depending on the level of infestation, PRM causes layer hens to suffer long-term stress due to sleep deprivation because of night disturbance, feather pecking, anaemia and increased mortality (Kilpinen, *et al.* 2005).

In order to assess the effects of the level of PRM infestation on layer welfare, a minimum of 10 traps were placed in seven layer houses (six intensive productions and one organic). Traps were checked weekly during 12 weeks and scored for degree of PRM infestation (Van Emous and Ten Napel, 2007). In five houses the quality of the plumage of layers was evaluated and signs of feather loss and feather pecking sored at the beginning and the end of the study. Weekly scores of productive parameters (egg production, mortality and mite treatment) were obtained from the owners for each layer house.

In four farms negative correlation (Spearman´s correlation, $p<0.05$) between infestation level and egg production and a positive correlation with mortality (Spearman´s correlation, $p<0.05$) were observed. Increased feather damage was present in every studied flock (t-Test, $p=0.002$, $t=-7.332$). Treatment for control of PRM usually took place when the infestation score was above 3.5.

In a 0 to 4 scale for infestation level, a score of 3.5 is related to heavy infestation. At this infestation level, treatments have been found to be of little impact and the mite population easily recovers because of a big stock of survivor mites. Increased feather damage and mortality and reduced productive rates can be determined by many factors but it is clear that PRM has a significant impact on layers. These effects are explained both by increased stress and negative physical effects (anemia) in the layer hens. We suggest that early treatments, at moderate infestation levels, can keep mite population low enough to significantly improve layer welfare.

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A MODEL FORECASTING THE *DERMANYSSUS GALLINAE* POPULATION IN LAYING HEN HOUSES USING MONITORING DATA, HOUSING TEMPERATURE AND DATES OF TREATMENT.

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Dermanyssus gallinae is a pest in egg production causing significant farm economic losses and reduced hen welfare and health. Currently, this pest is difficult to control. Integrated Pest Management (IPM), however, could improve the control of *D. gallinae* in a sustainable way (Sparagano *et al,* 2014). For implementation of IPM by layer farmers, an easy applicable population forecasting model is required (Peshin *et al.,* 2009). We developed an adaptive population dynamics model enabling to forecast the *D. gallinae*'s population dynamics in laying hen facilities pre – and post treatment and to determine and forecast the treatment effect requiring only monitoring data, housing temperature and dates of application of a treatment. We demonstrated the forecasting quality of the population model using three different monitoring methods; de MMS method (Cox *et al.,* 2009, the Simplified Passive tape Trap method (Roy *et al.,* 2014) and the Semi Attractive water Trap (Chiron *et al,* 2014).

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THE POTENTIAL OF AN INTEGRATED PRM STRATEGY IN PRACTICE

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The poultry red mite (PRM), *Dermanyssus gallinae,* is an important cause of welfare and health problems in laying hens, yet the control remains a challenge (Marangi *et al*., 2009; Sparagano *et al*., 2009). Combination of treatments in an integrated pest management (IPM) approach shows promising results. In this study, the effect of a combination of predatory mites, locally applied acaricides and a plant-based supplement was investigated under practical conditions.

In the present study, 31,000 hens were housed in 12 climate and light independent compartments equipped with enriched cages, 1 row aviary or 2 rows aviary. In each housing type 4 combination treatments were tested: 1) predatory mites + amitraz, 2) predatory mites + amitraz + plant supplement, 3) predatory mites + milbemectin, 4) predatory mites + milbemectin + plant supplement. Two species of predatory mites were used: *Androlaelaps casalis*, which was released in cultivating bottles attached to the housing system and *Cheyletus eruditus*, which was spread by hand throughout the housing system. The acaricides (amitraz or milbemectin) were applied locally through different types of traps, while the plant-based supplement was added to the drinking water of the hens. Both a visual score (Mite Monitoring System, MMS) and a counting of PRM in cardboard traps were performed to maintain an overview of the temporal evolution of its population. In addition, the predatory mite populations were monitored weekly.

Results show that the decrease of PRM over time was significantly higher for the triple treatment in the enriched cages. The same trend was seen in the aviaries although this was not significant. When treatment was stopped, a rapid increase of the PRM counts and scores was seen. However, even for the triple treatment, the effect was not sufficient to control the PRM infestation in the different housing types.

In conclusion, the combination treatment of predatory mites, locally applied acaricides and plant-based supplements shows promising results but further research and refinement is needed in order to make the methods applicable in large practical conditions.

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TROPICAL AND EMERGING DISEASES: NEW DEVELOPMENTS FOR TACKLING INSECTICIDE RESISTANCE

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A great many tropical and emerging diseases are transmitted by insects, including malaria, dengue, zika and lymphatic filariasis (mosquitoes), cutaneous and visceral leishmaniasis (sand flies), sleeping sickness (tsetse flies) and many more. Insecticides remain the most effective means of controlling vector borne diseases including malaria, where the extensive deployment of long lasting insecticide-treated bed nets (LLINs) have helped reduce malaria cases in Africa by half since 2000. However, only pyrethroids may be used for LLINs, which has led to a dramatic spread of pyrethroid resistance in malaria transmitting mosquitoes across the continent. The limited numbers of insecticides available and the speed at which insecticide resistance can take hold leads to fundamental questions about mechanisms of resistance, impact on vector control and ways to overcome insecticide resistance. Judicious use of insecticides is also critical for effective control. However, the quality of insecticidal applications is hampered by a lack of suitable field assays. In the past decade, great strides have been made in identifying genes associated with insecticide resistance in tropical disease vectors and in the development of new technology for monitoring and predicting resistance. This presentation explores recent developments and their potential impact on vector control.

PRELIMINARY RESULTS TOWARDS THE MOLECULAR CHARACTERIZATION OF VOLTAGE GENE SODIUM CHANNEL IN DERMANYSSUS GALLINAE ISOLATES

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In the last years, acaricide resistance in populations of Dermanyssus gallinae has been recurrently suspected, especially against some pyrethroids (Marangi et al., 2009). As already occurred in mite species Tetranychus urticae, such acaricide resistance is due to the nucleotides sequence variations of the Voltage Gene Sodium Channel (VGSC), gene coding the pyrethroid's target protein (Ilias et al., 2017). The molecular characterization of the VGSC gene and the possible polymorphisms involved in such resistance remain still unexplored in D. gallinae. Preliminary characterization of the D. gallinae VGSC gene (Deg-VGSC) sequence indicated that it is very different to a closely related mite species available in the GenBank (Varroa destructor) (Marangi et al., 2015).
In order to obtain the full length sequence of the Deg-VGSC gene, Metaseilus occidentalis, a mite species closely related to D. gallinae, has been used as reference sequence in order to design several primer pairs able to perform RT-PCR reactions for long fragments using the PolyA-RNA purified from Scottish isolates and total RNA purified from Italian isolates as templates.
The RT-PCR reactions provided PCR fragments from Scottish and Italian isolates corresponding to the start (about 1300 bp) and to the end (about 500 bp) of the Deg-VGSC gene, confirmed by sequencing.
To date, the start and the end of the Deg-VGSC coding gene sequence have been obtained for the first time. Subsequent experiments are in progress aimed at obtaining the rest of the whole VGSC coding gene sequence using new primers pair and high-quality PCR enzymes to get long fragments. Once the whole Deg-VGSC gene sequence will be obtain, the next step will be to screen VGSC sequences from populations of D. gallinae having various levels of sensitivity to pyrethroids in order to identify VGSC mutation(s) associated with pyrethroids resistance.

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THE BACTERIAL FLORA OF *DERMANYSSUS GALLINAE* AND ITS ANTIMICROBIAL RESISTANCE

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Microbiological examination of red mites present in poultry production facilities can have two aspects. One encompasses the ability to control the presence of pathogenic microorganisms that threaten the health of livestock and humans. A possible transfer of red mites through accessories, equipment, cardboard packaging and sale of poultry, mites become vectors by which the causative agents of the disease can spread. On the other hand, mites can be carriers of a non-pathogenic or opportunistic pathogenic bacterial flora that carries the resistance genes and, in the aforementioned manner, transfer them out of the production facilities. Administration of antimicrobial drugs for prophylactic purposes, as growth promoters or during therapy, leads to the formation of resistant strains. By excretion of these strains, they reach the ambient of a poultry house in which red mites are found. In the event that red mites come into contact with animal excrements, they become vectors that transmit the genes of resistance. In this investigation, hen feces and red mites were examined in order to determine whether the same antibiotic-resistant bacteria species can be found in two observed materials and to determine the antimicrobial sensitivity of isolated bacteria. The study showed that the same bacterial species (*E. coli, Enterobacter* sp.) can be found in both samples, feces and mites. We detected the presence of the same susceptibility of *Enterobacter* strains isolated from feces and mites. The study showed that resistance genes created using antibiotics in poultry production, hypothetical, can be transferred by mites, in and outside the facility.

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UNDERSTANDING POPULATION GENETIC AND ANTIGENIC DIVERSITY OF THE POULTRY RED MITE TO IMPROVE PROSPECTS FOR SUCCESSFUL VACCINE DEVELOPMENT

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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. However, *D. gallinae* spends the majority of its lifecycle hidden in crevices and cracks, only leaving to feed for short periods of time, typically during the night. This limited time spent on the host represents one of the biggest challenges faced in the control of *D. gallinae* by vaccination.

One major consideration for the development of a vaccine to protect chickens against poultry red mite is the extent and rate of occurrence of genetic diversity within mite populations. This project aims to gain an understanding of *D. gallinae* population structure and antigenic diversity relating to development of, and likely response to, subunit or recombinant vaccines. Genetic diversity will be studied on both an inter- and intra-farm level. Based on published work, five vaccine candidates shown to be capable of inducing mite mortality have been selected for multi-locus sequence typing (MLST; vitellogenin, hemelipoglycoprotein, paramyosin, tropomyosin and cathepsin D1), supplementing a single nucleotide polymorphism (SNP) based study of genome-wide diversity.

ENHANCED DELIVERY OF A PROTOTYPE POULTRY RED MITE VACCINE

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Dermanyssus gallinae is the most important ectoparasite affecting egg-laying chickens. Infested birds may suffer from anaemia, dermatitis, weight loss and decreased egg production. The scarcity of effective pesticides has contributed to a significant problem for the layer industry. Commercially available acaricides are not effective in the control of poultry red mite infestations due in part to increased parasite resistance. Additionally, acaricide use is gradually being discontinued as a consequence of public awareness and legislation against chemical residues on food products, and chemical release and accumulation in the environment. The development of an effective vaccine can decrease the occurrence and impact of *D. gallinae*, thereby improving the general health and welfare of layers without the use of acaricides.

Currently, prototype vaccines against *D. gallinae* have been delivered intramuscularly using adjuvants designed to produce high circulating IgG against the co-delivered antigen in mammals. Longevity of protection is particularly salient in the egg-laying sector where protection would need to be effective throughout a full laying cycle (ca. 1 year) following vaccination of immature birds.

Transfection vectors have been developed for genetic complementation of *Eimeria tenella*, prompting the notion that live-attenuated coccidial parasites could be used as effective vectors for the oral delivery of heterologous vaccine antigens to poultry. In cooperation with two PARAGONE partners - Norwegian Veterinary Institute and Moredun Research Institute - we propose to compare the delivery of a defined prototype *D. gallinae* antigen (cathepsin D1) in three systems, namely DNA vaccination, recombinant protein formulation in montanide and cytosolic, secreted, or membrane-tethered antigen expressed by the *Eimeria* vector. The immune responses, efficacy, and endurance of the effect of vaccination are being currently investigated.

IDENTIFICATION OF GUT ANTIGENS IN *DERMANYSSUS GALLINAE*

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The infestation of laying hens by *Dermanyssus gallinae*, is the biggest health and welfare issue in this sector of the poultry industry, with significant negative impact on efficiency and profitability. Chemical acaricides are not completely effective against these mites due to high levels of drug resistance and there is an urgent need for new methods of control including the development of effective vaccines. Several researchers have already shown that this is a feasible but difficult prospect (eg Harrington *et al.*, 2009; Wright *et al.*, 2009; Bartley *et al.*, 2015; Makert *et al.*, 2016).
We aim to generate tools and methods to identify mite proteins that could potentially be used in a vaccine. Because female *D. gallinae* are obligate blood-feeders we specifically target mite gut antigens, an approach used successfully to develop commercial vaccines against the cattle tick *Rhipicephalus microplus* (TickGARD®) and ruminant gastrointestinal nematode *Haemonchus contortus* (Barbervax®). We use three main criteria for selection 1) expression in the gut of feeding mites so that antibodies in the blood of vaccinated hens can target multiple stages; 2) membrane-bound on the surface, or secreted from mite gut cells, to maximise the chance of exposure to antibodies in the blood meal; 3) evidence of immunogenicity through ability to bind.
Because of their small size, it is extremely difficult to dissect the intestines of *D. gallinae* mites so starting material for this work was whole mites collected from UK farms. After mechanical disruption and homogenisation, several methods were used to generate protein mixtures enriched for integral and peripheral membrane proteins. These were analysed by 2D liquid chromatography mass spectrometry, and peptide sequences used to examine *D. gallinae* transcripts (Schicht et al 2013) resulting in the identification of ~1200 potential proteins. To identify immunogenic peptides and generate monoclonal antibodies, the 'enriched' fraction was used to bio-pan a synthetic phage library expressing immunoglobulin single-chain variable (VH+VL) fragments (scFv). After three rounds of selection 760 phage clones were amplified and screened by ELISA fraction, with 62 showing >3-fold binding to mite protein, compared to non-mite protein. These 62 high binders were tested for their reactivity with sectioned mite tissue by immunohistochemistry, with 19 found to bind specifically to the mite gut. Current and future work aims to 1) identify and characterise the protein targets of the 19 monoclonal scFv; 2) validate their localisation within the mite gut; and 3) test their efficacy as vaccine antigens.

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ABSTRACTS

Posters

BIOLOGICAL CONTROL OF THE CHICKEN RED MITE IN LAYER FARMS IN EUROPE: STATE OF THE ART AND PERSPECTIVES

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Almost every type of commercial agricultural production has pest organisms that thrive on it. Conventionally, these pests are treated with a chemical pesticide. Use of natural enemies to control pest populations is called biological control. This method is known since decades in crop protection and particularly in protected environment such as greenhouses. Control and reduction of pests can be further improved by using a combination of tools and methods; this is called Integrated Pest Management (IPM). Chicken red mite infestations are causing serious problems on almost every commercial egg production farm. Several species of natural enemies have been studied for their ability to control the red mite and a few of those are now commercially available. Among those are *Hypoaspis* spp., *Androlaelaps casalis and Cheyletus eruditus* (Lesna *et al*., 2009). Due to the biology and ethology of the red mite, an IPM strategy is likely to offer a promising solution for layer farmers. APPI has developed a unique strategy by combining the predatory mites TAURRUS (*C. eruditus)* and ANDROLIS (*A. calasis)* in one releasing protocol. Predators are released in batches between 3 to 6 times per flock cycle in specific locations using the abiotic preferences of the two species. This method is suitable for parent stock flocks, free range and aviary farms and is showing significant results on more than 200 farms in three European countries so far. The level of control over the red mite is likely to further increase with tactical combinations of biological control and other treatments.

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CONTROL OF RED POULTRY MITE (*DERMANYSSUS GALLINAE*) BY MECHANICAL EFFECT: THE CHOICE OF CURRENT IMPROVEMENTS OF FORMULATIONS, APPLICATION AND CONCEPT

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The application of products based on SiO2 is the most important alternative to the application of synthetic chemical acaricides in red poultry mite control, especially in Europe (Kilpinen and Steenberg, 2009; Schulz, 2014). However, neither SiO2 formulations in the form of water suspensions, nor diatomaceous earth, have so far provided the ultimate rational control of red poultry mite. There is a need for these to be improved and complemented. In lab tests we have found that a new formulation of SiO2 for mixing with water manifests a full lethal effect on *Dermanyssus gallinae* (in adult mites directly exposed for 1 minute or subsequently for 1 hour) in as soon as 3 days. In clinical conditions, the concept of application has been improved. We have achieved optimization of SiO2-based product application by combining silicates applied in water suspension and powder forms during the period of housing preparation, and when there is housing downtime in the warm period of the year. The SiO2 formulations were chosen after testing 20 powder formulations and 12 formulations applied by mixing with water. By combined application of the chosen SiO2-based products we achieved the highest degree of suppression with one treatment per year, or even complete eradication of *D. gallinae* from production facilities. According to integrated health care, poultry farms are instructed to use the housing downtime caused by infectious diseases to initiate a combined SiO2 treatment in order to eradicate or permanently suppress *D. gallinae* infestation, thus eliminating an important vector (Pavlicevic *et al*., 2017). We have also conducted lab and clinical tests of the biological efficacy of a new oil-based formulation that has a mechanical effect that prevents mites' respiration. In addition to high efficacy on directly exposed mites (100%), on unabsorbent surfaces (provided that the layer is not removed or corrupted) a high residual effect is achieved for over 3 months (90-100% per hour of subsequent exposure). New oil-based formulations can also be used for housing preparation in winter conditions. In situations when there is high flock infestation or continuous suppression during the production period, and when there is a shorter housing downtime, their use is more rational. Current improvements of application and formulations with mechanical effect can now provide a safe, rational and highly efficient red poultry mite control. However, they certainly do have their limitations. Complete and long-term success in *D. gallinae* control is dependent on the implementation of other elements of the control program including: adequate communication and engagement of poultry producers, thorough adaptation of environmental conditions, implementation of a complete technological procedure, application of acaricide products, monitoring, integrated health care, improvement of residue monitoring and introduction of biosecurity measures.

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AN INTEGRATIVE APPROACH TO THE MOLECULAR AND MORPHOLOGICAL IDENTIFICATION OF MITES ASSOCIATED WITH THE RED POULTRY MITE

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As broad spectrum pesticides are being withdrawn and sustainable control practices are increasingly adopted, accurate taxonomic identification is imperative for the evaluation of new acarine biocontrol agents (ABAs). While little is known on ABAs of the red poultry mite (RPM), compared to ABAs of plant feeding mites (Gerson *et al*., 2003), recent studies have identified potential ABAs for RPM (Lesna *et al*., 2012). The community of poultry growers, veterinarians and extension agents have yet to adopt this technology, partially because they lack the taxonomic expertise required to monitor the mite fauna before and after predator releases. Although the number of molecular taxonomy studies have increased substantially (Young and Hebert, 2015), species identification of mites is still based almost entirely on morphology and is dependent on traditional taxonomists. Without reliable DNA barcodes for identification, and with only few mite taxonomists left for accurate morphological identification, we are clearly facing a taxonomic crisis. DNA barcoding (Hebert *et al*., 2003) uses a standard marker (COI) to identify specimens following strict data quality and specimen management guidelines developed by the Consortium for the Barcode of Life (CBOL, www.barcodinglife.org). In the present study, we integrated molecular and morphological taxonomic tools with the aim of developing a library of mite DNA barcodes based on expertly identified specimens, to facilitate mite identification through DNA barcodes. Mites were collected from manure in poultry houses, bird nests, and from nearby non disturbed soils in 2015 and 2016 from Israel, France and Poland. Series of isomorphic specimens were imaged, placed individually in micro-plates in 95% ethanol and sent to the Canadian Center for DNA Barcoding (CCDB) for the sequencing. Following their return, mites were mounted on slides and 34 species were identified, belonging to the families (in parentheses no. of species per family) Laelapidae (4), Blattisociidae (3), Rhodacaridae (2), Digmasellidae (3), Melicharidae (3), Parasitidae (8), Macrochelidae (6), Ascidae (1) and Cheyletidae (4). A Lucid key for the identified species is being prepared and will be posted on the COREMI COST action FA1404 website.

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DESCRIPTION OF PROTEOMES FOR DIFFERENT FED AND UNFED STAGES OF THE POULTRY RED MITE (*DERMANYSSUS GALLINAE*)

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Poultry Red Mites (PRM) pose a threat to the egg industry worldwide. It causes serious economic losses due to reduction in egg production, higher costs (treatments and prevention), potential disease transmission and hen's welfare. In addition, producers are facing the lack of effective control tools because of the development of resistance against synthetic acaricides and withdrawal of authorized products through national and EU legislations. Vaccination could be seen as a novel and promising method for PRM control (Sparagano *et al*., 2014).

Mites collected from affected farms were sorted in four different categories according to feeding status (fed and unfed) and developmental stage (nymphs and adults). Each category included three pools of 100 snap-frozen mites each. After protein extraction and quantification, protein digests were analysed by reverse phase liquid chromatography coupled to mass spectrometry.

More than 3,000 proteins were identified. Proteins showing differences between groups were involved mostly in different metabolic processes and some of them were involved in reproductive processes or with structural functions.

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IN VITRO EFFECT OF PLANT ESSENTIAL OILS AS ACARICIDES AGAINST *DERMANYSSUS GALLINAE*

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The red mite, *Dermanyssus gallinae* (Dermanyssidae), is a periodic haematophagus ectoparasite of poultry, infecting also other bird species, mammals and even people (Zdybel *et al*., 2011). In recent years, resistance to poultry ectoparasites on chemicals commonly used to control has been repeatedly found by veterinarians Cencek *et al*. (2011). In addition, due to the growing interest of consumers in safe and non-food remnants of foodstuffs, the need to select and develop natural and effective alternatives has been recognized. The aim of this study was to test the effectiveness of essential oils against the poultry red mite, *D. gallinae*. Eight essential oils (derived from eucalyptus, lavender, thyme, rosemary, pine, clove, geranium, citronella) were tested for the acaricidal activity. The following concentrations were tested: 20%, 50%, 80%, 100% at a dose 0.28 mg/cm^2. The research is based on the method according to Zdybel *et al.* (2011) in modification.
The special plexiglas plates with veneer disc were used in order to imitate natural conditions.The solutions were spread on the surface of disc situated in a plastic chamber. Mites were collected on many farms in Poland and placed on the disc (minimum 100). All movable mites were used regardless of the stage of their development. The mortality of the exposed mites was measured after 48 h. The experiment was repeated twice. Positive and negative controls were used. All the oils reduced *D. gallinae* survival. The best results were observed for 80% rosemary oil (more than 90% mortality), 50% lavender (75% mortality), 100% thyme (80% mortality) and 80% clove oil (73% mortality). Plant essential oils described herein merit further study as potential *D. gallinae* control agents.

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IN VITRO EVALUATION OF THE EFFECTIVENESS OF COMMERCIALLY AVAILABLE ACARICIDES AGAINST THE POPULATIONS OF RED MITES (*DERMANYSSUS GALLINAE*) OCCURRING IN POLAND

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The control of poultry red mites, *Dermanyssus gallinae* (De Geer, 1778), is very difficult. One of the main reasons for this is that resistance of these parasites to acaricides appears. 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 2006-2008 an investigation was carried out in 32 battery cage farms of laying hens, localised in 14 Polish provinces. For the study, 9 acaricides were used containing the active substances: α-cypermethrin, phoxim, bendiocarb, fenitrothion, dichlorophos, diazinon and chlorpyrifos. Since 2015, a similar investigation has been carried out, but with the use of preparations currently available on the market. Their active substances are: avermectin, cypermethrin, permethrin, carboxylate, phenoxybenzyl, carbamate and silicon dioxide. In the current survey, 17 populations of *Dermanyssus gallinae* in battery cage farms from 7 provinces have been examined so far. The investigation was carried out by our previously described method (Zdybel *et. al*., 2011).

The efficacy tests of acaracides used in 2006-2008 showed that the most effective against the majority of red mite populations were preparations based on phoxim (Blaxime and Bye-Mite), and carbamate preparation (Ficam). Their mean efficiencies were 96.5%, 96.0% and 95.6%, respectively. In the current round of research, the most effective formulation was Ficam, and its efficacy against red mites from individual farms ranged from 54.2% to 99.0%.

DERMANYSSUS GALLINAE STATUS IN IRAN: AN EMERGING PROBLEM

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The Iranian poultry industry is the largest in the Middle East. In 2013 Iran was the 10th biggest producer of eggs in the world. *Dermanyssus gallinae* currently is the most important ectoparasite affecting egg layers in temperate and tropical countries worldwide. Due to Iran's suitable climate conditions, short complete reproduction cycle of *D. gallinae* and severe problems such as weight gain and egg laying capacity reduction caused by *D. gallinae*, controlling this ectoparasite is vital. The prevalence of ectoparasites on Iranian poultry production was investigated by different studies (Yakhchali et al., 2013). A massive infection of *D. gallinae* on birds, cages and the conveyor belts for eggs was obtained in seven different provinces of Iran. In Arak (dry province) 92.86% of examined poultry farms were infested with *D. gallinae* with prevalence (39.3%) significantly ($p<0.05$) higher in spring in comparison with winter. Humid and dry regions of North Iran were studied and the prevalence of *D. gallinae* was low (20%) in both regions. A study in Mashhad (dry province) shows *D. gallinae* was the most prevalent (45.83%) blood feeder mite in the breeder and caged layer flocks which caused 25 % decrease in egg production. Free range chicken backyards in Kermanshah (dry province) had 26.3% *D. gallinae* prevalence. *D. gallinae* has been controlled by continuous applications of acaricides. Cardboard traps containing acaricides were introduced as a successive device for collection and control of the poultry red mite. Concerning resistance development and chemicals still available for treatment of poultry, the importance of alternative products to control *D. gallinae* is highlighted. Eco-friendly ways to control *D. gallinae* have been studied in Iran and successful results using plant extracts were published (Barimani et al, 2016). Positive results for *Artemisia sieberi*–related essential oil, Traps containing carvacrol with 92% reduction in mite control, garlic extract with 96% success, cinnamon, eucalyptus and mint extract with 66.97, 80.85 and 90.19%, respectively has been showed. Plant preparation has high potential for more research as an alternative eco-friendly way to decrease *D. gallinae* population in the Iranian poultry industry.

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THE RESIDUE DEPLETIONS OF SPINOSAD AND ABAMECTIN IN EGGS OF LAYING HENS

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The poultry red mite, *Dermanyssus gallinae*, is one of the most economically important ectoparasites in poultry houses in many countries worldwide. Control relies mainly on the use of various synthetic acaricides including spinosad (SPN) and abamectin (ABM). However, there is no information on the existence/depletion of residues of both compounds in eggs following application in layers. The objective of this research was to determine the residue depletions of SPN and ABM in eggs of laying hens.

A total of 36 layers, 11-12 months old and weighing 1675 ± 151 g were used in this study. The animals were divided into 4 groups of 9 animals each such that the mean weight of animals in each group was similar. The layers were kept in individual cages during the whole study. Two different doses of SPN (3 and 6 g/m^2) and ABM (1.8 and 3.6 mg/m^2) were applied to hens kept in separated houses. ABM and SPN were applied in empty and stocked (George et al. 2010) 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 drug application. The eggs were collected individually on days 1, 2, 3, 5, 7, 8, 10, 12, 14, 20, 25 and 30 post application (p.a.) for each group. The eggs were broken and both the white and yolk were put in transparent lock bags and kept at -20 ºC until analysis. Residues of SPI and ABM in eggs were determined by High Pressure Liquid Chromatography.

Limit of quantifications were 1 ng/g for ABM and, 100 ng/g and 200 ng/g for SPN components, spinosyn A and spinosyn D, respectively. ABM was not detectable in white and yolk of any egg samples after low and high dose applications. While no SPN were found in egg white, it was detected in all yolk samples at both dosages beginning from day 2 p.a. The highest SPN levels in egg yolks were determined on day 10 p.a. (at low dose: 4.05 ± 0.88 μg/g and at high dose: 6.69 ± 1.92 μg/g). Relatively high residue levels of SPN (at low dose: 1.13 ± 0.51 μg/g and at high dose: 2.48 ± 0.87 μg/g) were detected even on day 30 p.a.

In conclusion, after application of ABM according to manufacturer's instruction, it was not at detectable levels (lower than 1 ng/g) in eggs p.a. On the other side, despite recommended for its high efficacy in the control of *D. gallinae* also in stocked cages (George et al. 2010), great attention should be paid to SPN-applications in layers because of high residue levels and slow depletion rates in eggs. Otherwise, the high levels of SPN-residues in the eggs would pose a serious risk in terms of public health. Further investigations are needed to re-evaluate effective and safer dosages for SPN in laying hens.

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RESULTS OF A PILOT STUDY REGARDING *DERMANYSSUS GALLINAE* IN THE GREEK LAYING HEN INDUSTRY

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*Dermanyssus gallinae,*also known as the poultry red mite (PRM), is a blood sucking ectoparasite, widespread in many parts of the world. PRM poses an increasing economic threat, especially for the laying hen industry, due to its haematophagous activity. It is responsible for reduction of the egg production (quality and quantity) and susceptibility of the poultry health status. Moreover, PRM has public health implications. The aim of this pilot study was to investigate the prevalence of *D. gallinae* infestation of the Greek laying hen industry and provide information on the importance of PRM control.

In our study, 12 Greek laying hen farms were visited in Central Macedonia (Northern Greece) and 5 cardboard traps (15X40cm) were placed in each farm in different sites, including beneath feed troughs, inside cage fittings and fastening clips, under egg conveyer belts and under manure belts. The traps (n=60 in total) were examined for the presence (counting and identification) of *D. gallinae.* According to our results, all farms were infected with this ectoparasite (100% prevalence). The average number (±SD) of mites per trap was 356±26. The results of this pilot study confirmed the widespread presence of PRM (100% prevalence). It is, therefore, evident that PRM is a major problem for the laying hen industry in Northern Greece and coordinated actions employing effective control strategies must be taken. An increase of *D. gallinae* prevalence rates may have an epidemiological impact on several animal and human diseases, as PRM can be a potential vector for several pathogens (One Health).

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