Università di Foggia

izs istituto zooprofilattico sperimentale della puglia e della basilicata

cost EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

1st COST CONFERENCE
and
MANAGEMENT COMMITTEE MEETING

on

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

COST Action FA1404

PROCEEDINGS

Bird-shaped painting from Daunian Pottery
(Apulia, VI century b.C.)

28th - 29th May 2015
FOGGIA, Italy

Content

PREFACE

COST Action FA1404 on "Improving current understanding and research for sustainable control of the poultry red mite *Dermanyssus gallinae* (COREMI)" aims to generate a synergic approach to improving the health, welfare and productivity of the million laying hens present worldwide, through more effective prevention and control of *Dermanyssus gallinae* , the Poultry Red Mite (PRM). This will be achieved via cooperation and networking between scientists, industries, farmers and other stakeholders from the different member states and people from different disciplines.

The 1st COST Conference and the Management Committee held in Foggia (Italy) 28-29 May 2015 was organized by the University of Foggia's Department of Agricultural, Food and Environmental Science (SAFE), the Experimental Zooprophylactic Institute of Apulia and Basilicata, and by the University of Bari's Department of Veterinary Medicine, in collaboration with the COST Core Group Members.

The choice of Apulia Region as the venue is due to the region's important traditions of research in Animal Husbandry and Health, thanks not only to the region's two Universities of Bari and Foggia, its Zooprophylactic Institute, and other Research Institutions but also thanks to its poultry farms traditional and not traditional and its qualified technicians and professionals.

The Event had a grand total of 150 participants and 20 speakers, from about 20 European and non-European countries. Italian and foreign experts participated on the following issues:

- **Theme 1- Developing alternative control measures**
- **Theme 2 - End users (One Health)-interdisciplinary approach**
- **Theme 3 - Genetic structure in a changing world**
- **Theme 4 - Epidemiology, pathology, geographical mapping and surveillance tools**

The 1st COST Conference and the Management Committee provided the opportunity for engaging in a constructive discussion, and above all for sharing the important results of scientific research. It also gave a precious opportunity to establish contact with other researchers interested in collaboration and COST membership, since the Action needs to open up new lifelines in order to continue addressing its objectives and meeting the needs of the poultry industry.
The Proceedings collect the abstracts of Invited Lectures presented at the 1st COST Conference covering the four Themes as well as the Openings by the Organizers and by the Chair, Olivier Sparagano.
The Organizing and the Scientific Committee wish to thank the authors for their contributions and the Sponsors for their support in publishing the Proceedings.

On behalf of the Scientific Committee:

Olivier Sparagano, Chair, UK;
Elias Papadopoulos, Vice Chair, EL;
David George (WG1), UK;
Monique Mul (WG2), NL;
Lise Roy (WG3), FR;
Antonio Camarda (WG4), IT;
Annunziata Giangaspero (STSM), IT

The Organizers

Annunziata Giangaspero[1], Maria Assunta Cafiero[2], Antonio Camarda[3], Marianna Marangi[1]

[1] Department of Agriculture, Food and Environmental Science, University of Foggia, Foggia, Italy.

[2] Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy.

[3] Department of Veterinary Medicine, University of Bari, Valenzano, Italy.

PROGRAM

May 27th, 2015

MC Members arrive and transfer to hotels

20.00 Welcome Cocktail (Chiostro Santa Chiara, Piazza Santa Chiara-Via Arpi, Foggia)

May 28th 2015

Auditorium "Santa Chiara",
Piazza Santa Chiara-Via Arpi, Foggia

OPEN PLENARY SESSIONS

08.30 Registration

09.00 Opening address (Annunziata Giangaspero, University of Foggia, Italy)

09.10 Welcome addresses from the Authorities

09.25 Presentation of Cost Action (Olivier Sparagano, Chair, Coventry University, UK)

Theme 1
Developing alternative control measures

Chairpersons: Eric Palevsky, Aida Kustura

09.40 Natural enemy R&D for Poultry Red Mite
Felix Wackers, Biobest, UK

10.10 Information on WG 1
David George, University of Northumbria, UK

10.20 Lessons to be learned from IPM inhorticulture
Tuomo Tuovinen, MTT Agrifood Research, Finland

10.40 Immunology and how it may assist in PRM management
Kathryn Bartley, Moredun Research Institute, UK

11.00 How knowledge on insect/mite molecular biology can contribute to Integrated Pest Management?
Pedro Hernandez, Centro de Investigaciones Biologicas, Spain

11.20 Coffee Break

Theme 2
End users (One Health)-interdisciplinary approach

Chairpersons: Helena Eriksson, Johan Zoons

11.40 One Health - an interdisciplinary approach. About hurdles and rewards
Wim van der Poel, Central Veterinary Institute of Wageningen UR, The Netherlands

12.10 Information on WG 2
Monique Mul, University of Wageningen, The Netherlands

12.25 Should the poultry red mite *Dermanyssus gallinae* be of wider concern for veterinary and medical science?
David George, University of Northumbria, UK

12.45 *Dermanyssus gallinae* in Romania: current situation regarding prevalence, monitoring and treatment.
Cristian Magdas, University of Cluj-Napoca, Romania

13.00 Lunch

14.00 Monitoring and prevention as a way to control poultry red mite (*Dermanyssus gallinae*) in organic layers.
Jutta Berk, Friedrich-Loeffler-Institut, Germany

Theme 3
Genetic structure in a changing world

Chairpersons: Susan Kabell, Jan Hubert

14.20 Characterizing and identifying litter mite communities – applications of DNA barcoding for PRM management strategies
Monica Young,University of Guelph, Canada

14.50 Information on WG 3
Lise Roy, University of Paul Valéry Montpellier, France

15.00 Genetic structure of PRM and dispersal patterns
Øivind Øines,Veterinary Institute of Olso, Norway

15.20 Molecular techniques applied on arthropod vectors – useful approach for mite monitoring and identification
Jan Chirico,National Veterinary Institute, Sweden

15.40 Understanding of the role of PRM in bacteria spread
Danjela Horvatek Tomic,University of Zagreb, Croatia

Theme 4
Epidemiology, pathology, geographical mapping and surveillance tools

Chairpersons: Veronika Maurer, Josè de la Fuente

16.00 Mesostigmata Dermanyssina mouthparts evolution: from predatorism to parasitism
Antonella Di Palma, University of Foggia, Italy

16.30 Information on WG4
Antonio Camarda, University of Bari, Italy

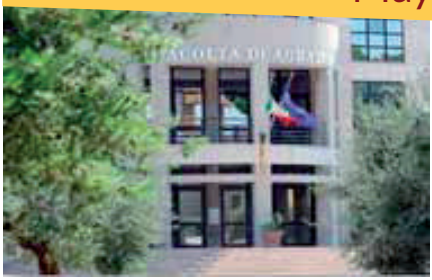
16.45 Routes of diffusion and persistence of viral pathogens in poultry farms and role of *Dermanyssus gallinae*
Teufik Goletic, University of Sarajevo, Bosnia and Herzegovina

17.05 Population dinamic of *Dermanyssus gallinae* in poultry farms
Lionel Zenner, VetAgro Sup, Campus Vétérinaire de Lyon, France

17.25 Final Discussion and Conclusion

20.00 Social Dinner

May 29th, 2015



Department of Agriculture
Via Napoli 25, Foggia

MANAGEMENT COMMITTEE (MC) MEETING (FOR MC MEMBERS, ONLY)

9.00 **Welcome to participants and Introduction**
Olivier Sparagano, Chair

- Adoption of agenda
- Approval of minutes and matters arising of last meeting
- Update

a. Status of Action, including participating countries
b. Action budget status
c. STSM status and new applications

- Promotion of gender balance and of Early Stage Researchers (ESR)

Administrative and Financial Remarks Update from the COST Association
Ioanna Stavridou, COST Office

Update from the Grant Holder
Jagdees Pabla, Coventry University, UK

- Follow-up of MoU objectives
- Progress report of working groups

WG1 -David George, University of Northumbria, UK
WG2 -Monique Mul, University of Wageningen, The Netherlands
WG3 -Lise Roy, University of Paul Valéry Montpellier, France
WG4 -Antonio Camarda, University of Bari, Italy
STSMs - Annunziata Giangaspero, University of Foggia, Italy

- Scientific planning
  a. Scientific strategy
  b. Action Budget Planning
  c. Long-term planning (including anticipated locations and dates of future activities)
  d. Dissemination planning (Publications and outreach activities)
- Requests for new members
- Non-COST applications to the Actions
- AOB
- Location and date of next meeting
- Summary of MC decisions
- Closing

13.00 Lunch
14.00 Departure

Open Coffee

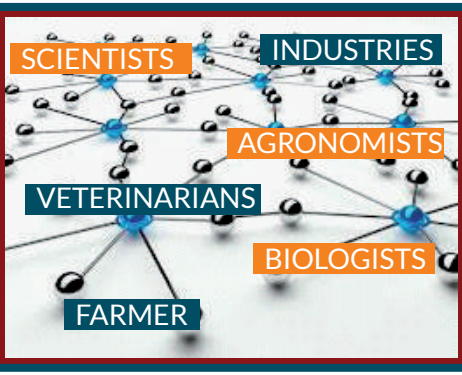

FOGGIA
Pdi Stª Cecilia da Capo
Pdi Stª cecilia
Pdi Monta di Pietrafitta
Via de Troia
Sª Mª del Carmine
Orto di Sagese
Capucini
Sª Marco
Pdi Stefano
Pdi Fontanelle
Pdi Monte Rozzi
Castiglione

WELCOME ADDRESS

Dear Director of the Department SAFE Prof. Agostino SEVI,
dear COST ACTION Chair Prof. Olivier Sparagano,
dear MC Members,
dear participants,
dear students,
on behalf of the organizing and Scientific Committee, it is my pleasure to welcome you all to this Conference.

The Italian Committee members of COST Action FA1404 are pleased to host here in Foggia **the very 1st COST CONFERENCE and the MANAGEMENT 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 approach to improving the health, welfare and productivity of the million laying hens present worldwide, through more effective prevention and control of *Dermanyssus gallinae* the Poultry Red Mite (PRM).



This will be achieved via cooperation and networking between scientists, industries, farmers and other stakeholders from the different member states and people from different disciplines.

The background and the COREMI project will be presented by the excellent Chair, Prof. Olivier Sparagano (University of Coventry, UK).
The choice of Apulia Region as the venue is due to the region's important traditions of research in Animal Husbandry and Health, thanks not only to the region's two Universi-

ties of Bari and Foggia, its Zooprophylactic Institute, and other Research Institutions but also thanks to its poultry farms traditional and not traditional and its qualified technicians and professionals.

Università di Foggia


Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente


Istituto Zooprofilattico Sperimentale di Puglia e Basilicata (IZS)


Università di Bari


Auditorium "SANTA CHIARA"

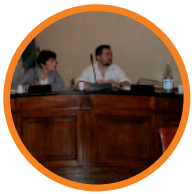
This Congress Plenary Session is taking place in this lovely Auditorium, which is the former church of Santa Chiara, originally built in the 1,500s and then rebuilt in 1743 after a devastating earthquake hit Foggia.
Tomorrow's Management Committee (MC) Meeting (for MC members only) will be held at the University of Foggia's Department of Science of Agriculture, Food and Environment.

As you can imagine, it is never an easy task to organise a Conference, and I can now assure you that it really is very hard work!
But we think that this experience is a special opportunity – and not just in scientific terms. From the very beginning, all those involved (the SC and we Local organizers) have shown so much willingness to work, and the result is that today we have a grand total of 150 participants and 20 speakers, from about 20 European and non-European countries.

As you can see, the conference schedule is very intense, and there are 4 alternating themes. Each theme will be introduced and presented by the chairpersons and Working Group Leaders.
However, the real star of this event is the Poultry Red Mite, *Dermanyssus,* which, as you now, severely affects poultries.

This isn't the first time that we have been obliged to talk about *Dermanyssus,*- there have been many occasions.

I remember that back in 2006 we were just a small group, but we met together right here in Foggia, at what was then the Faculty of Agriculture, to talk about our research, to carry out inspections, to look for the Red Mite.

Two years later we were in Cervia for another meeting. We were all certainly quite a bit younger, and there weren't so many of us, or rather we were spread out in small groups across Europe as we discussed this mite, which was causing - and continues to cause – so many problems for hens and the poultry industry.
However, we have gradually managed to create a growing group of scientists. And together we have even managed to obtain EU funding to create a network bringing us all together – at last about 70 scientists from 22 countries!
A great success because we are so many now and we're all here!
However, we're just at the beginning, because this is only our first COST Conference, This makes us forget all about the tiring work that went into organising the conference, because Foggia is now the Starting Line, and the most interesting stage is about to get under way.

There are so many individuals and organisations to thank for their invaluable support, all of whom have given their different contributions - at this difficult time - to the success of this important Event.

I would like to thank all the sponsors who have helped us to meet the organisational and other costs that the CE can't fully cover.
The Conference was made possible thanks to the support of local institutions (Banca del Monte Foundation, Order of Veterinary Surgeons, Foggia Municipality)

and Companies (APPI, Olmix, Bayer, Elanco, Zoochimica and Levanchimica).
I would like also to thank the Core Group colleagues for their constant support during the different stages of organising the scientific aspects, our many students who have been so willing to participate, the Agenzia Atena, who have always been there to help us as we struggled with the complexities of organising a major event. And a big thank to Grant Holder Jagdees Pabla for his outstanding help during the organizational stage. Since he originally comes from India, I would say that he has the patience of a modern day Gandhi!

We hope this Conference will provide us with an opportunity for scientific, cultural and personal development, and that you will also enjoy getting to know Foggia and the Daunia area.

Daunia has provided me with one of its symbols to use as the logo for this Conference: a stylised bird, which decorated hundreds of the objects used by the people of Daunia in the 6th century BC.
It is also the symbol of Foggia's Museum, which I invite you to visit during your stay here.

Foggia Province has wonderful landscapes, pretty old towns, archeological masterpieces, castles as well as outstanding beaches, and this is why I would like you to stay longer here.
Foggia is an old town but isn't Florence, Rome, Venice, Pisa or the other Italian cities. It was damaged first by the earthquake, and then by the bombs which fell during the Second World War, but it has a soul and the real beauty of this city is its people's warm hospitality.

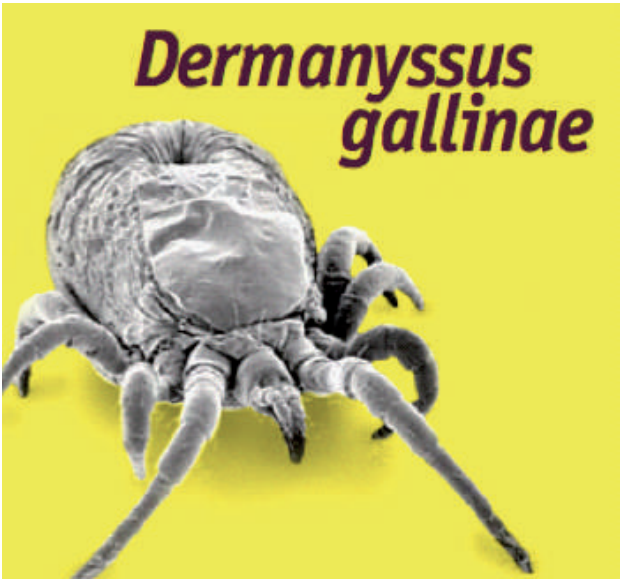
I'm sure that you're really going to appreciate this during your time in the city!
Thank you once again for being here today and for listening to me.

BUON LAVORO A TUTTI!
ON BEHALF OF THE ORGANIZERS
Annunziata Giangaspero

THE BACKGROUND and THE COREMI PROJECT

Presentation of the COST Action FA1404

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



Professor Olivier Sparagano
FA1404 Chair

Primary Aim

The overarching aim of COREMI is to generate a synergic / holistic approach to improve the health, welfare and productivity of the EU's 350 milion laying hens through more effective prevention and control of PRM

Secondary Aims

1. Stimulating research, education, exchange of knowledge and experience, and training of Early Career Investigatiors (ECIs)
2. Quick implementation of innovative ideas for PRM control as a result of the multidisciplinary and country wide networking community.
3. Communication and discussion of relevant research by organising workshops / Training Schools / Conferences...
4. Creating a multi-funcional website
5. Knowledge management and exchange (KME) with / to the scientific community, policy makers, primary producers, poultry breeding organisations, technology industries and Small and Medium Enerprises (SMEs)

Structure

22 Cost Countries represented so far

A Core Group:

- Chair (Olivier Sparagano, UK)
- Vice-Chair (Elias Papadopoulos, EL)
- WG1 Lead (David George, UK)
- W2 Lead (Monique Mul, NL)
- WG3 Lead (Lise Roy, FR)
- WG4 Lead (Antonio Camarda, IT)
- STSMs Lead (Annunziata Giangaspero, IT)

WGs and STSMs

Working Group 1

Developing alternative control measures

Leader: David George (UK); Vice-Leader: Ruedi Sweifel (CH)

Working Group 2

End Users (one Health) - interdisciplinary approach

Leader: Monique Mul (NL); Vice-Leader: Ekaterini Tiligada (EL)

Working Group 3

Generic structure in a changing world

Leader: Lise Roy (F): Vice-Leader: Fiona Tomley (UK)

Working Group 4

Epidemology, pathology, geographical mapping surveillance tools

Leader: Antonio Camarda (IT); Vice-leader: Danijela Horvatek Tomic (CR)

STSM coordinators

Leader: Annunziata Giangaspero (IT); Vice-Leader: Jose de la Fuente (SP)

Activities

Core Group Meetings

Management Committee Meetings

Conferences

Short - Term Scientific Mission (STSMs)

Training Schools

Dissemination

Olivier.sparagano@coventry.ac.uk

COREMI PROJECT 's **THEMES**

LESSONS TO BE LEARNED FROM IPM IN HORTICULTURE
Integrated management of poultry red mite

T. Tuovinen

Natural Resources Institute, Finland

Integrated Pest Management (IPM) has been adopted as the basis of plant protection practices in the EU (Directive 2009/128/EC, Anon., 2009). A short definition of IPM by FAO is: "*Integrated Pest Management (IPM) means the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically and ecologically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms.*"

The directive establishes a framework to achieve a sustainable use of pesticides by reducing the risks and impacts of pesticide use on human health and the environment and promoting the use of integrated pest management and of alternative approaches or techniques, such as non-chemical alternatives to pesticides. In horticulture, IPM principles have been partly applied in practice since the 1960's, although intensive use of pesticides is still commonplace. Horticultural IPM, and especially biological control in greenhouses, is motivated mainly by economic and practical drivers. These include the development of resistance to pesticides in many key pests, the withdrawal of many pesticides, and the strictly regulated use of remaining pesticides. Such drivers have forced many vegetable growers to adopt biological control.

EU countries are obligated to draw up National Action Plans (NAP) setting objectives and timetables to reduce risks and impacts of pesticide use (Anon., 2011). The main actions include:

1. Training and authorization of professional users of pesticides
2. Restrictions for sales of pesticides
3. Information on the risks of pesticides
4. Inspection of pesticide application equipments
5. Prohibition of aerial pesticide sprayings
6. Protection of aquatic environments and drinking water sources
7. Restricted use of pesticides in public areas
8. Measures to prohibit dangers of pesticide handling to people and environment
9. Measures to promote low-pesticide input pest management: IPM and organic farming
10. Harmonised risk indicators shall be established.

National plant protection authorities are liable of most of the actions. Paragraph 9 includes measures which the grower can choose and decide how to manage pests in the actual growing situation. These include at least the following elements:

1. Planning and prevention
2. Monitoring of pests
3. Use of economic thresholds to decide the need for action
4. Use of non-chemical measures instead of chemical pesticides
5. Precise and focused sprayings of pesticides
6. Minimized use of pesticides
7. Prevention of development of resistance to pesticides
8. Documentation, evaluation of the effects of plant protection actions, learning

These elements are also applicable in integrated management of the poultry red mite (PRM).

Horticulture:

Planning and prevention

- Soil improvement, new substrates, crop rotation
- Avoid sources of infestation, e.g. alternative cultivated and weed host plants of pests
- Healthy, certified plant/seed materials
- Other preventive methods: logistics, removing plant waste, use of insect nets, etc.

Monitoring

- Monitoring plan for various pests – key pests
- Visual observation of pests and symptoms
- Tapping net samples from vegetation
- Inspection of leaf samples
- Traps: glued traps, pheromone traps, traps with attractive odours, light traps
- Soil samples: nematodes, soil born fungi

Use of economic thresholds

- Density of a pest at which a control treatment will provide an economic return
- Depending a lot of the type of injury/ insect/ mite
- Action Threshold; Economic Injury Level EIL
- Difficult to present a numeric threshold applicable in various circumstances
- Standardized monitoring methods are needed

Non-chemical control measures

- Resistant varieties
- Preventive methods
- Classical biological control
- Conservation, maintaining biodiversity
- Active biological control: spreading of entomopathogenic fungi, bacteria and viruses, plant-parasitic nematodes, parasitoid insects, predaceous insects and mites

Precise and targeted control actions

- Timing of sprayings based on monitoring results
- Timing of introduction of biocontrol agents
- Developmental stage of target pests
- Local, focused sprayings, hot spots
- Outdoor: avoiding windy weather conditions
- Outdoor: use of wind-safe nozzles

Poultry (PRM):

Planning and prevention

- Construction of layer houses: avoiding mite-friendly structures
- Cleaning, disinfection when changing flocks
- Eliminate sources of infection, e.g. bird nests
- Healthy, mite-free new flock
- Planning of other preventive methods: logistics, inspections, sanitation
- Hazard analysis of critical check points HACCP

Monitoring

- Monitoring plan for PRM
- Visual observation: PRM hiding places
- Traps set up in strategic places
- Attractive traps? Pheromones?
- Automated mite counter?

Use of economic thresholds

- Zero tolerance in most cases
- No threshold values in practice
- First observations are most important
- Action needed immediately when observed – not to wait for ‘economic injury level’

Non-chemical control measures

- PRM-resistant hen breeds?
- Primary method: prevention
- Plant derived acaricides/repellents
- Other types of ‘soft chemicals’
- Biological control attempts: spreading of predatory mites, application of fungi

Precise and targeted control actions

- Timing of control actions
- Sprayings, distributions of chemicals
- Introductions of biocontrol agents
- Repetition of control measures
- Cleaning and mechanical control
- Localized control: hiding places, hot spots

Minimized use of pesticides

- Good spraying equipments for each crop
- Right nozzle type for each target and crop
- Lowest effective dose (but: risk of resistance)
- Adjusted amount of solution according to plant size
- Use of surface tension adjuvants
- Focused sprayings

Prevention of resistance development

- Follow the instructions of repetitive sprayings
- Use sufficient doses and concentrations
- Use of available alternative control measures
- Avoid consecutive applications of pesticides of the same mode of action (IRAC, FRAC, HRAC codes)
- Observe and register lack in effectiveness

Documentation, evaluation, learning

- Active notebook, updated data: monitoring results, actions taken, observations etc.
- Evaluation of the effectiveness of plant protection actions
- Analyses: If unsuccessful control, what went wrong? Why?
- How to manage next time?
- Learning from peer support farms and advisors

Minimized use of pesticides

- Applying acaricides to the right places

Prevention of resistance development

- Use of different types of acaricides in consecutive applications
- Use of alternative measures instead of acaricides
- Avoiding consecutive applications of acaricides of the same mode of action
- Observe and register lack of effectiveness

Documentation, evaluation, learning

- Active notebook, updated data: monitoring of PRM, actions against PRM
- Evaluation of the effectiveness of actions against PRM
- Analyses: How did we get PRM in the first place?
- How to prevent spreading of PRM? How to keep population low? How to get rid of PRM?
- Learning from peer support poultry farms

As shown above integrated management of PRM has many common elements with IPM in horticulture, especially in the greenhouse environment. Application of 'hazard analysis of critical check points' (HACCP) in planning PRM management (Mul and Koenraadt, 2009) is relevant also in greenhouse pest management. Better understanding of preventive measures in both sectors will help in reducing use of pesticides, and also minimize the need and cost of other control efforts. A comprehensive review of experimental and currently used control methods has been published recently (Sparagano *et al*., 2014).

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IMMUNOLOGY AND HOW IT MAY ASSIST IN THE CONTROL OF POULTRY RED MITE: A VIEW TO VACCINATION.

K. Bartley[1], H. Wright[1], J. Prichard[2], T. Küster[2], O. Sparagano[3], F. Tomley[2], A. Nisbet[1]

[1] Moredun Research Institute, Scotland, UK; [2] Royal Veterinary College, London, UK; [3] Coventry University, UK

Introduction

Vaccination against blood-feeding ectoparasites can result in effective and sustainable control (de la Fuente *et al*., 2003; Willadsen, 2004) and offers advantages including prolonged efficacy, freedom from chemical residues/environmental pollution and reduced risk of resistance. In previous studies we, and others, have shown that vaccination of hens is a feasible control strategy to control poultry red mite (Wright *et al*., 2009; Bartley *et al*., 2009 and 2012; Harrington *et al*., 2009) and the identification of suitable antigens for vaccine production is now a principal goal of research in this area.

Historically, vaccine candidate identification has been achieved using a "pragmatic approach", where extracts of native proteins are repeatedly subfractionated and tested *in vivo* for vaccine efficacy. Eventually, a complex mixture of proteins is distilled down into fractions containing relatively pure preparations of protective proteins that can be indentified using combined proteomic/bioinformatic technologies. A successful example of this pragmatic strategy is the identification of the Bm86 antigen, which is the protective component of the TickGard vaccine (Willadsen, 2004). In recent years, with a dramatic increase in publically-available genetic, genomic and transcriptomic data, a "rational approach" is often applied to antigen discovery. In this approach, suitable antigens are selected from DNA databases based on either orthology with protective antigens from other species of ectoparasite, or predicted function with the two caveats that, for this latter approach to be practically useful, we must: i) understand what molecules are truly essential to ectoparasite survival, and ii) demonstrate that these molecules are accessible to the host immune system (Willadsen, 2004). Other approaches, including immunoglobulin-based protein enrichment are also a valuable strategy. The strategies being developed by researchers at the Moredun Research Institute and the Royal Veterinary College and their current progress will be discussed.

The combined approach (Moredun Research Institute)

Previous studies determined that a PBS-soluble red mite extract (SME) was able to induce a serum-IgY response when injected into hens and, when this IgY was fed to mites *in vitro*, significant mite mortality resulted (Wright *et al*., 2009). When tested in a field trial, vaccination with SME reduced red mite infestation numbers by approximately 75% in a 4 month period (Figure 1). Recent efforts have focussed on identifying the immunoreactive protein components of the SME and testing their ability to induce mortality in mites fed on the blood from hens vaccinated with those proteins. The

Moredun strategy has combined elements of all approaches in order to identify vaccine candidates. We have subfractionated the SME and identified the protective subfractions through a process of vaccination and induced mortality in feeding mites. The immunoreactive proteins contained within these subfractions were identified using a combination of 2-D gel electrophoresis immuno-blotting (Figure 2) and immuno-affinity purification in concert with proteomic analyses and Mascot searches of a red mite transcriptomic database (Wright *et al*., 2011). Ten immunoreactive proteins were selected for further assessment as vaccine candidates using the following rational criteria: intensity of immune recognition; likelihood of exposure of the antigen to the antibodies in a blood meal; proposed function and known vaccine potential of orthologous molecules. Recombinant versions of the selected antigens have been produced and tested *in vitro*. The results will be discussed.

Figure 1. Mean mite trap numbers over a 4 month period following vaccination of hens and mite challenge. The control group (indicated by the red line) were injected with adjuvant only. The vaccinated group (indicated by the blue line) received SME prepared in the same adjuvant. The 1st trap data (25th Nov) was obtained approximately 2 weeks following challenge with live mites. Error bars represent the +/-SEM.

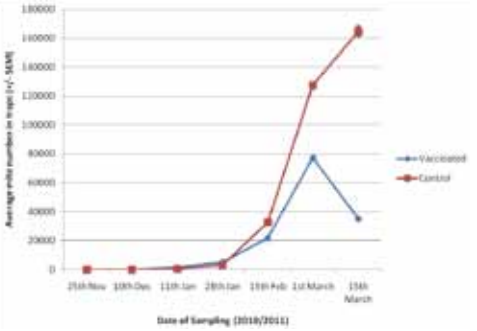
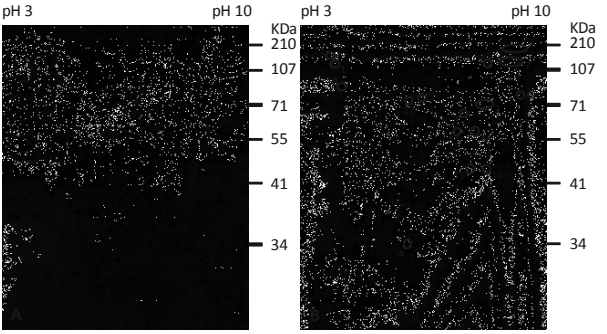


Figure 2. Two-dimensional gel and immunoblot analyses of a protective subfraction of SME. One replicate gel was immuno-blotted (panel A) and screened with serum form a hen vaccinated with subfraction proteins. Panel B is a replicate gel, stained with Coomassie, and the immuno-reactive spots selected for proteomic analysis are circled.



The "Immune-based and rational approaches" to selectively identify gut-membrane proteins (Royal Veterinary College)

Membrane proteins, particularly gut-associated membrane proteins, are considered to be ideal vaccine candidates for blood-feeding parasites, because they are readily accessible to ingested host antibody. The specific binding of antibodies to gut membrane proteins may interfere with that

protein function and hinder the mite's ability to digest and absorb nutrients from a blood meal. In addition, antibody binding may result in the activation of a targeted complement cascade resulting in physical damage to the gut-membrane, as is seen with the TickGard Bm86 antigen (Kemp *et al*., 1989). The RVC group have used a phage display biopanning technique to isolate antibodies that will be utilised to identify gut membrane proteins (Figure 3). Firstly, fractionation of red mite extract to enrich membrane proteins was performed. The membrane proteins were used as ligands for biopanning a phage display antibody library in order purify monoclonal antibodies specific for membrane proteins. The selection of monoclonal antibodies was further refined by immunohistochemical screening of sectioned mites and those shown to have gut-membrane specificity will be used in future experiments to identify gut membrane proteins.

To complement vaccine identification using the immune-based approach, RVC have also taken a rational approach to identifying membrane proteins. Red mite transcriptome data have been searched using several bioinformatic tools (Euk-mPLoc, CellPloc, TMHMM and TOPO2) to predict subcellular locations and transmembrane topography.

Figure 3. Schematic representation of the strategy employed by RVC to produce monoclonal antibodies that specifically recognise red mite gut-associated membrane proteins.

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HOW KNOWLEDGE ON INSECT/MITE MOLECULAR BIOLOGY CAN CONTRIBUTE TO INTEGRATED PEST MANAGEMENT?

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Integrated Pest Management (IPM) programmes are decision support systems aimed to manage pest damage by the most economical means, and with the least possible hazard to people and the environment. As any decision system, IPM must be built from accurate information to be efficient. Thus, the vast knowledge on pest biology and ecology acquired through research in recent decades, including the rapid advance of molecular biology and biotechnology, has been determinant for IPM development and success. However, much more effort must be made to incorporate biotechnology tools into IPM, and this can only be achieved through interdisciplinary collaboration.

Biotechnological tools, based for example on knowledge of pest/host physiology, host-parasite interactions and chemical ecology, that are available or in the pipeline for insect pest control, need to be developed for mites. Mites and ticks belong to Chelicerata, a subphylum of Arthropoda that is believed to have diverged during the Cambrian period, more than 500 million years ago (Dunlop, 2010). Consequently, their physiology does differ from that of insects, and knowledge acquired from insects cannot always be directly applied to mites. The recently sequenced and annotated genome of the two-spotted spider mite, *Tetranychus urticae*, the first complete genome of a Chelicerata sequenced, has revealed some unique peculiarities of mite physiology and has provided a highly valuable resource for its study (Grbić *et al.*, 2011). More recently, the transcriptome of several mite species, including poultry red mite (Schicht *et al.*, 2014), and the draft genome of a house dust mite, *Dermatophagoides farinae*, have been published (Chan *et al.*, 2015). Also, the draft genomes of two ticks, *Ixodes scapularis* and *Rhipicephalus microplus* are now available in the public domain. Basic research in mite and tick physiology will undoubtedly lead to the identification of new tools that will have practical implications for pest control.

One of the research lines of our laboratory is the study of insect and mite digestion and xenobiotic detoxification processes with the aim of contributing to the sustainability of current pest control methods, and the development of new methods for pest control. In addition, we are interested in the molecular identification of allergy-causing mites, and in studying the expression of mite allergens under different environmental conditions, with the aim of contributing to the standardization of allergenic extracts used for immunotherapy. Thus, we aim to develop and apply biotechnological tools for pest control and for the optimisation of mite-derived products.

At this conference, we will have the opportunity to address the gaps of knowledge on the poultry red mite that are needed to improve its control, recently reviewed by Pritchard *et al.*, (2015). Beyond that, we should make an extra effort to collaborate through the network with researchers working with species of mites and ticks of medical, veterinary and agricultural importance. This approach will clearly strengthen the network in its aim of improving current understanding and research for sustainable control of the poultry red mite. Indeed, a common research agenda for mites and ticks will clearly reinforce the chances of the network for further funding.

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ONE HEALTH, AN INTERDISCIPLINARY APPROACH

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Global change includes our socio-economic environment but also our biologic and our physical environment. Changes in the human way of live with increasing mobility and trade, changes in agriculture, animal husbandry, land use or wildlife result in increasingly more changes in the interactions between humans and animals, plants and other organisms of the ecosystem (Tylianakis *et al*., 2008). As part of the One-Health concept these changing interactions are subject of research but in many cases interactions between people and other organism populations such as animals and plants are studied separately.

Adopting the One Health approach, over the last few years there has been an increasing tendency to develop research project in which different fields work together on important health issues. In collaborative efforts between different internal and external research disciplines it is aimed to study the different interactions within ecosystems in a coherent way taking into account the many different relationships within these ecosystems. This approach should anticipate the development of interventions for the overall health improvement which might lead to opposite effects on the different elements within ecosystems. It is envisaged that a multidisciplinary approach in life sciences research will support a more effective protection of the health of people, animals and plants within the "One Health" concept. The ongoing "One Health" projects, integrate disciplines to enhance the overall scientific progress to be made for the quality of life.

In several dedicated projects researchers within Wageningen University have produced vision documents and developed a primary "One Health" strategy. In March 2015 the 3rd International "One Health" conference was held in Amsterdam and during this conference the Dutch National Centre for One Health (NCOH) was launched. The NCOH was founded by the Utrecht University and the Wageningen University and will be a virtual centre focussing on collaborations in at least four important One Health research themes, including infectious disease control and immunology. Another important Wageningen University initiative is the "Global One Health" approach, combining "Global Health" (Koplan *et al*., 2009) and "One Health". This wider multidisciplinary approach will also consider the health of animals, plants and sustainable ecosystems for human prosperity and not only for human health. There is a need to weigh all possible effects of interventions on the health of humans, animals, plants and the environment, while taking ecosystem sustainability into account. "Global One Health" will use multiple disciplines to seek transnational solutions for improving the health of humans, animals and plants worldwide.

Infectious disease can be controlled in two ways. Either by mitigation where preventing the disease, i.e. the clinical consequences of the infection (for the individual or the population), is the target. Or by eradication where at some geographical scale, e.g. regional or farm level, transmission of the infection is stopped sufficiently so that the infectious agent disappears from that region or farm. A

systems approach involving the quantification of human, animal and environmental factors in real systems and modelling these to identify additional variables that need to be tracked in order to answer management questions, can be very useful to assess the best disease control options in specific (artificial) ecosystems. For example: fighting poultry red mite is a complicated effort. Where eradication does not seem to be feasible, control strategies in poultry populations will have to focus on mitigation. In all cases farming practices, housing conditions and animal care taking needs attention. By using a systems approach, studies can be carried out to evaluate different control measures. A single bullet approach is unlikely to be effective, and a multidisciplinary approach will often be required, so that the chances for the emergence of drug-resistance, host expansion or other adverse effects are reduced.

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OF MITES AND MEN: SHOULD THE POULTRY RED MITE *DERMANYSSUS GALLINAE* BE OF WIDER CONCERN FOR MEDICAL SCIENCE?

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Parasitic bird mites present a significant threat to poultry production. Increasing reports of bird-mite attacks on humans (George *et al*, 2015) suggest that avian ectoparasitosis (gamasoidosis) may be of increasing medical concern. This may be of particular interest to those living or working in close association with poultry, as the species most often responsible are typically linked with domestic fowl (i.e. *Dermanyssus gallinae* and *Ornithonyssus sylvarum*). Though the threat that bird mites pose to poultry is relatively well understood, the risks posed to humans have been less well explored.

The potential health risk presented by gamasoidosis is exacerbated by the fact that bird mites such as *D. gallinae* can carry and transmit zoonotic diseases (Valiente Moro *et al*., 2009). Though the vector capacity of these mites is still an emerging science, mite-to-bird transmission has been demonstrated in a number of cases for *D. gallinae* (first reviewed by Valiente Moro *et al*., 2009 and later updated in George *et al*., 2015), increasing the likelihood that relevant diseases carried may also be passed from birds to mammals, humans included. Examples of disease spread to humans through bird mite vectors are rare in the literature, though transmission of spirochetes, rickettsiae, *Salmonellae*, *Bartonellae*, *Pasteurellae*, Sporozoa, hemogregarines, flagellates, and filariae have all been suggested (Litwin, 1961), with more recent evidence supporting acquisition of *Bartonella* via *Dermanyssus spp per se* (Melter *et al*., 2012).

The results of a recent review support that reported cases of gamasoidosis are increasing throughout the globe, with evidence that the majority of cases are linked to *D. gallinae*, and that mammalian companion animals and livestock may also be at risk (George *et al*., 2015). Further survey data from a subset of individuals afflicted by gamasoidosis confirm wide-spread occurrence, supporting the vector capacity of the mites responsible and adding Lyme disease to the expanding list of pathogens potentially transmitted by these mites (**Table 1**). Other issues identified through this survey included widespread misdiagnosis of gamasoidosis and (in some cases related) treatment failure and persistent infestation (**Table 1**).

Diagnosing gamasoidosis based on presenting symptoms (as is often undertaken) is inadequate and a suspected cause of large-scale misdiagnosis for similarly-presenting parasitoses such as scabies and pediculosis, general dermatitis or physiological conditions including delusional ectoparasitosis (George *et al*., 2015). Treatment is equally problematic, with species such as *D. gallinae* being hard to target and widely resistant to standard acaricides (Sparagano *et al*., 2014). Furthermore, the different mite species concerned display varied ecologies (with some living on hosts and others

residing off hosts and feeding intermittently) that necessitate divergent treatment approaches. In cases of human infestation, positive identification of species (or at least functionally similar groups based on life-history patterns) and recommendation of suitable treatment requires an understanding of mite taxonomy and ecology that many healthcare professionals and pest control organisations do not currently possess.

Overall it appears that although reported cases of gamasoidosis on humans remain relatively rare, *D. gallinae* represents a species of particular concern to medical health due to its reported genetic plasticity, occasional evidence of permanent infestations on mammals (e.g. Pampiglione *et al*., 2001), and high vector potential. Previous review suggests that incidences of gamasoidosis *per se* may be exacerbated by increased mite-novel host encounter rates and occurrence of certain 'risk factors' in the 'new' host, such as a breakdown in immune function (George *et al*, 2015). It follows that rising human populations, with anticipated increased incidences of immunocompromised individuals as a result of modern medical practises, may further promote gamasoidosis.

Though only preliminary, work in this area suggests that more attention should be given to gamasoidosis as a threat to human health, supporting that the mites responsible, and especially *D. gallinae*, could be considered an 'occupational hazard' to those working with poultry (Cafiero *et al*., 2011). Though cases of gamasoidosis have been reported since the 17th century (Toomey, 1921), documented in the leading medical literature since at least the 1920s (Anon, 1922) and reviewed twice in the last 15 years (Lucky *et al*., 2001; George *et al*., 2015), the full extent of gamasoidosis as a threat to human health has still to be explored.

Table 1. Results of a preliminary survey of suspected gamasoidosis in a selected internet user group. Method: Members of a selected internet user group (birdmites.org), were asked to complete a basic questionnaire on their experience of gamasoidosis. This user group was composed of global members afflicted with avian mites. Historically group membership ranged in the hundreds, though at the time of survey (Oct-Nov 2012) activity was limited to around 25 individuals. Questions, criteria and responses are summarised below. PCO = Pest Control Organisation.

Question	Criteria	Response	N
Has infestation been confirmed?	Confirmation required by a third party (healthcare professional, PCO or entomologist)	69% of respondents confirmed infestation	13
Duration of infestation	From onset of symptoms to present day (if on-going) or point of successful treatment	Average duration of infestation = 39 months (± 14.6 months SE)	13
Number of conflicting diagnoses	Diagnosis of condition other than gamasoidosis by a healthcare professional or PCO	Average number of conflicting diagnoses = 2.8 (± 0.3 SE)	12*
Treatments recommended and attempted	Only treatments prescribed by a healthcare professional or PCO	Pyrethroids (topical and premise) = 72%; DE = 27%; ivermectin = 27% IGR = 27%; esfenvalerate = 9%; cedar = 9%	11**
Treatment failure observed	Persistent symptoms post-treatment	100% of respondents reported treatment failure	12***
Suspected secondary health issues	Conditions arising post-infestation	Lyme confirmed in 3 respondents and suspected in 1 respondent *Bartonella* confirmed in 3 respondents and suspected in 1 respondent *Babesia* confirmed in 1 respondent General morbidity reported in most respondents	13

*information not extractable from one respondent. **Treatment type unknown in one respondent; treatment not yet attempted in one respondent. ***Treatment not yet attempted in one respondent. SE = standard error.

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DERMANYSSUS GALLINAE IN ROMANIA: CURRENT SITUATION REGARDING PREVALENCE, MONITORING AND TREATMENT

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The poultry red mite *Dermanyssus gallinae*, currently is the most important ectoparasite affecting laying hens in Europe, and the poultry red mite infestation still is an unsolved problem in poultry farms, because of acaricide resistance, legal regulations, residue risks, what limit chemical control of mites (Zenner *et al*., 2009; Schultz *et al*., 2014). This parasite is responsible for huge financial losses in poultry farming with estimated annual costs of €130 million throughout the European Union alone (Schicht *et al*., 2013). The prevalence of *D. gallinae* depends on flock systems: infestation rates of 4% in cage systems but 33% in alternative systems and 67% of backyard flocks, in different countries, *D. gallinae* prevalence rates can reach up to 80-90% (Sparagano *et al*., 2009; Schicht *et al*., 2013).

The most common form of control of *D. gallinae* in the poultry industry, is mainly based on acaricide applications, and carbaryl was the most widely used in the past and still used at present, followed by amitraz, permethrin and organophosphates, but only a few, or none of these compounds are specifically registered in some EU countries for use against the red mites (Marangi *et al*., 2012).

In Romania, the poultry industry has a tradition of almost 50 years, and according to a report of the Ministry of Agriculture and Rural Development, of March 31, 2014, in Romania there are nearly 80 million birds, with over 35 million laying hens (Anonymous, 2014). Although in Romania *D. gallinae* infestation in poultry, is an important problem with rates of infestation prevalence that can reach 90% (Magdaş *et al*., 2006) both in small family microfarms and intensive poultry farms, it is little investigated. The control of *D. gallinae* in layer farms and in the private yards, relies in the application of specifically registered organophosphate-based products, but also often the farmers use not specifically registered products (registered for use in agriculture or for other farm animal species) based on diazinon, amitraz, permethrin, cypermethrin or fipronil against the red mites. Treatment failures involve many times inadequate treatment protocols, the use of not specifically registered products and repeated, long term applications, leads to resistance in mites, Then, pesticides can accumulate in poultry meat, organs (Marangi et al., 2012) and/or eggs. It is known that continuous exposition to pesticides can have consequences for human health, especially for pregnants with the consequent premature deliveries. Cardio-circulatory diseases and infarction, prostate cancer, genetic mutations, neurotoxic disorders can be also associated to pesticide exposition in humans (Gamlin *et al*., 2007; Singh *et al*., 2011).

In the organic layer farms plant extracts based products are used. Many studies were made on the effects of essential oils, plant extracts, oriental medicinal plant extracts and desiccant dusts against red mites (Kim *et al*., 2007; George *et al*., 2010; Magdaş *et al*., 2010; Gorji *et al*., 2014; Steenberg and Kilpinen, 2014). Advantages of these naturally occurring products include low mammalian toxicities, short environmental persistence and complex chemistries (limiting the development of

pest resistance against them). These plant extracts are the main option for poultry red mite infestations, in the organic layer farms, where the conventional synthetic acaricides are forbidden to use, and much more, essential oils and plant extracts may be used in poultry farms without withdrawal period. Such control methods will have a positive impact on the transition to sustainable egg production, and will allow a reductio in the use of persistent broad - spectrum synthetic acaricides.

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MONITORING AND PREVENTION TO CONTROL POULTRY RED MITE (*DERMANYSSUS GALLINAE*) IN ORGANIC LAYER FARMS

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Introduction

Dermanyssus gallinae, the poultry red mite (PRM), is highly relevant with respect to both animal welfare and economics in laying hen housing. The prevalence of *D. gallinae* in Europe and other parts of the world is very high and cause high economics losses which estimated by €130 million per year for the EU egg industry (Sparagano et al., 2014). In semi intensive farming systems such as barns, free range or organic farming often a higher prevalence rate could be observed due to better hiding places for *D. gallinae* in these more complex housing systems. Due to developments of resistances and current and future expected legal regulations, treatment options are limited in the EU, especially in organic production systems. Alternatives to chemical acaricides for control of D. gallinae are silica products, which are based on silicon dioxide. The acaricidal effect of such products is attributed to sorptive properties of the particles, which result in the mite's death by desiccation after absorbing lipids from their cuticle (Mewis and Ulrichs, 1999; Akbar *et al*., 2004; Schulz et al., 2014). Silica products have a low oral toxicity (Islam *et al*., 2009) and there is no safety concern for silicon dioxide intake up to 1500 mg SiO_2 per day for humans (EVM, 2003). Furthermore, they are registered and approved as a food additive (EFSA, 2009) and silica products can be applied in the presence of hens at high infestation with *D. gallinae* in practice.
In practice, there is a large variety of methods to apply silica products. Thus, we investigated schedules of application of silica products in organic layer farms. In addition, we tested the efficacy of some products under field conditions throughout laying period.

Material and Methods

The study included 17 organic layer farms located in seven Federal States of Germany. Flock sizes varied between 500 and 3000 hens and housing conditions differed regarding construction (massive or mobile houses) and equipment (multi-tier or single-tier systems). Infestation with poultry red mite was determined by the use of mite traps in the 1st, 12th and 24th week after housing of young hens in the laying farm. The mite traps used was a modified development of traps as described by Safrit and Axtell (1984) consisting of a plastic tube with a corrugated paper inside. The number and distribution of mite traps in the house was carried out as described by Cox *et al.* (2009). At the above mentioned intervals the 36 traps were collected, then frozen and up to a number of 350 mites per trap PRM were counted. In the case of higher numbers, the number of PRM was estimated by

weighing the content of traps. Approximately 20 % of the weight was counted in order to extrapolate the total number of PRM. Additionally, in 15 of the 17 farms at the 24th week a visual monitoring was carried out according to Cox et al. (2009) at 36 control points to compare both methods. The Mite Monitoring Score (MMS) varied from score 0 (no visible PRM) up to score 4 (visible accumulation of PRM with an area over 1 cm²).
For each farm we recorded whether the farm applied a preventive treatment with silica prior to housing of hens, whether farms used an own monitoring method to determine the mite infestation, and whether mite control treatments occurred during the survey period.
First of all, the results of the infestation survey were subjected to an exploratory data analysis. In order to compare the different farms the absolute numbers of PRM and the relative change rate in the number of PRM from the second to the third sampling was calculated. For this, the absolute change in the number of mites was determined from the second to the third sampling and calculated in relation to the number of PRM at the second sampling. This resulted in the relative increase between the second and the third time of the survey. The relationship between mite monitoring and average rate of change in number of mites was tested using the t-test. Effects of mite monitoring (yes/no) on the relative change of PRM infestation was tested using Mann-Whitney-U-Test.

Results

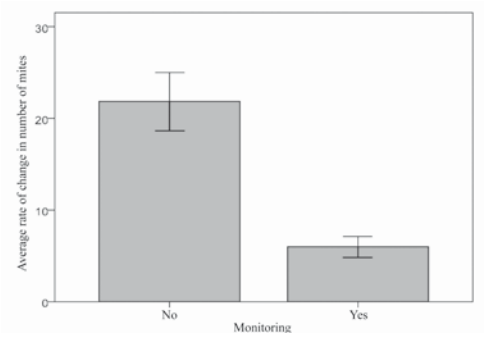
Altogether, 11 of the 17 included farms preventively applied silica and eight famers carried out their own monitoring method. Application of the silica in presence of the hens was carried out in six farms (Table 1). In case of an increased number of PRM during the laying period they could respond promptly due to this monitoring. A total of seven farms neither did a monitoring nor did apply silica in the presence of laying hens.

Table 1 Prevention, monitoring and curative use of acaricides in the farms

Measures	Monitoring by the farmers		Prevention		Curative use of acaricides during the laying period	
	Yes	No	Yes	No	Yes	No
Number of farms	8	9	11	6	6	11

In the farms without monitoring the number of mites increased significantly between the second and third survey sampling for more than 20 times, while on farms with monitoring an increase of 5 times was found (Fig. 1, t-test, $p < 0.001$).

Fig. 1 Relationship between monitoring and average rate of change in number of mites



The average number of PRM per trap varied depending on the farm and the date of sampling. For the first sampling time, at the beginning of the laying period, the average number of mites per trap varied between 0 and 123. At the second sampling time the average number of mites per trap varied between 0 and 2.200, and increased up to values between 0 and 8.500 at the last sampling time.
The comparison of visual monitoring by MMS (Cox et al., 2009) and monitoring by our modified mite traps showed that with these traps small mite infestations could be detected in contrast to MMS.

Conclusions

In investigated organic layer farms different control strategies and mite infestation monitoring program were applied. Some farms used silica as preventive and acaricides as treatment. The obtained results revealed that monitoring and preventive silica applications are important components for successful mite control and management. Farms with curative use of acaricides did not show a lower mite infestation compared to farms without such measures. The reason for this might be that highly infested farms may more readily use curative application than farms without obvious mite infestation. For the planning and application of control measures, it is important to have knowledge about the PRM infestation levels on farm for future management measures. The modified mite traps used in this study are proved to be practicable and can be recommended for the determination of mite infestation in laying houses.

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CHARACTERIZING AND IDENTIFYING LITTER MITE COMUNITES – APPLICATIONS OF DNA BARCODING FOR PRM MANAGEMENT STRATEGIES

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Inventorying species and assessing community structure using traditional approaches can be a challenging task, especially for taxa which are difficult to morphologically identify. However, the use of DNA barcoding (Hebert et al., 2003) can facilitate rapid biodiversity assessments of fastidious taxa by delineating species using molecular operational taxonomic units (MOTU's) (Layton et al. 2014; Smith et al. 2009). The following is a case study where this approach was used to assess the community structure of terrestrial mites across several substrate and habitat types in subarctic Canada (Young et al., 2012).

A standardized sampling program was implemented, where mite communities were sampled in ten localities from seven substrates; moss, soil, litter, woody debris and lichens (Cladina spp., Peltigera leucophlebia, Parmelia/Hypogymnia). Approximately 500 mL of material from each substrate was collected, and extracted using modified Berlese funnels into 95% ethanol (EtOH). As well, two transects of five pitfall traps (with 95% EtOH) were deployed at each site and visited every 3 days over a total period of 9 days.

The specimens in each sample were sorted into morphospecies, and up to five specimens of each were selected for sequence analysis. These specimens were identified to a family level, and oribatid mites were identified to genus. Each specimen was photographed and subsequently placed in a well containing 50 µl of 95% EtOH in a 96 well microplate. Collection details for each specimen together with its taxonomic assignment and its photograph are linked in the Barcode of Life Datasystems (BOLD – www.boldsystems.org). All subsequent laboratory analyses were performed at the Canadian Centre for DNA Barcoding (CCDB – www.ccdb.ca)

Tissue lysis was performed on the whole specimen, and an automated, silica membrane-based DNA extraction protocol was performed using 3 µm glass fibre filter plates (Ivanova et al., 2006), coupled with 0.45 µm glass fibre plates as an additional step to recover the specimen vouchers (Porco et al., 2010). DNA amplification and sequencing reactions were performed following standard protocols (http://ccdb.ca/resources.php) using a cocktail of LepF1/LepRI and LCO1490/HCO2198 primers. Failed amplification reactions were further processed using the MLepF1 and MLepR2 primers. DNA extracts were placed in archival storage at −80°C at the Biodiversity Institute of Ontario. Vouchered specimens were stored in 95% EtOH or slide mounted in Canada balsam, and deposited at both the Biodiversity Institute and the Canadian National Collection of Insects, Arachnids and Nematodes.

Contigs were assembled and edited using CodonCode Aligner v. 3.0.1, and aligned in MEGA 5.03 (Tamura et al., 2007). Each sequence with a length greater than 500 base pairs (bp), less than 1%

ambiguous sites (Ns), and lacking stop codons was assigned a Barcode Index Number (BIN) by BOLD (Ratnasingham and Hebert 2013). Because BINs are highly congruent with biological species (Ratnasingham and Hebert 2013), they allow for global comparison of specimens lacking species designations using a transparent and reproducible method.

In total 8240 specimens (approximately 14% of the total catch) were selected for analysis. Barcode sequences were recovered from 6365 specimens with an overall sequencing success rate of 77.2%. However, taxonomic bias in sequence recovery was detected, with a high of 80.4% in Sarcoptiformes and a low of 68.2% in Trombidiformes; this is likely a reflection of primer binding. These sequences represented 899 BINs.

Using BINs allowed us to assess various aspects of the diversity of mites, including the completeness of sampling, species richness, and faunal similarity between mite communities in several substrates and sites. In a relatively short time frame we were able to document almost three times as many species in subarctic Canada than previous surveys conducted using traditional morphological identifications (Danks, 1981). This framework facilitates rapid and detailed assessment of mite communities in any geographic setting, such as characterizing beneficial mite communities associated with healthy poultry farms absent of Poultry Red Mite infestations.

This study serves as the foundation for a DNA barcode reference library of mites. Traditionally, DNA barcode reference libraries are assembled by sampling local fauna from museum collections that have been expertly identified by taxonomists (Hebert et al., 2013). However, museum collections of mites are often slide mounted, or stored in non-DNA friendly fluid, to facilitate morphological identification. With this non-destructive method in which DNA is extracted prior to detailed morphological assessment, the voucher specimens remain a valuable resource for future taxonomic research, particularly since specimens are already partitioned into MOTU's and can be easily retrieved for morphological study. As the DNA barcode reference library of mites progresses, an increasing proportion of specimens can be assigned to family, genus, and even species by their BIN identity.

Ultimately, litter mite communities in poultry farms can be characterized by high-throughput DNA barcoding methods in the absence of species identifications. Once baselines are determined for various community structures, future analysis could see rapid assessments of bulk samples by way of Next Generation sequencing technologies.

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GENETIC STRUCTURE OF POULTRY RED MITE AND DISPERSAL PATTERNS

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Genetic structure of an organism may reveal information of the epidemiology and may give hints on how a pest is spread, which is important with respect to assessment of the infection pressure, but also important when resistance to treatment develops. We have performed a study of the poultry red mite *Dermanyssus gallinae* and distribution of haplotypes with an emphasis of mite sampled in layer farm production sites in Norway. We also attempted to compare these variants with wild mite sampled outside infected layer farms and if these had a genetic relationship to the mite collected in layer farms. Molecular investigations do not identify mite collected from the wild birds to be *D. gallinae*. Analysis of layer mite, revealed two predominant haplotypes variants (B9 and A16) in Norwegian layer farms. Most farms seem to have only one or a few dominating haplotype variant(s), with examples of the same haplotype reemerging after reinsert of birds in an infected house. When statistically analyzing the distribution of mite haplotypes found in a layer houses, with respect to the origins of the birds, significant associations are identified. Our data on the dispersal pattern in our data, suggests that PRM in Norway commonly harbor a population of only a few haplotypes and with a limited spread of these mite, and seems not to be associated with the wild fauna.

MOLECULAR TECHNIQUES APPLIED ON ARTHROPOD VECTORS – USEFUL APPROACH FOR MITE MONITORING AND IDENTIFICATION?

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Identifying species of medical and veterinary important arthropods as parasitic mites and vectors of infectious agents by using keys based on morphological features is both difficult and time-consuming. When regarding Dermanyssus gallinae, Brännström et al. (2008) studied whether population genetic markers such as analyses of ribosomal RNA (rRNA) genes by the internal transcribed spacers (ITS1 and ITS2) as markers could be used to detect genetic variation between closely related species (Berrilli et al., 2002). Brännström et al. (2008) applied this approach to study whether there was a possible spread of D. gallinae and mites containing pathogens from wild bird populations into different egg-laying operations. Moreover, Öines and Brännström (2011) used the same markers, and the mitochondrial cytochrome c oxidase subunit I gene, COI (Hebert et al., 2003), to study routes of transmission of D. gallinae in Norway and Sweden. Since then, several research groups have applied such molecular techniques to test interrelationships among different Dermanyssus spp based on morphological and molecular characters, where the latter was more informative (Roy et al., 2009a) and describe phylogenetic relationships and phylogenetic inferences in populations of D. gallinae both on national and international levels (Marangi et al., 2009; Marangi et al., 2014).

The standardized DNA barcodes are a 658-bp region of COI (Hebert et al., 2003) opened a window of opportunity to identify arthropod vectors to species and through metabarcoding (Taberlet et al., 2012) even quantify the different proportions of species within bulk samples containing high numbers of different, e.g. mosquito species (Lilja et al., in prep). Information gathered from DNA barcodes can be used beyond taxonomic studies and may have far-reaching applications within the fields of biosecurity within the area of medical- and veterinary entomology, including vector ecology, by early identification of invasive arthropod pest- or vector species. In 2006, an outbreak of bluetongue virus, affecting ruminants vectored by biting midges (Culicoides spp), was introduced to the Netherlands by unknown routes (Mintiens et al., 2008) and was then further spread to many countries in northern Europe (Carpenter et al., 2009; Sternberg et al., 2010). By that time, only three Culicoides species were described in Sweden, therefore, a barcoding research program was initiated and 31 of the 37 species found were in agreement with the morphological determination (Ander et al., 2013).

Beside the above mentioned monitoring and identification applications of molecular techniques, there are some additional or corresponding areas where molecular approaches has been applied on studies of D. gallinae. These studies regarded host range, dependence of ecological parameters and the unclear patterns of sharing haplotypes in different mite populations (Roy et al., 2009b), phylogeny (Roy et al., 2010) and in studies on presence and development of resistance to different acaricides and related mechanisms (Roy et al., 2009c; Bartley et al., 2015).

Endosymbionts in arthropods is widely studied, mainly in insects, but recently also in Acari. De Luna et al. (2009) isolated bacteria from D. gallinae that could be further investigated to explore their possible role for the fitness of the mite, with available molecular techniques.

Interesting immunological approaches, affecting hosts as well as the mite itself, has been described by Bartley et al. (2009) and Harrington et al. (2009a; 2009b; 2010a; 2010b) and will most probably exploit the molecular tool-box to reveal novel findings within this important area of research.

In conclusion, this shows that the molecular techniques are promising tools that gives an opportunity to continue studying different biological aspects of the mite as the knowledge need to be expanded and further research is needed.

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UNDERSTANDING OF THE ROLE OF PRM IN BACTERIA SPREAD

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Dermanyssus gallinae, poultry red mite (PRM), is an obligatory haematophagous ectoparasite of both domestic and wild birds. The mite has been reported to parasitize on numerous bird species and is considered as one of the major problems in poultry industry all over the world, more frequently in free range systems, but also in cage units, especially in laying hens facilities (Fiddes *et al*., 2005; Guy *et al*., 2004; Hamidi *et al*., 2011; Sparagano *et al*., 2014; Zenner *et al*., 2009). The infestation of PRM in poultry results in stress, loss of body weight, decreasing egg production, anaemia and, in severe cases, in mortality (Hamidi *et al*., 2011).
Recent investigation showed that bacterial microflora related to the PRM depends on environmental factors (poultry farming practices) and geographic isolation, but also, to some degree, the stability of bacterial populations was observed within a specific time scale (Valiente Moro *et al*., 2011). PRM can also represent a potential vector of several pathogens including different bacteria and viruses (Chikuba *et al*., 2008; Eriksson *et al*., 2010; Hamidi *et al*., 2011; Huong *et al*., 2014).
To detect pathogens in vectors, especially the non-culturable bacteria, molecular methods are useful, such as denaturating gradient gel electrophoresis (DGGE) and temporal temperature gel electrophoresis (TTGE) (Valiente Moro *et al*., 2009), but on the other hand, it is very important to select the proper method for the preparation of the PRM samples and for the isolation of DNA (Desloire *et al*., 2006).

Salmonella and PRM

Bacteria *Salmonella* Enteritidis (SE) is still a major cause of food-borne disease worldwide. Eggs and egg products are the most often identified food vehicles in the *Salmonella* outbreaks, and SE is the most often isolated serovar from human infections. Numerous investigations confirmed the relation between PRM and SE (Hamidi *et al*., 2011; Valiente Moro *et al*., 2007a, b; Valiente Moro *et al*., 2009). SE can be found in the PRM both in populated and depopulated units, what represent the possible threat for currently present poultry flocks, but also for further ones to be housed in the same units (Valiente Moro et al., 2007b). If the SE is present in PRM on the populated farm, the clinical signs may not occurred in poultry, but nevertheless, the presence of SE could pose a risk to public health through Salmonella-positive mites being squashed on eggs (Hamidi *et al*., 2011). Transovarial and transstadial transmission of Salmonella by PRM was reported previously (Hamidi *et al*., 2011; Valiente Moro *et al*., 2007a), as well as the multiplication of SE inside previously infected mites (Valiente Moro *et al*., 2007a), what clearly stated that PRM can serve as biological vector of SE and natural carriage of these bacteria in poultry premises (Valiente Moro *et al*., 2009).

Campylobacter and PRM

The most frequently reported food-borne illness in the European Union (EU) is campylobacteriosis, caused by *Campylobacter jejuni* or *coli*. Raw or undercooked poultry meat represent one of the most important source of human infection (Anonymous, 2014). Even if this is zoonosis of significant socio-economic impact both on humans and poultry, the investigations or articles related to PRM and Campylobacter are scarce. Van Sauers and Burt (2009) concluded that the PRM does not carry *Campylobacter spp.* and thus the infection of the mites cannot be correlated with the *Campylobacter* status of the farm.

Avian chlamydia and PRM

Avian chlamydiosis is the disease of enormous importance in different cage and aviary birds, as well as in pigeons flocks, but it also represent a dangerous zoonosis related to poultry, especially turkeys and ducks (Dickx and Vanrompay, 2011; Hulin *et al*., 2015; Teske *et al*., 2013). PRM can be found in companion bird's flocks, especially in pigeons, budgerigars and canaries (George *et al*., 2015). The investigation conducted by Circella *et al.* (2011) clearly showed a relation between PRM and *Chlamydia psittaci*, causing severe infection in canary flock. In this case, the authors stated that *D. gallinae* could have played an important role predisposing the canaries to the development of chlamydiosis, by inducing anaemia and debilitation. Investigations that could reveal the relation between PRM and chlamydial species in poultry production weren't conducted recently.

Other pathogenic bacteria and PRM

In the recent study conducted by Huong *et al*. (2014) it was demonstrated that *Mycoplasma synoviae* (MS) and *Mycoplasma gallisepticum* (MG), two important poultry pathogens, can be prevalent in and transferred by PRM in chicken flocks. Valiente Moro *et al*. (2009) found that PRM carried other pathogens such as *E. coli*, *Shigella* sp., and *Staphylococcus*. Also a zoonotic agent, *Mycobacterium* spp., was detected in PRM, and the authors also suggested the possible transovarial and transstadial transmission of this pathogens by *D. gallinae* (De Luna *et al*., 2008). Bacteria *E. rhusiopathiae* was isolated from PRM (from the integument as well as from the interior of the mites) found on the hen farm with clinical signs of erysipelas (Chirico *et al*., 2003).
Older studies revealed the isolation of *S.* Gallinarum (Zeman *et al*., 1982) and *Listeria monocytogenes* (Grebenyuk *et al*., 1972) from PRM, but also the transmission of *Pasteurella multocida* (Petrov, 1975) and *Coxiella burnetii* (Zemskaya and Pchelkina, 1967) were reported (George *et al*., 2015)

Since the effective measures for control of PRM in poultry farms still does not exists, it is important to be aware of the role of PRM as reservoir hosts of different pathogenic bacteria. Also, their persistence in poultry units between flock cycles is of great significance, as a source of infection for the subsequent flocks.
Molecular tools, based on PCR and sequencing, should be used for detailed characterization of pathogenic microorganisms, as well as for determination of bacterial communities associated with PRM, what potentially can lead to progress in the knowledge of PRM vectorial role, monitoring and biological control methods.
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MESOSTIGMATA DERMANYSSINA MOUTHPARTS EVOLUTION: FROM PREDATORISM TO PARASITISM

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Dermanyssina represents the most species-rich, ecological diverse and geographically wide spread cohort of Gamasida. They are found worldwide and include free living predaceous or fungivorous soil inhabiting forms, as well as pollenivores and facultative or obligatory external or internal parasites of vertebrates and invertebrates (Evans, 1992; Alberti and Coons, 1999; Lindquist *et al.*, 2009).

The gnathosoma is certainly one of the most distinctive features in mites and the adaptations of these mouthparts to different nutritional patterns are enormous and comparable to the ones observed in insects (Evans, 1992; Alberti and Coons, 1999). Several hypothesis suggest that fluid feeding is a derived condition in mites; and observations among gamasid mites suggest that ingestion of particulate food could be the plesiomorphic condition (Evans, 1992; Walter and Proctor, 1998).

The gnathosoma is composed basically of two main components: the cheliceral frame and the infracapitulum (=subcapitulum). The cheliceral frame is a membranous part where the chelicerae are inserted, in a movable way, by means of the "cheliceral sheaths". The infracapitulum is located ventrally to the chelicerae and is mainly composed of the palpcoxae that meet the tegulum dorsally located (terminology after Evans and Loots, 1975). The resulting gnathosomal tube is divided into a dorsal and a ventral part by the cervix (=subcheliceral plate), which connects the mesial walls of the palpcoxae. Dorsally, the chelicerae lie over the infracapitulum, which contains the mouth and the pharynx. The mouth is defined as the opening at the base of the labrum leading into the pharynx. The region anterior to the mouth is called "pre-oral channel". The labrum is an unpaired projection covering the pre-oral channel and the mouth. Hence the infracapitulum is a cone like projection with the pre-oral channel at the tip and the chelicerae dorsally located (Alberti and Coons, 1999). The pre-oral channel dorsally sealed by the labrum and posteriorly connected to the pharynx and the esophagus, is responsible for sucking up fluids. In free living species, the labrum and the lateral walls of the pre-oral channel are provided with numerous tiny ridges or processes that are considered to act as a sieve preventing the entrance of solid particles of food. Such system is reduced in species that live on blood or pre-orally liquefied food (Evans, 1992; Evans and Loots, 1975; Flechtmann *et al.*, 1994; Di Palma *et al.*, 2006).

The infracapitulum presents ventrally an infracapitular gutter with characteristic transverse denticulate ridges and a well developed structure: the tritosternum. At the tip of the infracapitulum there are paired projections: the laciniae and the horn-like corniculi plus a pair of salivary styli where the secretions of salivary glands discharge (Gorirossi- Bourdeau, 1956; de Lillo and Aldini, 1994; Di Palma *et al.*, 2006).

Plesiomorphically the chelicerae show a terminal chela and are composed of three articles: a basal (first) article where protractor and retractor muscles are inserted, a middle (second) article that ends

with a fixed digit of the chela and a movable digit (third article) ventrally located to the fixed one. The ancestral function of the chelicerae was probably to grab and manipulate the food to the mouth, but capability of piercing, cutting and ripping the food developed rapidly (Evans, 1992; Alberti and Coons, 1999).

Predatory Dermanyssina, usually grab their prey by means of their chelicerae, hence the cheliceral digits cut an opening into the prey and the alternating action of the chelicerae and their chewing activity together with the secretion of the salivary glands, through the salivary styli (Gorirossi-Bourdeau, 1956; de Lillo and Aldini, 1994; Nuzzaci *et al.*, 1996; de lillo *et al.*, 2001; Di Palma *et al.*, 2006), result in partial digestion of the prey. Sclerotized corniculi and palps help in holding the prey during feeding activity (de Lillo and Aldini, 1994; Di Palma *et al.*, 2006). The fluid food flows between and around the chelicerae to reach the pre-oral channel bordered by the lips. However the amount of fluid produced during feeding activity may exceed the amount that the mite can swallow thanks to the pharyngeal pump, hence the overflow reaches the posterior region of the gnathosoma where the bases of the first legs and the folded cuticle redirect it laterally and anteriorly to reach the tip of the infracapitulum passing through the infracapitular gutter and the tritosternum (Wernz and Krantz, 1976). Cheliceral morphology, shape and dentition have been correlated with the type of prey and even with the possibility, for predators, to switch to plant-feeding (Buryn and Brandl, 1992; Flechtmann and McMurtry, 1992a,b; Adar *et al.*, 2012).

Obligate phytophagous are rare among Dermanyssina and almost exclusively on fungi, pollen or nectar. They show stout chelicerae with few teeth in the fixed digit and a concavity in the movable (Fam. Ameroseiidae). It is assumed that fungal hyphae and spores are chopped or punctured while held in the concavity (Alberti and Coons, 1999).

The chelicerae of the facultative bloodsuckers still have some of the characteristic of the predators (powerful claws and well developed teeth) and feed mainly on a mixed diet: i.e. they take blood by gnawing dried clots formed at the sites of scratches on the host body (Lagutenko, 1962).

Zooparasites in Dermanyssina show adaptations that are obvious in obligate blood feeders (Krantz, 1978; Evans 1992). The chelicerae appear to be the most adaptive in this respect and specialized for host penetration. They tend to be elongated, slender, more or less pointed without conspicuous teeth and involved in host piercing (Lagutenko, 1962; Radovsky, 1969; Nuzzaci and de Lillo, 1995; Phillis, 2006). Moreover they might develop a concavity in the inner (paraxial) surface so that when placed together they form a channel (sometimes held together by a coupling device) while the terminal chelae (fixed digit) become more or less reduced. The functional morphology of such channel is still controversial: i.e. either for piercing or a tube for sucking up fluids, or both function at the same time (Lagutenko, 1962; Radovsky, 1969; Phillis, 2006). On the other hand the strainer system in the pre-oral channel tend to be absent so as the number of denticles in the infracapitular gutter and the tritosternum may be vestigial or absent (Evans and Till, 1965; Radovsky, 1969; Evans and Loots, 1975; Evans, 1992; Lindquist *et al.*, 2009). Finally the apical paired projections (laciniae) are poorly developed as well.

Species strongly adapted to parasitism show a vestigial fixed digit while the stylet like movable digit shows strong teeth and a pronounced capacity for tearing or piercing the host tegument to allow the feeding (Lagutenko, 1962; Nuzzaci and de Lillo, 1995; Phillis, 2006).

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POPULATION DYNAMIC OF DERMANYSSUS GALLINAE IN POULTRY

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The poultry red *mite Dermanyssus gallinae* is a cosmopolitan ectoparasite of both wild and domestic birds. It is one of the most economically important pests of laying hens, especially in European countries (Chauve, 1998). The primary host of *D. gallinae* is poultry. Its economic impact is partly due to the downgrading of eggs stained by the blood of squashed mites, but also, during the course of a massive infestation, the hens also suffer for anaemia which is further responsible for a reduction in their egg production and can lead to the death of the birds. Moreover they can also act as a vector for several poultry pathogens including Salmonella spp., avian spirochetes, fowl poxvirus and equine encephalomyelitis virus (Valiente Moro *et al*., 2005). Finally, this mite may also attack mammals including man (Auger *et al*., 1979).

D. gallinae spend the greater part of their lifetime away from the birds and are usually found in nest boxes or sequested in the cracks and crevices of the poultry house where they seek refuge when not feeding. The life cycle of *D. gallinae* involves 5 stages: egg, larva, protonymph, deutonymph and adults stage feeds on birds, usually at night (Wood, 1917; Kirkwood, 1968). On these, only the nymph and adult stages feed on birds, for 30-180 min at a time. After mating, an adult female lays 1-9 eggs every 12-24h following a blood meal. Female will feed and lay eggs several times during their lifetime, laying around 30 eggs in total. Under favorable conditions (28-30 °C), larvae emerge from eggs within 2-3 days and molt into the protonymph 1-2 days later without feeding. The protonymph is hematophagous and needs to feed before molting, where the resulting deutonymph then has to take another blood meal in order to molt into a male or female adult. This life cycle can progress very rapidly if a host is available and temperature and hygrometry are suitable, which is usually the case in poultry facilities. But *D. gallinae* can survive for long period without feeding, allowing these mites to survive in poultry houses between flocks.

D. gallinae is difficult to eradicate from infested farms dues to its short lifecycle, high reproduction rate and, in some cases, increasing resistance to commonly used acaracides that are becoming less efficient.

To help further development of control strategies (use of sanitizing empty poultry houses between flocks, acaricides, alternative strategies) we developed a population model for *D. gallinae* that allows to test *in silico* the efficacies of control methods (Huber, 2011). In this model the life cycle of *D. gallinae* has been simplified in 3 developmental stages where the non-feeding egg and larva stages are grouped, the feeding juvenile proto and deutonymph stages form the “nymphs” stage and adults are also combined into a stage (Figure 1). Two different values of intrinsic fecundity (3 and 8 eggs / female/ laying) were tested. Starting with only a single adult leads to a stationary population is reached after 6 months for 3 eggs and with oscillation after 2.5 months for 8 eggs (Figure 2).

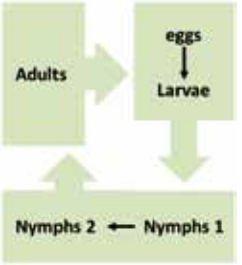

Figure 1.

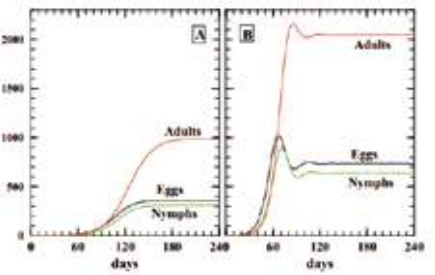

Figure 2. A. Intrinsic fecundity of 3; B. Intrinsic fecundity of 8.

Mechanical cleaning and sanitary clearance on the mite population were both applied in the model at day 150, about one month after the stationary state of the model is reached. The mechanical cleaning consists in the elimination of accessible *D. gallinae* at the time of application, leaving other stages intact. Two situations were tested: the first where only the adults are eliminated (Figure 3A) and the second where both nymphs and adults are eliminated (Figure 3B). In the first case, the impact is weak with a short recovery time (about 30 days) to the initial state for all stages. In the second case, the population drop is more pronounced and the recovery to the stationary state was much longer (more than 60 days) suggesting that diligence is an important factor in the success of mechanical house cleaning to manage *D. gallinae*, even if it only could lead at best to a mid-term effect. Sanitary clearance represents the situation experienced during empty period between flocks in a poultry house where feeding stages are unable to take a blood meal. Mites only survives and it leads to a complete disappearance of eggs, to a concomitant increase of nymphs within 10 days, followed by a slow decrease of nymphs due to mortality as well as a rapid drop in the number of adults, also due to mortality. But it only results to a temporary disappearance of adults, eggs and larvae. Nymphs are the most starvation-resistant stages of *D. gallinae* as they can survive without a blood-meal for 28 weeks (Kirkwood, 1963). Simulation demonstrates that increasing the length of the sanitary clearance phase alone had no effect on the recovery time (Figure 4).

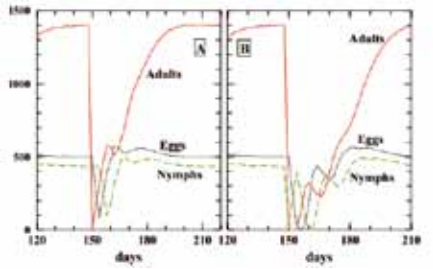

Figure 3.A.Effect of mechanical cleaning at day 150. A. all adults are removed; B. all nymphs and adults are removed.

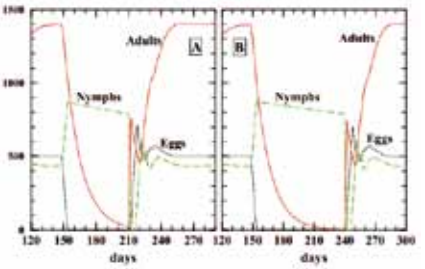

Figure 4. Effect of a sanitary clearance of 60 days (A) or of 90 days (B).

Other means for controlling *D. gallinae* population were subjected to this model using computer simulation as different acaricide protocols which correspond to kill a proportion of nymphs and adults during the persistence of the treatment, once or with repetitions. This modeling approach to describe the effect of current control strategies could also be used to test the effects of new and alternative control strategies, whilst simultaneously performing cost-benefit analyses for the strategies considered.

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