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Understanding the Biology & Control of the Poultry Red Mite, *Dermanyssus gallinae*

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Abstract

*Dermanyssus gallinae*, the poultry red mite (PRM), is a blood feeding ectoparasite capable of causing pathology in birds, amongst other animals. It is an increasingly important pathogen in egg-layers and responsible for substantial economic losses to the poultry industry worldwide. Even though PRM poses a serious problem, very little is known about the basic biology of the mite. Here we review the current body of literature describing red mite biology and discuss how this has been, or could be, used to develop methods to control PRM infestations. We focus primarily on the PRM digestive system, salivary glands, nervous system and exoskeleton and also explore areas of PRM biology which have to date received little or no study but have the potential to offer new control targets.

Keywords: Dermanyssus gallinae, poultry red mite, biology, anatomy, control, mode of action
1. Introduction

Dermanyssus gallinae, the Poultry Red Mite (PRM), belongs to the Order Mesostigmata which incorporates many mite species that vary considerably in morphology and behaviour. Many species are phytophagous, saprophagous or predatory free living species (Koehler, 1999; Gerson et al., 2008) whilst others, including PRM, have obligatory parasitic behaviour.

PRM is a haematophagous ectoparasite of poultry and wild birds (Kristofik et al., 1996; Brannstrom et al., 2008), requiring blood meals to develop into the last 3 subsequent stages of its life cycle as well as for development of eggs during oviposition (See figure 1). Predominately females feed on blood several times during their lifetime though it has been reported that males may blood feed intermittently (Chauve, 1998). Whilst PRM feeds primarily on birds, it is cosmopolitan in its choice of host and has been reported to be capable of feeding on rodents (Bakr et al., 1995; Lucky et al., 2001; Abd El-Halim et al., 2009) and humans (Beck, 1999; Rosen et al., 2002; Bellanger et al., 2008; Collgros et al., 2013;) though these are most likely accidental hosts and do not sustain a complete PRM life cycle. PRM has been
implicated as a transmission vector for several significant animal pathogens, including some that are zoonotic. PRM-mediated transmission between hens has been shown directly for *Borrelia anserine*, fowl poxvirus and eastern equine encephalitis virus (Chamberlain & Sikes, 1955; Shirinov *et al.*, 1972; De Luna *et al.*, 2008; Valiente Moro *et al.*, 2009). The transmission of *Salmonella* spp. between birds by PRM has also been demonstrated and moreover the bacteria can be transmitted by mites transovarially to their progeny, rendering PRM a potential reservoir for zoonotic salmonellosis (Valiente Moro *et al.*, 2007). Human cases of salmonellosis have been significantly reduced in recent decades however there is still an industry-wide requirement for safer and better defined vaccines against salmonellosis (Desin *et al.*, 2013). The potential of *D. gallinae* to harbour and transmit pathogens therefore appears to be an important and emerging problem.

Pathology due to PRM in parasitized birds is variable depending on infestation rates. Symptoms from host birds most notably include a decline in general bird health due to lack of sleep and increased self-pecking (Kilpinen *et al.*, 2005). Severe PRM infestations can lead to more serious effects such as cannibalism, anaemia and in
some cases even bird death (Chauve, 1998; Kilpinen, et al., 2005). The most economically damaging symptom of PRM infestations is the reduction in egg laying amongst hens as well as a decline in egg quality (Chauve, 1998; Cosoroaba, 2001).

Many controls against PRM, such as the use of chemical acaricides and silica dusts, are often sold as broad spectrum substances for controlling a range of farmyard and domestic pests. Reports of PRM resistance to acaricidal drugs containing amitraz, carbaryl and permethrin (Zeman & Zelezny, 1985; Beugnet et al., 1997; Marangi et al., 2009), allied with genetic variation between red mite populations (Brannstrom, et al., 2008; Potenza et al., 2009; Roy & Buronfosse, 2011) suggest there is an urgent requirement for research to uncover more specific control strategies. Detailed knowledge of *D. gallinae* biology and behaviour is comparatively underrepresented in the literature given its commercial impact; this was estimated, for instance, in 2005 to cause €130 million per annum economic loss in Europe alone (Van Emous, 2005).

Here we present a brief overview of the basic understanding of PRM biology with specific regard to how this relates to current and potential future controls and their modes of action. We provide microscopic imagery of internal morphology, currently
lacking in the existing literature, and discuss the types of control that target PRM systems at a cellular and systematic level. It seems increasingly likely that control of PRM will require the application of integrated approaches, a concept we discuss against the backdrop of current ineffectiveness of the existing standalone controls. PRM is yet to be managed efficiently in large scale commercial farming facilities, which leaves an open platform for the introduction of a range of control options and potential for a standardised integrated control management.
2. External Morphology

*D. gallinae* thrives in environments of high (at least 70%) humidity whereas it does poorly in arid conditions because it cannot fully retain moisture (Nordenfors *et al.*, 1999) despite being externally protected by an exoskeleton (see Di Palma *et al.* (2012) for detailed diagrams). A dorsal exoskeleton shield covers the length of the idiosoma (body) and is not gender specific. Ventrally however, females present two separate shields; a genitoventral shield spanning posteriorly from leg pairing II and a smaller, more rounded anal shield. Males possess a single, smaller ventral shield comprised of a seemingly fused joining of the genitoventral and anal shields (Di Palma, *et al.*, 2012).

The exoskeleton of acari is made of chitin, a tough and resilient polymer. In an unmodified state, often seen in the larval stage, chitin is translucent and comparatively flexible. Hormones secreted through pores trigger the polymerisation of chitin which is mixed with various protein families and phenolic compounds creating a sclerotized layer. The sclerotized cuticle offers a stiff layer which defines the mite’s body shape, aids with muscle attachment and limits water loss (Evans & Till, 1979; Hackman, 1982). Sclerotized cuticle can be identified by a
brown/yellowish area, often covering the whole of the outer adult body and is replaced during each moulting stage as it cannot be extended during mite growth.

The outer part of the mite exoskeleton, known as the epicuticle, consists of a layer of wax which further limits water loss, and a cement layer which protects the cuticle from external abrasion. Red mite controls, such as silica dust (Maurer & Perler, 2006) and diatomaceous earth powder (Kilpinen & Steenberg, 2009), seek to dry out these outer layers and kill PRM through desiccation. Lipid removal through adsorption is thought to be due to the surface migration of fatty molecules into the hollow crystalline structure of the dust particles (Ebeling, 1971) which also interrupt the lipid layers through physical sheering (Vincent et al., 2003). These inert dust particles act via a chemically neutral mechanism and are not associated with any forms of resistance to mite controls, however their use can be limited by environmental conditions including very high humidity (>80%) and high levels of environmental dust within farming units (Kilpinen & Steenberg, 2009). Refinement of materials selected for dusting could possibly have potential to extend the longevity of this type of control, as could the use of dusts in liquid form. Schulz et al (2014),
however, reported no overall significant difference between liquid and dust form silica-based controls.

There are prospects to develop novel control methods for PRM based on the use of entomopathogenic fungi. Fungi produce extracellular chitinases which when introduced to PRM chitin-rich hydrophobic coats can kill mites via desiccation (St et al., 1996). Fungi exhibit delayed pathology within PRM allowing for its wide dissemination, thus eliminating large mite populations (Tavassoli et al., 2008; Tavassoli et al., 2011). Beauveria bassiana has proved to be effective against PRM more than 10 days post exposure (Steenberg & Kilpinen, 2003) whilst Trichoderma album (Kaoud, 2010) and Metarhizium anisopliae fungi (Tavassoli, et al., 2011) are efficient at high spore concentrations as new acaricides. The use of parasitic fungi as a way to control PRM infestation could however generate downstream environmental disequilibrium, since entomopathogenic fungi are generally not specific for PRM and may affect other naturally existing insect populations.

Heat treatment is also regularly used to reduce PRM populations in egg laying units in Norway and The Netherlands (M. Mul et al., 2009). Heating hen houses to a
recommended 55°C kills PRM though it is suggested that high mite mortality also occurs at 35°C (Tucci et al., 2008). Heat treatment between flocks is not recommended for controlling PRM by itself but as part of an integrated approach (M. F. Mul & Koenraadt, 2009).

3. Digestive tract

The mite digestive tract is a comparatively well studied part of the anatomy of several species including the storage mite *Lepidoglyphus destructor* (Erban & Hubert, 2011), the house dust mite *Dermatophagoides farina* (Dumez et al., 2014), the sheep scab mite *Psoroptes ovis* (Hamilton et al., 2003) and a range of synanthropic species (Erban & Hubert, 2010). In combination these studies provide an outline of the general anatomy of mites (Mehlhorn, 2001), although the specific physiology of PRM, which are haematophagous mites, may be substantially different.

It is largely accepted that the ‘general’ mite digestive tract is organised into three recognisable parts; the foregut, midgut and hindgut. The foregut comprises the pharynx and oesophagus extending posteriorly from the gnathosoma to the midgut.
Active food movement occurs through the oesophagus of PRM (J. Pritchard, personal observation) presumably via the action of pharyngeal dilator muscles and valves as has been demonstrated for *P. ovis* (Mathieson & Lehane, 2002).

The midgut, or ventriculus, and its associated caecae are thought to be primarily responsible for PRM digestion as is for other haematophagous mites. The midgut is located proximally between the third leg pairing and dorsally to most other internal soft tissue including the malpighian tubules (see figure 2a+b). In unfed mites, the midgut appears reduced in size but in engorged mites it expands to fill most of the body cavity (Evans, 1992; Nisbet & Billingsley, 2000) as would be expected of a haematophagous parasite that ingests large blood meals. Enlargement of the midgut creates an increased surface area for digestive processes and also reduces the distance of the midgut and caecae from internal organs that depend on nutrient transport from the gut.

Acari midgut digestive cells are generally classified into three types (anterior midgut cells, caecal cells and posterior midgut/hindgut cells) based on their function and location. Anterior midgut epithelial cells contain large vacuoles and go through a
state of cytoplasmic degeneration whilst digesting food (Brody et al., 1972; Coons, 1978). In engorged mites, these cells detach from the gut mucosa and are able to engulf ingested material within the gut lumen becoming swollen and highly vacuolated. The presence of intracellular large vacuoles that contain material of a similar density to that seen in the gut lumen suggests that food digestion is carried out at least in part intracellularly (Mathieson & Lehane, 2002). The autophagic-lysosomal pathway is the most likely way that intracellular digestion occurs and is thought to be initiated by the action of parasite endopeptidases such as Cathepsin D and Cathepsin L (Nisbet & Billingsley, 2000). Vaccination of poultry with recombinant PRM Cathepsin D or Cathepsin L induces anti-Cat D or anti-Cat L specific IgY immunoglobulins and when these are ingested by PRM in an in vitro feeding system, they cause increases in mite mortality (Bartley et al., 2012). Most likely these IgY antibodies bind directly to secreted Cathepsins D and L in the lumen of the mite gut however vaccine-induced immunity is believed also to cause damage to the gut barrier through direct binding of immunoglobulins to membrane-bound proteins, even though complement induced antibody upregulation may be required (Kemp et al., 1989; Bartley, et al., 2012).
PRM have six caecae extruding distally in a lateral manner, four anterior and two posterior, all connected to the midgut in parallel to the third leg pairing (see figure 2a+b). Caecal epithelial cells in various mite species are densely packed with lysosomes, smooth endoplasmic reticulum and mitochondria, all indicative of high metabolic activity related to digestive enzyme activity. Brody et al (1972) proposed that the lack of visible particulate material in the caecae of the house dust mite *D. farinae* indicates that caecal cells secrete enzymes which are used for digestion in the anterior midgut. However Erban and Hubbert (2011) demonstrated that midgut and caecal-wide hydrolysis of fluorescent substrates by several proteolytic enzymes occurred in the storage mite *L. destructor*. Given the significant expansion in size and large volume of blood found in the caecae in engorged PRM (see figure 2a+b) we suggest its caecae are also actively involved in food digestion.

The start of the hindgut in PRM is defined by the junction of two large malpighian tubules at the posterior end of the midgut (see figure 2). Posterior midgut cells and hindgut cells in several species of mite have been shown to be apically-basally elongated with large microvilli (Brody, *et al.*, 1972; Mothes-Wagner, 1985). It is
believed the hindgut in mites is involved in water reabsorption and nutrient uptake, though the mechanism is yet unclear. Water reabsorption creates a black food bolus in PRM (J. Pritchard, personal observation) as seen also in *D. farinae* (Brody, *et al.*, 1972). Berridge and Gupta (1967) hypothesised that active transport of ions from the rectal papillae of the blow fly into intercellular spaces causes an osmotic gradient and thus water moves from the lumen to the hemolymph through osmosis. Further understanding of water reabsorption in PRM could help identifying potential targets for control.

The peritrophic membrane is another potential future target for control; its presence has, however, neither been confirmed nor rejected in PRM. The presence of a peritrophic membrane in some mites is well defined such as in the flour mite *Acaris siro* (Hughes, 1950; Sobotnik *et al.*, 2008) but seemingly absent in others (Coons, 1978). The peritrophic membrane is a lamellar structure of chitin and associated structural proteins, which surrounds the food bolus protecting the gut against pathogenic microorganisms and compartmentalising food for digestive activity. Sobotnik *et al.* (2008) reported that the ingestion of calcofluor (which binds chitin in
the membrane) and diflubenzuron (inhibits chitin synthesis) reduces *Acaris siro* population growth. Interfering with chitin or the chitin associated proteins could be a viable and safe method for PRM control since these molecules are absent in birds and mammals. In haematophagous arthropods peritrophic membranes have been suggested to protect epithelial cells against sharp edged haemoglobin crystals that form with blood meals (Berner *et al*., 1983; Eisemann & Binnington, 1994). In several species of ticks the membrane has been described in great detail (Matsuo *et al*., 2003; Zhu *et al*., 1991) however as Eisemann & Binnington (1994) have noted, targeting the peritrophic membrane in arthropods presents immediate difficulties. This includes the possible destruction of antibodies and effector molecules from vaccinated hosts within the proteolytic environment of the gut as well as the necessity of a repeated control action every time a new peritrophic membrane is formed during a new blood meal.

Proteins associated with the PRM midgut are not normally exposed to the avian immune system during mite feeding so the bird host does not generate a natural antibody response to them. These ‘concealed’ gut antigens within the PRM
therefore have potential to be selected as targets for vaccination as antibodies from
vaccinated bird hosts would be taken up in a mite blood meal. Immunising hosts with
gut-derived concealed antigens has proven successful for development of the
vaccine TickGARD® (Hoechst Animal Health; Australia) against the midgut-
expressed BM86 protein of the cattle tick *Rhipicephalus microplus* (Willadsen *et al.*, 1995). Though no homolog to BM86 has been found in PRM the same strategy has
recently been pursued using other internally expressed proteins (Arkle *et al.*, 2008;

4. Nervous system

Acari, including PRM, have a clustered region of nervous tissue known as the
synganglion in the anterior section of the idiosoma, just anterior to the midgut (see
figure 3). In PRM this central nervous mass is separated into two regions, the supra-
oesophageal nervous mass and the sub-oesophageal nervous mass. In agreement
with Serverino *et al* (1984) we describe four pairs of pedal ganglia extending distally
from the supra-oesophageal mass (Figure 3a), each ganglion connecting to each of
the eight legs of the mite. The sub-oesophageal mass (figure 3b) is bisected longitudinally by the oesophagus and surrounded by fat tissue.

Chemical acaricides against PRM predominantly target neurotransmitters and synapses between neurons within the synganglion tissue (see figure 4). These substances classically target the voltage-gated Na\(^+\) channels of pre-synaptic axons, propagating a continually depolarised membrane leading to loss of action potential and eventually mite paralysis. Mites that cannot move to find food or escape environmental factors eventually die. Sprayed acaricides are most likely taken up via sites of gaseous exchange in the PRM principally through the stigmata, located adjacent and dorsally to coxae II and III, through the peritreme branching network, into the haemolymph and finally through to the synganglion tissue. Mite synganglion tissue is reported in several mite species to be covered in an acellular sheath of neural lamellae which allows access of nutrients and other compounds (Coons & Axtell, 1971; Woodring & Galbraith, 1976). A rind of perikaryon (neural somata) cells further surrounds a central neuropile of axons and dendrites (Severino, et al., 1984) where it is likely PRM neurological controls are mostly active.
PRM populations are known to be resistant to earlier generations of neurological pesticides, such as dichlorodiphenyltrichloroethane (DDT) and the pyrethroids (Zeman & Zelezny, 1985; Beugnet, et al., 1997). DDT is now banned for pesticidal control within the EU (UNEP-Chemicals, 2006) as it accumulates to high concentrations in food chains, persists in the fatty tissues of animals and humans, and is associated with risk of several chronic illnesses (Orris et al., 2000; Eskenazi et al., 2006). Pyrethroids are no longer used extensively with the exception of permethrin, a 3rd generation synthetic compound with activity against insects and acari (Blagburn & Dryden, 2009). Pyrethroid resistance has been reported in the important mite species *Varroa destructor* (Unit, 2013) and *Sarcoptes scabiei* (Andriantsoanirina et al., 2014). The use of pyrethroids has also been associated with increased numbers of *Tetranychus urticae* due to its toxicity against predatory mites of this species (Penman & Chapman, 1988).

Other commercially popular pesticides include organophosphates such as Phoxim (Bayer, Germany), which target acetylcholinesterase, a hydrolytic enzyme required for acetylcholine hydrolysis and cross-synaptic signal termination. Acetylcholine is
essential for neuron-to-neuron excitatory signal transmission thus inhibition of signal
termination by Phoxim overloads receptors with too much acetylcholine preventing
recovery of post-synaptic neuron potential. 50% Phoxim (Byemite®, Bayer, Germany) shows acaricidal effect on all stages of PRM as well as on egg
development (Meyer-Kühling et al., 2007), although resistance may have already
arisen in some natural populations in Poland (Zdybel et al., 2011). Post-synaptic
acetylcholine receptors are also targeted by naturally derived essential oils and
spinosyn A via competitive inhibition. Conversely, these compounds hinder
acetylcholine binding so no post-synaptic signal is produced. Spinosad acaricides
are a mixture of the compounds spinosyn A and D. Unlike spinosyn A which binds
post synaptic acetylcholine receptors, spinosyn D targets GABA (gamma-
aminobutyric acid) receptors (Orr et al., 2009). Focusing acaricidal controls on two
different target receptors of acetylcholine and GABA reduces the chance of natural
resistance of mite populations to spinosad controls. The neurotransmitter GABA
acts, in contrast to acetylcholine, by inhibiting excitatory signals. This suppression is
enhanced by abamectin/ivermectin controls which stimulate GABA release in pre-
synaptic neurons and enhance its post-synaptic binding to GABA receptors. This
induces hyperpolarisation of post-synaptic membranes via increased flow of chloride ions thus affecting downstream signalling capabilities.

Due to the conserved nature of acari and insect neural pathways several acaricides are effective against many co-inhabiting species. The use of such substances, albeit practical, increases the risk of ecological disequilibrium. In addition the concurrent use of controls that target similar pathways increases the likelihood of resistance selection to multiple controls as has been seen in other insects and arthropods (Acevedo et al., 2009; Fernández-Salas et al., 2012)

5. Salivary gland proteins

Salivary gland proteins in haematophagous arthropods, including many acari species, have been shown to have biological functions in blood feeding. These proteins can influence blood flow through antihemostatic properties (Champagne, 2004), interact with host immune cells to cause immunomodulation (Schoeler & Wikel, 2001; Titus et al., 2006) and eliminate bacteria in the feed by displaying antimicrobial properties. Salivary proteins of the cattle tick *Rhipicephalus annulatus* have been suggested as potential alternative vaccine candidates (Shahein et al., 2013) as
there is concern that ‘concealed’ or ‘hidden’ antigens from tick guts such as the
BM86 vaccine TickGARD may not be effective in species other than *R. microplus*
their hosts for days or weeks at a time. This prolonged period of feeding requires the
production of bioactive lipids and proteins in the salivary glands which are used to
cement the tick to the biting site as well as to fight host immune-regulation,
haemostasis and inflammation (Steen *et al.*, 2006; Francischetti *et al.*, 2009). It is
possible that PRM salivary gland proteins are taxonomically related to known tick
salivary gland proteins, however PRM feeding time is much shorter. A recent
publication on sequencing the PRM transcriptome identified 24 potential salivary
proteins likely to be involved in blood digestion (Schicht *et al.*, 2013) some of which
have hypothesised anti-bacterial functions.

Secreted proteins in the saliva of the honey bee mite *V destructor* damage insect
haemocytes and prevent aggregation formation that occurs in host wound healing
(Richards *et al.*, 2011). The requirement of *V. destructor* populations to feed multiple
times on the same host is reflected in PRM behaviour, although it is unclear whether
PRM feed repeatedly on the same open wound similarly to. If this is the case anti-
healing proteins may be a viable control target when present in PRM. More likely
targets however are secreted salivary proteins with anti-microbial function since
pathogens are ingested with blood meals regardless of feeding time duration.
Studies into anti-microbial salivary proteins in ticks (Yu et al., 2006; Liu et al., 2010)
as well as other arthropods (Titus, et al., 2006) should benefit further PRM research.
6. Alternative and novel targets

Mechanical and sensory inhibition

Mites do not have eyes but sense their environment through hair-like appendages called setae, normally clustered at the palpal or tarsal extremities. In general, setae sense vibration, heat, moisture, CO$_2$ or chemical cues generated by hosts or potential mates. PRM setae in the forelegs and palps play important roles in both olfactory and mechanical sensing (Cruz et al., 2005) as evidenced by increased movement of PRM in response to small vibrations and increases in environmental heat, suggestive of the presence of a passing host (Kilpinen, 2001). Kilpinen (2001) demonstrated that PRM exhibit increased heat-induced movement 2-10 days post-feeding compared to mites fed 1 day before or fed >10 days before. Interestingly this correlates to the physiology of blood digestion in PRM suggesting that hungry mites 2-10 days post feeding exert more energy on host finding, but after 10 days they become more static to conserve energy. PRM undergo a stasis-like diapause if no host is present or if the temperature drops, which is reflected by seasonal variations reported in PRM numbers (Nordenfors & Hoglund, 2000).
The potential of utilising CO\textsubscript{2}, olfaction, and micro-vibrations in control strategies discussed below.

**Disrupting mating behaviour using micro-vibrations**

Both females and males of various species of insects produce and react to micro-vibrations thought to be involved with mate attraction. Predominantly this behaviour has been studied in tree and plant parasitising species including the American grapevine leafhopper *Scaphoideus titanus* (Mazzoni *et al.*, 2009), the southern green stink bug *Nezara viridula* (de Groot *et al.*, 2010), the Asian citrus psyllid, *Diaphorina citri* (Rohde *et al.*, 2013) and the southern pine beetle *Dendroctonus frontalis* (Aflitto & Hofstetter, 2014). Studies have shown conspecific vibration patterns such as those from competing males (Mazzoni, *et al.*, 2009; Rohde, *et al.*, 2013) or heterospecific patterns such as those from a predator (de Groot, *et al.*, 2010), can alter male behaviour resulting in reduced mating events.

PRM are a colony-developing species and therefore mating may simply be a random process or pheromone-dependent (Entrekin & Oliver, 1982; Koenraadt & Dicke, 2010), rather than directed by vibration. Mite activity is increased when PRM are
exposed to substrate-borne microvibrations at 2 kHz (Kilpinen, 2005) however this has not been suggested to be directly related to mating behaviour. Further work into PRM reproductive behaviour and vibration sensing is needed to understand if this could be a potential route for population control.

**Use of carbon dioxide / mite traps**

*D. gallinae* initially remain static in the presence of CO$_2$ although after 2 minutes exposure they display higher rates of movement compared to those of unexposed PRM (Kilpinen, 2005). This correlates to the behaviour of other haematophagous arthropods such as mosquitoes and ticks where CO$_2$ induces increased movement based on evolution of host seeking behaviour. CO$_2$ producing traps can be used as attractant controls as demonstrated by Garcia and others (Garcia, 1962; Newhouse *et al.*, 1966; Wilson *et al.*, 1972). Carbon dioxide has also been considered for control of several species of phytophagous mites that feed on stored crops (White & Jayas, 1991; Conyers & Bell, 2003). Using levels of 50-60% CO$_2$ in enclosed storage units reduces mite numbers significantly by asphyxiation however the use of CO$_2$ at these levels is not appropriate for PRM control in farming units housing poultry flocks. The use of local CO$_2$ gradients to attract PRM into the vicinity of an
already established PRM trap could be a potential alternative approach. Cardboard traps coated in compounds with acaricidal properties have proved to be a simple but effective control measure in trials in Sweden (Chirico & Tauson, 2002). Implementation of CO$_2$ producing products for large scale control does remain speculative given the dangerously high levels that would be required for larger farming units. More appropriate would be their implementation in an integrated approach using multiple control methods.

**Predators and olfactory perception**

Olfactory receptors in PRM are suggested to play a role in mite survival since PRM remains initially and transiently motionless upon sudden CO$_2$ concentration increase (Kilpinen, 2005). The CO$_2$ increase possibly mimics the presence of potential predators. Consistently higher levels of CO$_2$, however, induce PRM movement, suggesting perhaps a situation when their immediate risk of danger ceases to exist. PRM colonies that are openly exposed to hen flocks in illuminated areas are quickly pecked and presumably eaten (J. Pritchard, personal observation) thus explaining why PRM usually inhabit dark enclosed spaces and are nocturnal feeders. Use of intermittent light regimes has shown to vary mite numbers captured in studies carried
out in Poland (Sokół et al., 2008) however application of lighting regimes in poultry houses varies between countries and such maybe subject to poultry welfare laws.

Several predatory species including *Hypoaspis miles*, *Hypoaspis aculeifer*, *Amblyseius degenerans* and *Phytoseiulus persimilis* are able to feed on *D. gallinae*, though feeding success as part of experimental PRM controls have proven to be dependent on environmental conditions and absence of alternative prey (Lesna et al., 2009; Ali et al., 2012; Lesna et al., 2012). The predatory mite *P. persimilis* feeds predominantly on the spider mite *Tetranychus urticae* and has been shown to be attracted to volatile compounds produced by plants fed on by *T. urticae* (Drukker et al., 2000; De Boer & Dicke, 2004). A hypothetical PRM control could be, for instance, the addition of such predator attractants to areas typically inhabited by PRM.

*D. gallinae* themselves are affected by volatile compounds, most notably repellent substances (Soon-Ill et al., 2004; George, Olatunji, et al., 2010; George, Sparagano, et al., 2010). Plant derived essential oils are shown to possess repellent and even lethal characteristics of which garlic and thyme oils appear to be the most effective.

As reviewed by George et al. (2014) naturally derived essential oils benefit from low
mammalian toxicity and short environmental persistence indicating their potential future use as part of integrated control strategies.

Conversely, little research has been carried out into mite attracting compounds. Zeman (1988) showed attraction of PRM to host-derived bird surface skin lipids which is postulated to be part of the evolution of PRM host-detection. Furthermore *D. gallinae* have been shown to release pheromones which attract other PRM causing mites to cluster together, most likely for protection (Entrekin & Oliver, 1982; Koenraadt & Dicke, 2010). How repellent or attractant compounds are used in future controls would require further research. The study of attractants to be employed in mite traps, repellents to be employed in densely populated areas and mechanical constraints would be beneficial for the development of integrated control strategies.

**Embryogenesis**

Adult female PRM are oviparous, laying 3-4 eggs after mating. Oviposition time varies with temperature but is suggested to be on average 1-3 days at 20-45°C (Maurer & Baumgartner, 1992; H. Nordenfors, *et al.*, 1999). Embryo development requires various compounds including proteins, sugars and lipids which are secreted
from both ovarian and extra-ovarian tissues. These compounds include vitellogenin, the precursor for the yolk protein vitellin, an essential nutrient during early embryogenesis (Seixas et al., 2012). A range of proteases involved with the hydrolysis of vitellin, leading to yolk degradation, have been isolated in eggs of the cattle tick *R. microplus* (Logullo et al., 1998; Sorgine et al., 2000; Seixas et al., 2008) and targeted via vaccination. This has led to reduction in tick fecundity and next generation egg weight in ticks fed on the blood of vaccinated bovine hosts (da Silva Vaz et al., 1998; Seixas, et al., 2008). Of these proteases vitellin-degrading cysteine endopeptidase (VTDCE), a Cathepsin-L like protein, is the most active enzyme. Comparative study into embryogenesis in PRM is lacking, but homologues to Cathepsin-L have been identified through suppression subtractive hybridization (Bartley, et al., 2012). Wright et al (2011) identified vitellogenin in PRM as the protein with the highest difference in expression between cDNA libraries of fed and unfed mites. Due to the increase in expression Cat-L and vitellogenin in fed mites it is plausible that Cat-L like proteases could play a part in PRM vitellogenesis. Huntley et al (2004) describe a vitellogenin homologue in the sheep scabies mite *P. ovis* to be highly immunogenic to the host. It is hypothesised *P. ovis* may induce allergic
response to aid feeding and thus pre-vaccination of allergens such as vitellogenin may inhibit the induction of pro-inflammatory IgE antibodies and influence mite feeding. Success of PRM control is often measured at population level through total mite numbers, egg counts, analysis of rates of oviposition and development of early stage PRM. Embryogenesis and its associated molecules such as vitellin are therefore suggested as attractive potential future control targets.

The Haemocoel / Immune system

Jasinskas et al (2000) reported the ability of immunoglobulins specific to a range of tick proteins to cross from a blood meal to the haemolymph of the lone star tick *Amblyomma americanum* through the midgut epithelium. This proof of concept in ticks suggests there is a possibility of raising antibodies against essential proteins for ticks/mites present in the hemolymph and fat body. The acari immune system is composed of phagocytising haemocytes and anti-microbial peptides such as defensins and lysozymes. The midgut is the primary site for destruction of bacterial and viral pathogens which are ingested with a blood meal, but if these microbes successfully traverse the midgut epithelium, then defensins and lysozymes are secreted into the haemolymph and fat body (Ceraul *et al.*, 2003; Simser *et al.*, 2004;
Taylor, 2006). Lysozymes in astigmatid mites can function in both defence and also in digestion when microbes are used as a secondary food source (Childs & Bowman, 1981; Erban & Hubert, 2008). Greater understanding of PRM lysozymes and the cells that contain them could contribute to novel controls against the mites by affecting the ability of the mite to process ingested pathogens that may affect or be transmitted by PRM, as demonstrated for ticks (Simser, et al., 2004).

Infection of PRM with bacteria has been shown by Valiente Moro et al (2009) who demonstrated that *Salmonella enteritidis* can enter the PRM haemolymph/reproductive organs and infect protonymphs via transovarial passage. Valiente Moro et al further demonstrated the negative effect of bacterial infection on PRM fecundity, with only 31% oviposition in infected PRM compared to 68% oviposition in control PRM. This suggests that targeting the PRM immune system and thus affecting their ability to cope with pathogens such as *S. enteritidis* in the reproductive organs could be explored.

Subolesin, a tick homologue of the mammalian akarin family of proteins, is associated with the upregulation of innate immunity in various tick species (Zivkovic
et al., 2010) and is proposed to be a transcription factor involved in multiple cellular processes (De la Fuente et al., 2008). Harrington et al (2009) showed that immunisation of chickens with recombinant Aedes albopictus subolesin increased fed PRM mortality by 31% compared to control groups, suggesting that a potential PRM subolesin orthologue may be a target for control. RNA interference of the subolesin gene in ticks has shown varying efficacy in terms of how well ticks are able to control bacterial infections. Zivkovic et al (2010) demonstrated that RNAi knock-down of subolesin in ticks increased infection by Francisella tularensis but decreased infection by Anaplasma marginale. Whether by means of immunological repression resulting in increased bacteria loads or affecting other PRM systems, subolesin would make an interesting target for further vaccine studies against PRM.

7. Integrated Control Strategies
The efficiency of PRM control is dependent on many factors including substances employed, farm layout, mite population numbers and environmental factors. Future improvements to PRM control therefore will likely require integrated strategies such as the Hazard Analysis and Critical Control Points (HACCP) method laid out by Mul
and Koenraadt (2009). The efficacy and longevity of new control strategies, such as the introduction of vaccines or novel acaricides, are likely to be affected by specific farming practices and methods of animal husbandry (Harrington et al., 2011) and will require careful planning. For example, introduction of novel acaricides to a system using natural predators of PRM may also affect the predator species as well as *D. gallinae* (Harrington et al., 2011).

8. Concluding remarks

The variable nature of control strategies taken by each farmer, ongoing changes in caged poultry regulations and the rapid emergence of acaricidal resistance, suggests that PRM will continue to be a major problem to the global egg-laying industry. Understanding PRM biology is essential for developing improvements to current biological controls and should be at the forefront of any future PRM research. In this short review we have identified several biological targets that offer potential for possible future controls against PRM including embryogenesis, food digestion, sensory perception and predatory intervention. The current lack of a single
commercial control methodology means that research into these fields would be of

everous benefit to the poultry industry and commercial sector.
9. Acknowledgements

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10. References


Figure 1: The life cycle of *Dermanyssus gallinae*. The life cycle of PRM can be completed in 7 days, from egg to adult (Maurer & Baumgartner, 1992) although 14 days is more usual. Commonly only females of the protonymph, deutonymph and adult stages feed on blood, though males have been known to feed. Female adults typically lay clutches of 4-8 eggs with a maximum of 30 eggs total in their life time. Larvae have 6 legs (not 8 as the other stages) and all stages live off the host, feeding intermittently for short periods at a time.

Figure 2 Comparison of the PRM digestive system in blood fed (2a) and unfed (2b) mites. Mites were observed at x100 magnification from the dorsal side. Gnth – Gnathosoma (mouthparts), Os – Oesophagus, Ca I-III – Caeca I-III, Mp – Malpighian tubules, Hg – Hindgut. The PRM digestive tract extends from the gnathosoma posteriorly through the oesophagus, midgut and caeca and ending in the hindgut. Most blood digestion occurs in the much expanded three caecal pairings (Ca I-III) and central midgut (Mg) (Figure 2a). Malpighian tubules elongate longitudinally along the idiosoma connected to the anterior hindgut (Figure 2b). These are involved in nitrogenous waste collection and osmoregulation. Waste leaves through the
posterior hindgut and through the anal opening (not shown). Note: mite body shape increases and gets rounder during feeding and the digestive tract completes most of the body cavity of the PRM when full (Figure 2a) compared to that of an unfed mite (figure 2b).

**Figure 3: The synganglion tissue (brain) of the PRM.** Longitudinal sections of 10µm thickness observed at x200 magnification. Sections were stained with 1:100 anti-Cathepsin-D chicken IgY (kindly donated by Dr Alisdair Nisbet) then 1:1000 goat anti-rabbit IgG HRP and counter stained with haematoxylin. Pg I-IV – Pedal ganglion 1 to 4, SpCNM – Supra-oesophageal central nervous mass, Sb – Sub-oesophageal mass, Es – Oesophagus. The PRM synganglion tissue, as in all acari, is divided by the oesophagus into two connected masses – the supra-oesophageal mass (Figure 3a) and the sub-oesophageal mass (Figure 3b). Figure 3a shows the supra-oesophageal central nervous mass connected to 8 pedal ganglia extending distally to each corresponding leg. Figure 3b shows the sub-oesophageal mass, comparatively more rounded, split by the oesophagus extending longitudinally down though the centre.
Figure 4: Neurological targets for acaricidal controls against *D. gallinae*. Pesticides and other controls affect either the transmission of acetylcholine (secreted from an excitatory neuron shown in red) required for excitatory signals or gamma-aminobutyric acid (GABA) (secreted from an inhibitory neuron shown in blue) which are the predominant inhibitory neurotransmitters in the nervous system. Competitive inhibition of acetylcholine and GABA through binding to post-synaptic receptors is a common mode of action for acaricides. An alternative mode of action is the binding and inactivation of the enzyme acetylcholinesterase, which is required to hydrolyse acetylcholine and end signalling, thus leading to overstimulation. Several pesticides bind to and over stimulate the voltage gates Na+ channels in the presynaptic axon. These mechanisms aim to induce paralysis and consequently lead to death in red mite through excitotoxicity and overstimulation in neural pathways or conversely through transmission inhibition.