Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Fakulteten för veterinärmedicin och husdjursvetenskap
Faculty of Veterinary Medicine and Animal Science

Poultry red mites in Swedish laying hen flocks – occurrence and efficacy to a selection of acaricides

Röda hönskvalster i svenska värphönsbesättningar- förekomst och känslighet mot ett urval av akaricider.

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Department of Animal Nutrition and Management, nr 611
Independent project/Degree project/Master's thesis 30 hp
Agronomprogrammet Husdjur
2017

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Credits: 30 hec
Level: A2E- masterarbete
Course title: Degree project in Animal Science
Course code: EX0551
Programme/education: Agronomprogrammet – Husdjur

Place of publication: Uppsala
Year of publication: 2017
Cover picture: Name of photographer
Number of part of series: 711

Online publication: http://stud.epsilon.slu.se

Keywords: Poultry red mite, *Dermanyssus gallinae*, laying hen, efficacy, occurrence

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Abstract

The poultry red mite *Dermanyssus gallinae* is a blood-feeding ectoparasite commonly found in poultry facilities. It has an adverse effect on health and welfare of laying hens around the world. A common method used for control of *D. gallinae* is the use of acaricides (pesticides used against mites). There are few acaricides available for control of *D. gallinae* today and concerns are raised regarding reduced effect of these due to development of tolerance in the mites. There are limited information regarding the occurrence of *D. gallinae* in Swedish layer flocks and efficacy in *D. gallinae* to acaricides. The aims of this study were therefore to investigate the occurrence of *D. gallinae* in laying hen flocks in different housing systems in Sweden by the use of questionnaires and collection of mites in traps. In addition, a test was conducted in laboratory environment where the efficacy of *D. gallinae* to the acaricides phoxim and cypermethrin was examined. The data from the survey, with a response rate of 38%, showed that the prevalence of *D. gallinae* is widespread throughout Sweden in all types of housing systems and is causing varying degrees of problems for Swedish egg producers and their hens. The questionnaire indicated that 63% of the producers had mites in their layer flocks. Of those reporting presence of mites, 73% experienced some sort of problem related to the mites. The most common problems linked to mites were bloodspots on the eggshell, stressed hens, personal being attacked, feather pecking and increased mortality in hens. According to the questionnaire survey the majority, 77%, of the egg producers, used control measures against *D. gallinae* during on-going production. The most common measures were use of silica based preparations, the acaricide based compound Baymite®, combined with dry cleaning, wet cleaning or combined wet- and dry cleaning. Fifty-seven percent of the producers used control measures against *D. gallinae* between production cycles, i.e., in empty houses. The most common methods then were use of Baymite® and silica based preparations combined with dry cleaning. Measures against mites during ongoing egg production or between production cycles were considered to be effective. Fifteen percent of the producers reported that the mites had disappeared and 72% that the problems with mites had decreased, whereas 12% experienced that the control methods had poor or no effect. In the mite count study, mites were recovered from traps placed in 46 different units (unit defined as a laying hen facility enclosed by solid walls) in 25 farms. The material used when monitoring the degree of mites was traps made of pre-cut white semi-transparent rectangular pieces of “corrugated plastic” (100 x 70 x 2 mm) with transverse funnels. Results of the mite count shows that the majority, 65% of the egg producers units had a low abundance of mites, 11% of the units had abundant amount of mites, 9% had moderate abundance and 15% had no mites detected in their units. In the efficacy test the two acaricides phoxim and cypermethrin were used in the dilution recommended by the manufacturer for use in poultry. The result from the efficacy test showed that phoxim inactivated all mites from 15 out of 18 flocks and that cypermethrin inactivated all mites from only 7 out of 18 flocks after 48 hours.

Sammanfattning

Det röda hönskvalstret *Dermanyssus gallinae* är en blodsugande ektoparasit som är vanligt förekommande i värphönsbesättningar. *Dermanyssus gallinae* har en negativ inverkan på hönsens hälsa och djurvälfärd. En vanlig metod för bekämpning av *D. gallinae* är användning av akaricider (bekämpningsmedel mot kvalster). Idag finns få akaricider tillgängliga för bekämpning av *D. gallinae* och det finns farhågor att resistensutveckling hos kvalstren försämrat preparatens effektivitet. Information om förekomst och känsligheten hos *D. gallinae* mot förekommande akaricidpreparat i svenska värphönsflockar är mycket begränsad. Syftet med studien var därför att undersöka förekomsten av *D. gallinae* hos värphönsflockar i olika inhysningssystem i Sverige genom en enkätundersökning och insamling av kvalster med hjälp av fällor. Dessutom utfördes ett test i laboratoriemiljö där kvalstrets effektivitet mot akariciderna foxim och cypermetrin undersöktes. Data från kvalsterenkäten med en svarsfrekvens på 38% visade att förekomsten av kvalster är spridd över hela landet och finns i alla typer av produktionssystem. I enkäten angav 63% av äggproducenterna att de för närvarande har kvalster i besättningarna och hela 73% av producenterna med kvalster i sina flockar upplevde någon form av problem kopplad till kvalsterförekomsten. De vanligaste problemen var blodprickar på skalen, stressade hönor, att personalen blir angripen, problem med fjäderplockning och ökad dödlighet hos hönsen. I enkäten angav majoriteten, 77% av producenterna att de använde bekämpningsmetoder mot kvalster under pågående produktion. De vanligaste behandlingsmetoderna var kiselbaserade preparat, det akaricidbaserade preparatet Baymite® i kombination med torrengöring, våtrengöring eller kombinerad torr och våtrengöring. Femtiosju procent av producenterna använde bekämpningsmetoder mot kvalster mellan omgångar dvs. i tomma hus. De vanligaste behandlingsmetoderna då var Baymite® och kiselbaserade preparat i kombination med torrengöring. Behandlingsmetoder mot kvalster under pågående produktion eller mellan omgångar ansågs vara effektiva. Femton procent angav att kvalstren försvunnit, 72% att kvalsterproblemet minskat och 12% ansåg att bekämpningen haft dålig eller ingen effekt alls. Vid kvalsterräkningen, återfanns kvalster i fällor från 46 olika djurutrymmen (med djurutrymme menas här ett utrymme avgränsat med täta väggar) från 25 gårdar. Det material som användes vid kontroll av kvalsterförekomst var fällor av halvgenomskinliga rektangulära bitar av veckad plast (100 x 70 x 2 mm) med tvärgående kanaler. Resultatet från kvalsterräkningen visade att majoriteten, 65% av äggproducenterna hade ringa förekomst av kvalster i sina djurutrymmen, 11% hade riklig förekomst, 9% hade måttlig förekomst och 15% hade inga kvalster påvisade i sina djurutrymmen. Resultatet från effektivitetstestet visade att brukslösning av foxim inaktiverade samtliga kvalster från 15 av 18 hönsbesättningar, medan cypermetrin inaktiverade samtliga kvalster från endast 7 av 18 besättningar efter 48 timmar.

1 Introduction

The poultry red mite (PRM), *Dermanyssus gallinae* is a blood-feeding ectoparasite with a severe impact on laying hens. It is considered to be the economically most devastating ectoparasite in laying hens in Europe (George *et al*., 2015) due to its adverse effect on production, health and welfare of the laying hen (Kilpinen *et al*., 2005; George *et al*., 2015). *Dermanyssus gallinae* subsists on sucking blood from its host and multiplies very quickly. It can survive in poultry facilities for long periods without its host and is easily spread by the host or indirectly by various fomites. This distinctive feature makes them particularly hard to eradicate and once established in the poultry premises they tend to multiply again after treatment (Marangi *et al*., 2012). *Dermanyssus gallinae* causes several problems in laying hens such as stress, itching, reduced egg quality, anaemia, increased mortality and may be a potential risk of spreading infectious agents (George *et al*., 2015). It occasionally harms other mammals such as rodents, rabbits, cats, dogs, horses and humans (George *et al*., 2015). Dermatitis, caused by *D. gallinae* bites, can be a problem for workers in poultry premises infested with *D. gallinae* (Gavrilovic *et al*., 2015; Rosen *et al*., 2002). The ability of *D. gallinae* to carry zoonotic agents (Valiente Moro *et al*., 2009) is of concern and the mites should be considered as a potential human health problem (George *et al*., 2015). The control of *D. gallinae* is mainly depending on synthetic acaricides (mite pesticides). Repeated and long-term use of acaricides has resulted in development of acaricide resistant populations of *D. gallinae* (Abbas *et al*., 2014). Due to this the effectiveness of available acaricides is rapidly decreasing (Abbas *et al*., 2014). In Sweden, Baymite® (Bayer Animal Health, Bayer Healthcare AG, Leverkusen, Germany) is the only acaricide approved for treatment during the production cycle, i.e., with birds in the premises. Development of resistance is usually a consequence of natural selection and the result of selection of individuals that are genetically predisposed to survive an acaricide (Gullan & Cranstone, 1994). It is therefore a considerable risk that mites will develop acaricide resistance when only one chemical compound is being used (Nordenfors, 2000).

1.1 Background

Animal welfare concern led to the ban of the battery cages in Sweden in 1999 and the European Union in 1 January 2012 (European Commission, 1999). Today all layers in EU, including Sweden, are housed in alternative systems providing access to nest, perches and litter (SFS 1988:539 9 §). In January 2016 there were 7.7 million laying hens in Sweden according to the Swedish Egg Association (personal message A. Jeremiasson 2016-02-04). Egg producers with a minimum of 350 laying hens are included in the statistics. Of the layers in Sweden (Jan 2016), 17.5% are kept in furnished cages providing 600 cm^2/bird. Furnished cages are designed to allow hens to perform certain behaviours such as scratching and dust bathing, resting on perches, and laying eggs in seclusion (Abrahamsson & Tauson, 1997).

In Sweden, (Jan 2016), 82.5% of the laying hens are kept in floor housing systems. The majority of the floor housed layers (72.8 % of the Swedish layers) are housed in multi-tier systems, also called aviaries (Wall *et al*., 2016). The rest of the floor-housed layers are housed in single tier systems, also referred to as traditional floor system or in Jansen system (an aviary system that complements the traditional floor system with tiers). The majority of the floor-housed layers are kept indoors and the eggs are classified as barn eggs. An increasing percentage of the floor-housed layers are in organic egg production (15.3%) implying that the production follows organic standards demanding organic feed and access to an outdoor area. Approximately 3% of the floor-housed layers are free range, which means that they follow conventional regulations regarding housing and feed but have access to an outdoor area during the whole year (personal message A. Jeremiasson 2016-02-04). The structure of Swedish egg production has changed substantially over the years and there is a trend towards an increased Swedish layer stock, fewer egg producers and larger flock sizes. There is an ongoing trend in Sweden, with a reduction of layers in furnished cages and corresponding increase in layers in floor housing systems (Lannhard-Öberg, 2015).

1.2 Aim

The most recent Swedish survey on the occurrence of *D. gallinae* in laying hen flocks was done over 20 years ago, before the ban on battery cages came into force (European Commission, 1999). The aims of this study were therefore to investigate the occurrence of *D. gallinae* in different housing systems for laying hens in Sweden and to investigate the efficacy of *D. gallinae* to two selected acaricides.

More specifically:

- By use of questionnaires and collection of mites in traps, investigate the proportion of infected flocks in different housing systems and estimate the degree of infection of *D. gallinae*.
- In the laboratory investigate efficacy to the acaricides phoxim and cypermethrin in *D. gallinae* trapped in Swedish layer flocks.

2 Literature review

2.1 Dermanyssus gallinae

2.1.1 Description

Dermanyssus gallinae (De Geer 1778), more commonly known as the poultry red mite (PRM), is a blood-feeding ectoparasite (Pritchard *et al*., 2015). *D. gallinae* is an arthropod belonging to the class Arachnida, order Mesostigmata and the family Dermanyssidae (Moss, 1978). The adult female

is around 0.75mm long in an unfed state and in a fed state 1.5mm long (Sikes & Chamberlain, 1954). The mite is oval to pear-shaped and the male is slightly smaller than the female. Depending on the feeding status the colour can vary from grey, brown/red to black but newly fed mites are bright to dark red. The larvae and unfed nymphs are smaller than the adult mites and are transparent (Nordenfors, 2000; Sparagano *et al*., 2014). The mite has a dorsal exoskeleton shield covering the body (idiosoma), which is not gender specific. Females have two separate ventral shields, a genitoventral shield and a smaller rounded anal shield. The male has a single ventral shield consisting of a fusion between both the genitoventral and the anal shields (Sparagano *et al*., 2014; Di Palma *et al*., 2012). The exoskeleton is made of a tough and elastic polymer called chitin. The polymerization of chitin is triggered by hormones secreted through pores and mixed with phenolic compounds and proteins to form a layer of sclerotin (Pritchard *et al*., 2015). The sclerotized layer is hard and provides protection and maintains the body shape of the mite (Hackman, 1982). It also limits water loss and supports attachment of muscles (Pritchard *et al*., 2015). The mouthparts (chelicera) are long and whip like and terminate in small scissors-like structures (chelae) (Baker, 1999). The nymphs and adult mites have four pairs of legs (coxa) whilst the larva has only three pairs of legs located in the front part of the body (Baker, 1999).

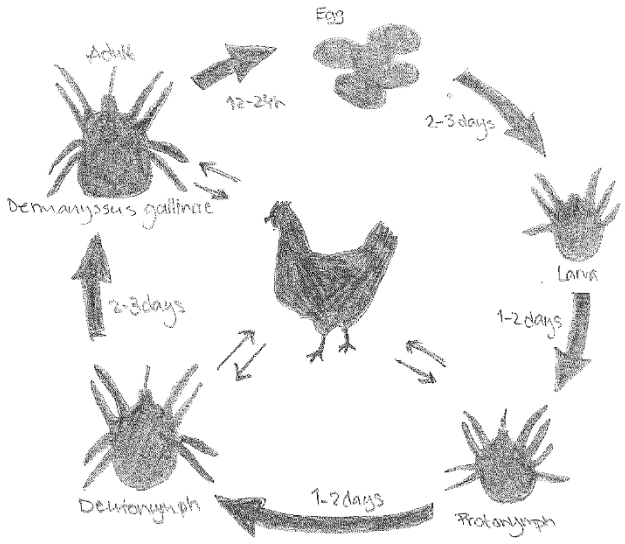

Figure 1. Life cycle of *D. gallinae*. Modified from Sparagano, *et al*. 2014.

2.1.2 Life cycle and biology

The life cycle of the *D. gallinae* (Figure 1) is normally about 14 days (Sparagano *et al*., 2014) but can be completed in only 7 days (Maurer & Baumgärtner, 1992). Females of the two nymph stages and adult females feed on blood. Males on the other hand feed only occasionally while the larvae do

not feed at all (Chauve, 1998). The adult females lay clutches of about 4-8 eggs and can during a lifetime produce a maximum of 30 eggs (Pritchard *et al*., 2015).

Dermanyssus gallinae spends most of its time in hiding places such as cracks, crevices and nests in poultry premises, near its host (Kilpinen 2001; Brännström, 2010). The mites use a combination of chemical signals, and response to carbon dioxide, temperature and vibration to locate the host (Sparagano *et al*., 2014). *Dermanyssus gallinae* is mainly feeding at night and is only present on the host for about 1-2 hours to feed. After feeding *D. gallinae* returns to hiding places where they aggregate together by response to thigmokinesis (movement or inhibition of movement in response to contact stimuli) and pheromones (Sparagano *et al*., 2014) to mate and lay their eggs (Nordenfors *et al*., 1999). *Dermanyssus gallinae* is haplodiploid and arrhenotokus meaning that the males are haploid and develop from unfertilised eggs and the females are diploid and develop from fertilised eggs (Cruickshank & Thomas, 1999). In vitro mites are able to feed and reproduce at temperatures ranging from 10 to 37°C and the most favourable temperature for juvenile development lies between 25 and 37°C with an optimal relative humidity at 65-70%. Lower temperatures are more restrictive and temperatures > 45°C and at < -20°C seem to be lethal (Maurer & Baumgärtner, 1992). In an in vitro study by Nordenfors *et al*. (1999) female mites were able to lay eggs at temperatures ranging from 5 - 45°C and the largest number of eggs were observed at a temperature of 20°C and a relative humidity of 70%. The development of larvae and protonymphs were only seen at temperatures ranging from 20 to 25°C. Mites held at temperatures ranging from 5 to 25°C were observed to survive for up to 9 months without feed (Nordenfors *et al*., 1999).

2.1.3 Prevalence

Dermanyssus gallinae is one of the most widespread mites around the world and there are reports on prevalence of *D. gallinae* in a number of countries (Sparagano *et al*., 2009). In northeast Tunisia *D. gallinae* was found in both layer farms and breeding farms with a prevalence of 34% (Gharbi *et al*., 2013). In Iran 39% of the layer farms were infested with *D. gallinae* (Yakhchali, 2013). The presence of *D. gallinae* has also been reported in France (Chauve, 1998), Switzerland (Morgenstern & Lobsiger, 1993), China (Wang *et al*., 2010), Kenya (Mungube *et al*., 2008) and Norway, (Øines & Brännström, 2011). United Kingdom, Italy, the Netherlands, Japan, Morocco, Serbia and Montenegro have claimed that *D. gallinae* is present in 80-90% of layer farms (Sparagano *et al*., 2009). In Sweden, Höglund *et al*. (1995) found infestations of *D. gallinae* in only 6% of flocks in conventional cages, 33% of flocks in floor housing systems indoor and 67% of the backyard flocks. This can probably partly be explained by differences between production systems in opportunities for the mites to hide in cracks and crevices and evade chemical control methods (Sparagano *et al*., 2009).

2.1.4 Hosts

Dermanyssus spp. has a wide range of hosts involving a variety of different bird families such as Galliformes and Passeriformes (e.g. domestic fowl (*Gallus gallus*), wild canary (*Serinus canaria*), great tit (*Parus major*), starling (*Sturnus vulgaris)*, great spotted woodpecker (*Dendrocopos major*), Egyptian vulture (*Neophron percnopterus*), European roller (*Coracias garrulus*) and duck (*Bucephala albeola*) (Roy *et al*., 2009). However, molecular analysis has shown that *D. gallinae* from wild birds are genetically different from *D. gallinae* in layer farms (Brännström *et al*., 2008).

Dermanyssus gallinae occasionally attacks non-host animals staying nearby poultry facilities such as cats and dogs (Ramsay *et al*., 1975; Declerq & Nachtegaele 1993; Grant, 1989), horses (Mignon & Losson, 2008) and humans (Collgros *et al*., 2013). Under controlled laboratory conditions *D. gallinae* have been shown to feed on both mice and rabbits (Chamberlain & Sikes, 1955). *Dermanyssus gallinae* have also been recovered from goats (Dorny *et al*., 1994) and house mice (*Mus musculus*) (Allymehr *et al*., 2012). However, reports like these do not necessarily confirm infestation of these species and *D. gallinae* may have been present on goats/mice without feeding from them.

2.1.5 Diagnosis and monitoring of infestation

Because of the life cycle of *D. gallinae* and their ability to hide, small populations of *D. gallinae* can be difficult to find and pass undetected (Pavlicevic *et al*., 2007). Signs of infestation of *D. gallinae* are findings of spots or clumps of mites on feeders and other equipment, bloodspots on eggs and workers getting bitten (Marangi *et al*., 2012). One way to monitor and/or diagnose infections of *D. gallinae* in a poultry flock is to use traps of corrugated cardboard. The traps should be placed where mites are known to aggregate e.g., close to nest boxes or on perches (Nordenfors, 2000).

2.1.6 Clinical effects

Infestations of *D. gallinae* have various negative effects on the welfare of hens. An adult mite ingests approximately 0.2 µl blood (Sikes and Chamberlain, 1954) and severe infestation of *D. gallinae* may lead to anaemia and in extreme cases even death due to the severe blood loss (Kilpinen, 2005; Marangi *et al*., 2012; Sparagano 2014). Mite infestations may also cause a considerable stress to the hens. Kowalski & Sokol (2009) found that infestations of *D. gallinae* caused an increase in plasma corticosterone, adrenaline and a decrease in β - and γ - globulins indicating development of somatic and psychogenic stress reactions in the hens. Mite infestations can also lead to disturbed sleep patterns for the hens due to increased need for e.g., head scratching during night, and increased preening behaviour during the day (Kilpinen, 2005). There have also been reports on severe feather pecking and cannibalistic behaviour among hens due to infestation. Other

problems associated with infestation are increased feed consumption, poor growth, reduced egg quality, due to shell thinning and bloodstains on eggshells arising when eggs are rolled over fed mites (Cosoroaba, 2001; Wojcik *et al*., 2000; Sparagano *et al*., 2014; Chauve 1998; Marangi *et al*., 2012; Mul *et al*., 2009).

In addition to direct effects of the mite on the hens, *D. gallinae* may also serve as a vector and reservoir for infectious agents. Several bacterial and viral agents such as avian paramyxovirus type 1, *Erysipelothrix rhusiopathiae*, *Pasteurella multocida*, *Salmonella* Gallinarum and *Salmonella* Enteritidis have been isolated from *D. gallinae* (Arzey, 1990; Chirico *et al*., 2003; Valiente Moro *et al*., 2007; Zeman *et al*., 1982; Grebenyuk *et al*., 1972). Transmission between birds has not been confirmed for all agents isolated from mites.

Infestations of *D. gallinae* do not only affect hen welfare but also have implications on human health such as itching and skin irritation on workers (Rosen, *et al*., 2002). There have been increasing number of reports on temporary attacks of *D. gallinae* on humans in private households due to wild birds nesting closely to the households (Gavrilovic *et al*., 2015; Rosen *et al*., 2002). It may be suspected that problems like these go unreported and that human cases therefore may be underestimated and/or misdiagnosed (Collgros *et al*., 2013). Therefore, dermatitis caused by *D. gallinae* should be of increasing medical and veterinary concern (George *et al*., 2015). There is also a possibility of *D. gallinae* acting as a vector by carrying and transmitting zoonotic agents such as *Bartonella Quintana* to humans although this needs to be further elucidated (Melter *et al*., 2012).

2.1.7 Transmission

Mul & Koenraadt (2009) considers it likely that wild birds building nests on poultry premises or adjacent to it can be a source of infection of *D. gallinae.* However, according to Oines & Brännström (2011) there is no current data supporting that wild birds in Sweden act as a reservoir for infection of *D. gallinae* in layer farms. The transmission and spreading of mites between poultry facilities in Sweden are most likely due to synantropic factors such as, through transport vehicles, indirectly through workers and equipment or through disposable materials such as egg trays from the packing centres (Brännström *et al*., 2008; Oines & Brännström, 2011).

2.2. Control methods

Presently, measures to reduce populations of *D. gallinae* worldwide consist mainly of sanitising empty poultry houses between flocks and the use of acaricides (Huber *et al*., 2011). In Sweden however, the most common method to control *D. gallinae* infestations in poultry facilities is the use of silica based preparations (Hermansson & Odelros, 2011). Field studies have shown that these methods may have a limited effect due to that mite

populations often tend to recover after temporary suppression (Nordenfors & Höglund, 2000).

2.2.1 Control with silica based preparations

Silica based preparations mainly consist of silicon dioxide and have no known poisoning effect to hens or humans but there is a risk of silicosis when inhaling silica if used in a powdered form (Mul *et al*., 2009; Schulz *et al*., 2014). The main advantage in the use of silica is the capacity to immobilise the mite. This is done through adhering to the mite's body and tarsal parts of the legs, preventing its mobility (Mul *et al*., 2009). The absorptive properties of silicon dioxide cause death to the mite by dehydration through absorption of lipids from the mite cuticle. Silicon dioxide has a non- chemical mode of action and are non- selective for physiological derived resistance. Therefore, the development of resistance is considered to be unlikely (Chauve, 1998; Mul *et al*., 2009; Schulz *et al*., 2014).

2.2.2 Control with acaricides

Acaricides are pesticides that kill mites and ticks. It is a conventional control method against mites and the use of different acaricide compounds is widespread around the world (Marangi *et al*., 2012. Acaricides are classified in several ways such as mode of entry in the targeted pest, chemical structure, source, organic or synthetic. Control of mite infestations in poultry farms mainly consists of the use of synthetic acaricides see Table 1 (Giangaspero *et al*., 2012). There are several synthetic acaricides shown to be effective against *D. gallinae* such as organophosphorus compounds (e.g. phoxim, metrifonate, malathion), synthetic pyrethroids (e.g. cypermethrin, permethrin, flumethrin) and carbamates (e.g. carbamyl, bendiocarb) (Nordenfors, 2000; Einstein *et al*., 1994; Zeman & Zelezny, 1985). However, in order for the compounds to be effective in combating mites it is important that they come in contact with the mite (Abdel-Ghaffar *et al*., 2009). In common for all of these synthetic acaricides is that they induce paralysis and death to the mite by means of different mechanisms of action (Giangaspero *et al*., 2012). Organophosphorus compounds (OP's) work as cholinesterase inhibitors and acts to irreversibly inhibit the metabolism of acetylcholine. This is done by OP's attaching to the enzyme cholinesterase which then in turn cannot break down the acetylcholine. Due to this acetylcholine accumulates and causes rapid muscle twitches and induce paralysis (Einstein *et al*., 1994; Urquhart, 1987; Hoy, 2011). Pyrethroids work as neurotoxins and interact by binding to the voltage-gated sodium channel protein in the nerve cell membranes. This process disrupts the function and normal transmission of nerve impulses and leads to paralysis and eventual death (Salish, 1989; Einstein *et al*., 1994; Davies, 2007). Carbamates are similar to the OP's cholinesterase inhibitors, but reversibly inactivates the enzyme acetylcholinesterase and are less toxic than the OP's (Einstein *et al*., 1994).

Table 1. Acaricide types and mode of entry

Mode of entry	Chemical family	Active ingredients
Acetylcholinesterase inhibitors	Organophosphates	Phoxim, Metriphonate, Malathion
Sodium channel modulators	Pyrethroids	Cypermethrin, Permethrin, Flumethrin
Acetylcholinesterase inhibitors	Carbamates	Carbamyl, Bendiocarb

January 1st, 2000, EU's regulation of maximum residue limit (MRL) came into force to protect consumers against residues of veterinary drugs in food. At that time the most effective acaricide being used in Sweden was metriphonate (Neguvon® vet). Following the new regulation metriphonate was withdrawn due to toxicity and environmental concerns (Bartley, 2015) and the possibility to effectively combat ectoparasites became limited (Beck-Friis, 2000; Bartley, 2015).

Between the years 2000 and 2009 there were no pharmaceuticals approved for use in on-going egg production cycles in Sweden, and the major control against ectoparasites at this time was silica based preparations (silicone dioxide) (Chirico, 2005). On the 28th of June 2004 Baythion E® (phoxim) was licensed for use in empty facilities and no layers were allowed in the facility in the week following treatment (Secher, 2004). Baythion E® was withdrawn from the Swedish market on the 23th of August 2008 and was banned for use from the 1st of January 2010. This was due to that no company or EU member state indicated an interest in taking over the role of participant for Baythion E® and the substance was therefore removed from the Review Programme and not included in Annexes I, IA or IB to Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. The decision was based on the Commission's Decision 2007/565/EG (Kemikalieinspektionen, 2008). In 2009 the EU agency for medicinal products, the EMEA, had determined and approved the MRL value for the active substance phoxim. This resulted in that Baymite® (phoxim) on the 13th of January 2009 was given permit for use during production cycles in laying hens as a measure to control *D. gallinae* (Läkemedelsverket, 2009). At present, in Sweden (January 2016) the only approved acaricide against *D. gallinae* is Baymite® (personal message. H. Eriksson 2017-05-29).

2.2.3 Acaricide resistance

Whenever acaricides are used extensively such as misuse or overuse against mite populations, the chance of a resistance case arises considerably and results in the survival of resistant individuals and faster evolution of resistant populations (Chapman, 1997). The definition of acquired resistance is "a resistance that results from heritable decreases in efficacy to drugs with the passage of time" (Chapman, 1997; Abbas *et al.*, 2014). Controlling

populations of *D. gallinae* with repeated and long-term use of the same acaricides may result in development of resistance in mites (Abbas *et al*., 2014). There are over 35 compounds suggested for controlling red mite infestations but not all of them are suitable due to food safety reasons such as toxic residues in meat and eggs and concerns about environmental effects (Chauve, 1998; George *et al*., 2009; Marangi *et al*., 2012). Egg producers' incorrect use of acaricides such as too low concentration and incorrect treatment schedule as well as ineffective and illegal use of chemicals around the globe has contributed to a fast spread of acaricide resistance (Sparagano *et al*., 2009; Mul & Koenraadt, 2009). This has also resulted in increased infestation rates of *D. gallinae* (Sparagano *et al*., 2009) making the control of infestations even more challenging (George *et al*., 2015). Resistance to cypermethrin, malathion and permethrin has been observed in the UK (Fiddes *et al*., 2005). Resistance to permethrin has been shown in Sweden (Nordenfors *et al*., 2001), France, (Beugnet, 1997) and in the Czech Republic (Zeman, 1987). In Italy observations have shown a multiple resistance to permethrin, carbamyl and amitraz (Marangi *et al*., 2009). There are some management procedures that can be done to prevent further development of resistance to acaricides. These include regular drug monitoring test of resistance, use of different combinations of acaricides, rotational use of acaricidic groups with different actions of mechanism and finally, use of effective acaricides only (Chauve, 1998; Abbas *et al*., 2014). It is also important to combine these control methods with other management procedures such as good cleaning routines and to use biosecurity measures to minimise the spreading of mites (Abbas *et al*., 2014).

2.2.4. Alternative control measures to synthetic acaricides

In addition to preserving the effectiveness of existing synthetic acaricides there is an urgent need for development of alternative controls measures (Bartley, 2015). This is due to the development of acaricide resistance of *D. gallinae* and the rapid decrease of synthetic acaricides approved for use in layer flocks (George *et al*., 2015). Examples of alternative control measures under development are heat treatment, design of housing systems, lighting regimes, plant derived products predatory mites and vaccines (Mul *et al*., 2009).

3 Materials and methods

This project comprised three parts. In the first study information of the *D. gallinae* situation in Swedish layer flocks were gathered by sending a questionnaire to egg producers. Secondly, the actual degree of infestation was monitored by placement of mite traps in some 54 flocks. Finally, some of the trapped mites were tested for efficacy to phoxim and cypermethrin in laboratory tests.

3.1 Questionnaire

In order to gather information about *D. gallinae* in layer flocks in Sweden a questionnaire (see Appendix 1) was designed in the web-based tool Questback (www.questback.se). The survey was carried out during August 25th to November 30th 2015. The questionnaire was distributed to all Swedish egg producers (according to the register at the Swedish Egg Association) - by e-mail to 234 egg producers and by mail to about 80 egg producers all over Sweden. The questionnaire enabled for producers to send in their answers anonymously and no question was mandatory. In addition, in the web-based questionnaire there were conditions posted on certain issues implying that attendant questions were depending on producer's answer.

3.2 Sampling of mites

When answering the questionnaire egg producers were invited to take part in a survey monitoring whether mites were present in their flocks or not by use of mite traps. Producers who agreed to take part gave their contact information when answering the questionnaire. The material used when monitoring the degree of mites was traps made of pre-cut white semi-transparent rectangular pieces of "corrugated plastic" (100 x 70 x 2 mm) with transverse funnels (Wellplast AB, Munka Ljungby, Sweden), modified after Nordenfors *et al*. (1999). The mite traps were sent to producers together with a cover letter, sampling instructions and a referral that included questions regarding e.g. how long time eggs had been produced at the facility, production system and year of installation, and the number of hens. Producers participating in the sampling of mites could get answers to the degree of mites in their flocks if giving their contact information, otherwise the samples could be submitted anonymously. According to the instructions, the traps were to be spread out evenly over the unit's entire length and height (unit was defined as a laying hen facility enclosed by solid walls and therefore hens separated by nets were thus in the same unit). The placement of traps was chosen on the basis of experience from previous surveys in similar systems, were mites are known to aggregate, particularly in nest and roosting places and varied depending on the type housing system (Nordenfors & Chirico, 2001). In the furnished cages the traps were placed under the nest lining. In the Jansen system the traps were placed in the front edge of the nest. In other systems (single or multi-tier) the traps were fastened around both the upper and lower perches by plastic straps. All units at the farms were to be sampled including those that were currently empty. The sampling period was set to seven days and thereafter the traps were to be put in a double set of zipper bags and sent to the laboratory. Mites were trapped for two purposes - mite counting and for use in efficacy test. Specific procedures when sampling and handling mites aimed for the different purposes are described in separate sections below.

3.2.1 Sampling procedure for mite counting

For the sampling of mites, the number of traps was adjusted according to the flock size with 1 trap per 1000 layers. However, the minimum of traps per unit was set to 10 and the maximum 35. At the laboratory at SVA (National Veterinary Institute) the traps for counting of mites were stored at -20°C for at least 24 hours to kill all mites and thereafter the producer identity was encoded. If mites were found in the traps, the degree of mites was estimated.

3.2.2 Sampling procedure for efficacy test

In addition, two traps per unit were sent out for collection of mites for the efficacy test. These were put up in the same way as the other traps (according to instructions). However, after the seven-day sampling period these traps were put in a separate double set of zipper bags and sent to the laboratory. At the laboratory at SVA mites for efficacy test were stored at 4°C and later on used in the test for mite efficacy to acaricides.

3.3 Mite counting

After killing possible mites by freezing, the traps were counted and the removal of mites from each trap were done by cutting open all the funnels and scraping out the mites. The mites from each trap were poured on to a Petri dish (Petri Dish, PS 145x 20mm, with vents, Greiner Bio-One) and sectioned into eight compartments were one section was counted and multiplied by eight. The mites were spread evenly in the petri dish and if the number of mites were less than 500 the mites were counted as individuals using a stereomicroscope. When the number of mites exceeded 500 the numbers of mites were instead estimated according to Nordenfors & Höglund (2000) by volume using a measuring tube calibrated and graded for 500, 1500, 2000, 3000, 4000 and 5000 mites with a measuring accuracy of 500 or 1000 depending on the mite abundancy. The grading of severity of mite infestation was done by dividing the total number of mites by the number of traps per flock, generating a mean number of mites per trap. The degree of mites was graded depending on the amount as “No mites detected” when 0 mites/trap was recovered, “Low abundance” when there was between 1-1000 mites/trap recovered, “Moderate” when there were 1001-2500 mites/trap recovered and “Abundant” when there was >2500 mites/trap recovered.

3.4 Efficacy test

To investigate the efficacy in *D. gallinae* to phoxim and cypermethrin the commercial products Baymite® (Bayer Animal Health, Bayer Healthcare AG, Leverkusen, Germany) and Intermitox® (Interhygiene, Cuxhaven, Germany) were used. The *in vitro* tests included 18 isolates of *D. gallinae* from Swedish laying hen flocks. The mites were sent in by producers monitoring possible mite infestation in their laying flocks by the use of traps, as previously described. Prior to the test, the traps with mites were kept in a refrigerator at 4°C between 1 to 19 weeks. Two tests with different methods were used to test mite efficacy to the chosen substances.

Method 1

The two acaricides phoxim and cypermethrin were tested in different concentrations. For phoxim the two dilutions used were 10ml in 2.5l water (2%), in accordance with recommendations from the manufacturer, and 7.5ml in 2.5l water (1.5%). For cypermethrin the dilutions tested were, 20ml (2%) in 1l water, 10ml (1%) in 1l water, and 7.5ml in 1l water, 10ml in 1 l water was the dilution recommended by the manufacturer. Plates of 3mm thick corrugated cardboard (Boxon Pak AB) were impregnated with the different concentrations of phoxim and cypermethrin dilutions and tested by the method described by Nordenfors *et al*. (1999). Briefly, the impregnated strips were cut in to small round pieces with a diameter of 5 mm and placed in each well of a 96-welled flat-bottomed Immulon® 1B ELISA-plate (Thermo Scientific, Rochester, USA). One set of tests for each of the 18 isolates tested for phoxim consisted of three plates (one plate for control, and one plate for each concentration of the acaricide). For cypermethrin one set of tests consisted of four plates (one plate for control, and one plate for each concentration of the acaricide). The set of tests were tested once with no replicates. One mite was separately added in each well of the plates sealed with micronic-lids. The plates were kept in an incubator at 20°C and 70 % relative humidity. The survival of mites was measured by monitoring the activity or inactivity of mites. Activity was defined as leg movements and inactivity was defined as immobility when observing or tapping on the plate. The mites were observed for approximately 5-10 minutes after 48 hours at 10-20x magnifications using a stereomicroscope.

Method 2

The two acaricides were used in the dilutions recommended by the manufacturers for use in poultry, i.e., 10ml in 2.5l water (2%) phoxim and 10ml in 1 l water (1%) cypermethrin. The dilutions were prepared according to manufacturer's instructions and poured in spray bottles. Filter paper circles (Schleicher & Schuell, Dassel, Germany) with a diameter of 125mm were put in sterile 145mm petri dishes. Phoxim and cypermethrin dilutions and a control (water) was sprayed on the respective plates at 15 cm distance with a fine mist. The procedure was repeated three times within 1 min to moisten the filter paper evenly and mites were then added to the dish. Mites from 18 poultry laying flocks were used and 40 mites from each isolate were added to each dish. After sealing with a lid and parafilm tape (Parafilm "M" Laboratory Film, Pechiney Plastic Packing, Chicago) the petri dishes were kept at room temperature on a tinplate with a Vaseline barrier. One set of tests for each of the 18 isolates consisted of three dishes (one dish for control, and one dish for each of the acaricides). The set of tests were tested once with no replicates. The survival of mites was measured by monitoring the activity or inactivity of mites. Activity was defined as leg movements and inactivity was defined as immobility when observing or tapping on the plate. The mites were

observed according to Nordenfors *et al*. (1999) after 48 hours under 10-20x magnifications using a stereomicroscope.

3.5 Statistical methods

Descriptive statistics were used to summarise the results of the amount of mites found and the answers given in the questionnaire. Associations between the given answers in the questionnaire were investigated and normally distributed data were analysed using Fishers´s exact test. When data were not normally distributed Chi-2 test, Ordinal logistic regression or Kruskal-Wallis equality-of-populations rank test was used. The descriptive statistics were mainly performed using Excel and the statistical tests were performed using Stata Statistical Software (Stata Statistical Software: Release 13.1, 2010; StatCorp LP, College Station, TX). were analysed

4 Results

4.1 Questionnaire study

Out of 314 invited egg producers, 120 answered the questionnaire where 10 responded by mail and 110 by e- mail, implying a response rate of 38%. When presenting the results of the questionnaire the frequency of answers on each question is expressed as N=number of producers answering.

4.1.1 Participating producers and flock information

Of the respondent egg producers (N=119) 81% were located in the southern part of Sweden (Götaland), 13% in the middle part (Svealand) and 6% in the northern part (Norrland). The majority, 78%, had produced eggs on the specified property for more than 10 years (N=118). Only one producer had produced eggs for less than a year, 13% in 1-5 years and 8% in 6-10 years.

The number of units per farm ranged from 1-9 (see Figure 2, N=112), where the majority 42% had one (47 producers) or two units with layers (39 producers). In total 225 units were reported from these 112 producers.

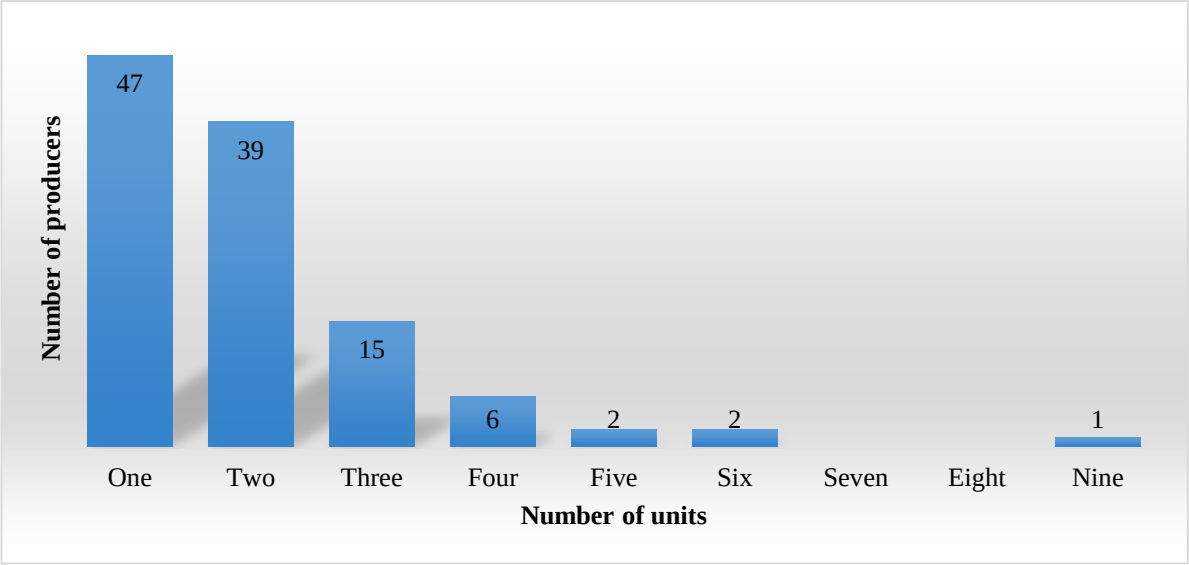

Figure 2. Number of producers with a certain number of units per farm, reported by 112 different producers.

In total 112 producers specified the kind of eggs produced in their units, and these producers had in total 225 layer units (see Table 2). The most common egg production was "Barn egg production in aviary system" which represented 33% of the reported layer units (75/225), followed by “Furnished cages” with 23% of the units (51/225) and “Barn egg production in traditional floor system" with 19% of the units (43/225). "Organic egg production in aviary system" and "Organic egg production in traditional floor system" accounted for 16% (36/225) and 6% (13/225) of the layer units, respectively. "Aviary system with free range" and "Traditional floor system with free range" accounted for 2% (5/225) and 1% (2/225), respectively, of the layer units. The representation of production systems among egg producers followed the same pattern, with “Barn egg production in aviary system” being most frequently represented 39% (44/112 egg producers) and “Free range with traditional floor housing” being the least represented system 2% (2/112 egg producers). The majority (82%) of the 112 producers had only one type of egg production system, 14% had two different production systems and 3% had three different production systems (not in Figure).

The number of layers in 185 units reported by 91 producers varied greatly (see Table 3). The largest number of birds per unit was found in “Barn egg production in aviary system” (range: 1300-50000, median: 16300 mean: 18246 (n=68) and in units with “Furnished cages” (range: 1200-50220, median: 12720, mean: 15434, n=43). In “Organic egg production in traditional floor system” (range: 1500-19000, median: 3700, mean: 6761, n=9) and “Barn egg production in traditional floor system” (range: 720 to 20024 median: 6000 mean: 6317, n=32) the numbers of layers per unit were lowest. In “organic egg production in aviary system” the spread was from 3000 to 18000, median: 9000 and mean: 10882 (n=28). In “aviary system

with free range" there were only three units where the numbers of animals were specified as two with 4300 animals and one with 19640 animals. In "traditional floor system with free range" there were only two units where the number of animals were specified, one with 9000 animals and one with 14100 animals.

Table 2. Number of units (layer flocks) with a certain production and housing system reported by 112 egg producers with a total of 225 units.

	Units n=225	**%**	**Producers n=112**	**%**
Barn, aviary system	75	33	44	39
Barn, traditional floor system	43	19	25	22
Organic, aviary system	36	16	21	19
Organic, traditional floor system	13	6	9	8
Free range, aviary system	5	2	3	3
Free range	2	1	2	2
Furnished cages	51	23	33	29

Table 3. The median and mean number of animals per unit and production systems reported by 91 producers with a total of 185 units.

	Min layers per unit	**Max layers per unit**	**Median size layer unit n=185**	**Mean layers per unit n=185**
Barn, aviary system	1300	50000	16300 n=68	18246 n=68
Barn, traditional floor system	720	20024	6000 n=32	6477 n=32
Organic, aviary system	3000	18000	9000 n=28	10882 n=28
Organic, traditional floor system	1500	19000	3700 n=9	6761 n=9
Free range, aviary system	4300	19640	4300 n=3	9413 n=3
Free range	9000	14100	14247 n=2	14100 n=2

Furnished cages	1200	50220	12720 n=43	15434 n=43

4.1.2 Occurrence of *D. gallinae*

In total 107 producers responded to the question about presence of mites in their laying flocks (see Figure 3). One respondent did not provide information about number of years as egg producer and was therefore not included in Figure 3.). Fisher's exact test showed a significant difference in the distribution of answers regarding the mite occurrence and number of years as a producer (p=0.001). Of the 107 producers, 64% (68/107) answered that they presently had mites on their farms, 27% (29/107) that they previously had mites on their farms but not at present, 6% (6/107) answered that they had never had mites on their farms, and 4% (4/107) did not know if their layers had mites or not. Producers responding that they did not know if they had mites (4 in total) were only found in the group of producers with egg production for more than 10 years. A larger proportion of those who had kept layers for 10 or less than 10 years compared to those who had had layers for more than 10 years answered that they never had experienced mites on their farms (6 vs. 1 producers).

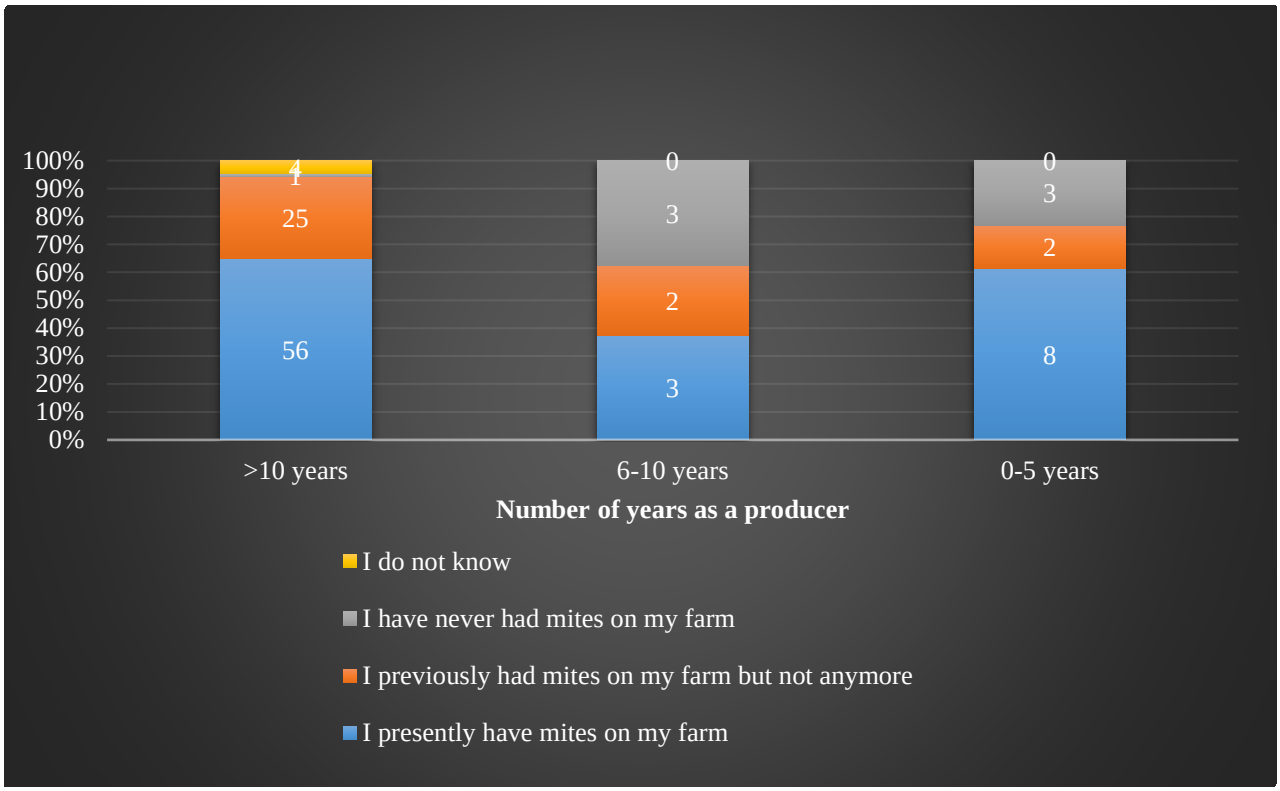

Figure 3. The number of producers who answered, “I presently have mites on my farm”, “I previously had mites on my farm but not anymore”, “I have never had mites on my farm” or “I do not know” to the question “What is the presence of mites on your farm like? In total 107 producers answered the

question and their answers were divided into three categories dependent on their number of years in egg production.

Fisher's exact test showed a significant difference in the distribution of answers regarding the mite occurrence and number of units on a farm (p=0.002). There were no producers with more than 2 units who answered that they had never had mites, (see Figure 4). There were a larger proportion (84%) of producers with more than 2 units who answered that they presently had mites compared to those who had one (55%) or two (63%) units. There was a higher proportion (43%) of those who had one unit who answered that they previously had mites but not anymore, compared to those who had 2 (21%) or more than 2 units (16%).

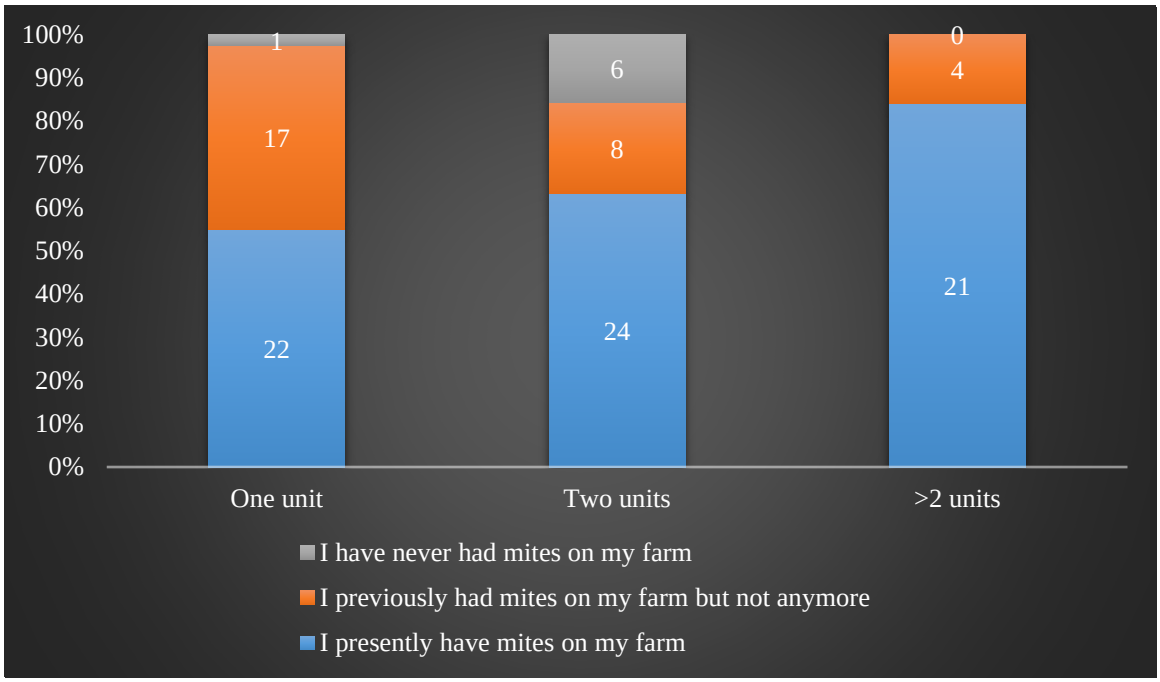

Figure 4. The number of producers who answered, “I presently have mites on my farm”, “I previously had mites on my farm but not anymore” or “I have never had mites on my farm” to the question “What is the presence of mites on your farm like? In total 103 producers answered the question and their answers were divided into three categories dependent on their number of units.

Of 92 producers with only one kind of production system, 88 gave an answer to whether they presently had mites or not (not in Figure). According to Fisher´s exact test the answers differed significantly (p=0.005) between farmers with different housing systems. Producers with "Organic egg production in aviary system" answered more often that they never had had mites, compared to farmers with other systems 31% (4/13) of producers with “organic egg production in aviary system” compared with 4% (3/75) of those who had other systems). Of the 75 producers with other systems 32% (24/75)

stated that they previously had mites but not anymore, compared to 1/13; 8% of the producers with “organic egg production in aviary system”. However, the majority of producers in both “Organic egg production in aviary system” and other systems answered that they presently had mites 54% (7/13) of the producers with “Organic egg production in aviary system” compared with 60% (45/75) of those with other systems).

4.1.3 Problems caused by *D. gallinae*

Of the 101 producers that responded to the question “Do you consider mites being a problem today (autumn 2015)?” 34% answered that they had problems now and then, 19% that they had major problems, 20% that they had small problems and 28% said that they did not have problems. Of the 29 producers reporting previous problems with mites on their farm but not anymore, 69% answered to have had no problems today, 17% had small problems today, 7% had problems today now and then and 3% had major problems today (and one did not answer this question). Not all of the 97 producers that presently or previously had mites on their farm, gave an answer to what kind of problem they consider to be associated to the presence of mites. However, of the 72 producers that did answer (see Figure 5), 55 (76%) had problems with bloodspots on eggshells. Moreover, 31 (43%) indicated personal being attacked by mites, 12 (17%) increased mortality in the hens, 32 (44%) hens become stressed, 22 (31%) associated increased feather pecking with mite occurrence, 3 (4%) incidence of pecking/cannibalism and 5 (7 %) increased feed consumption. One producer indicated that mites caused impaired general condition of the hens and one that the mite presence lead to eggs being heavily soiled.

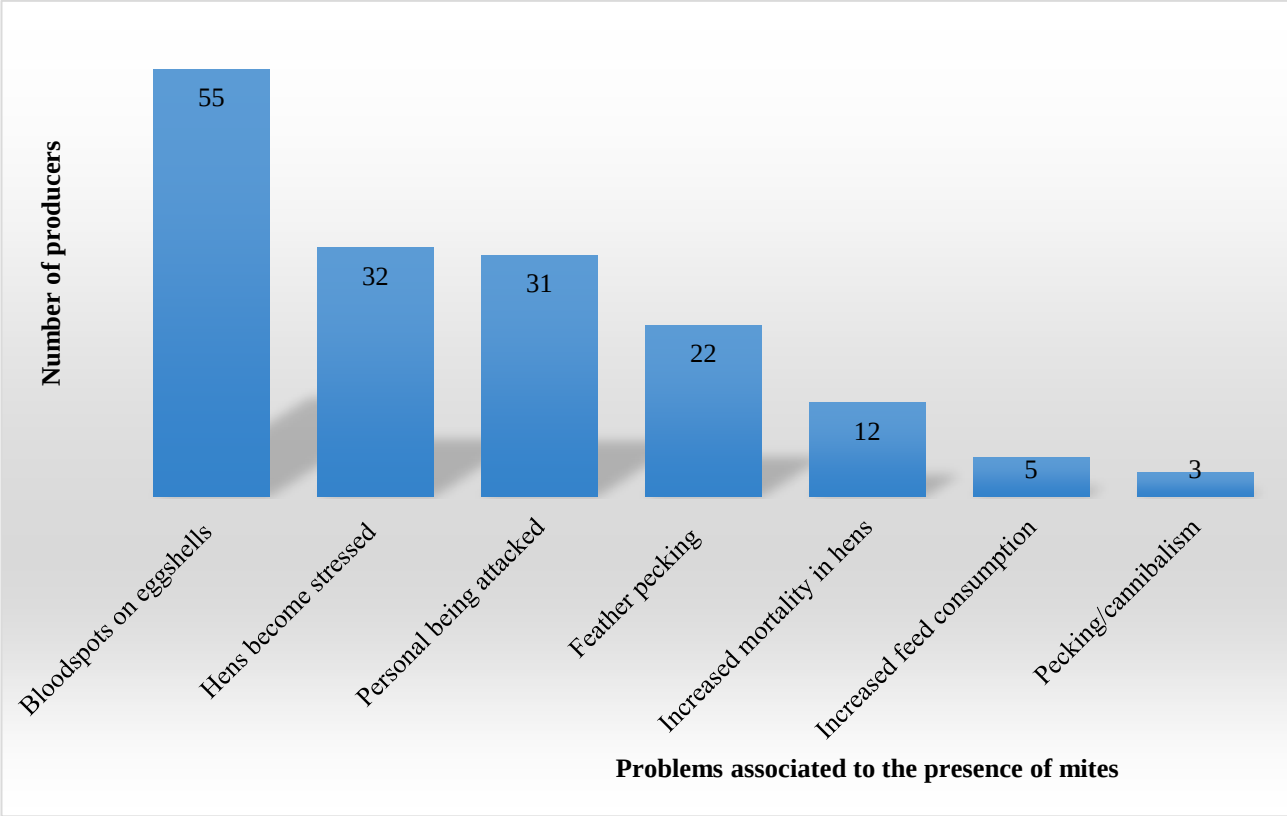

Figure 5. Problems associated to the presence of mites reported by 72 different producers.

4.1.4 Control during ongoing production

On the question regarding which control measures that had been used against mites during ongoing production during the last 5 years, 106 producers responded. Of these 106 producers 77% answered that they applied measures to suppress mites during ongoing egg production, 46% used measures to control mites in all flocks and 31% in some but not all flocks. Of the 82 producers who treated for mites during ongoing production, 41% applied dry cleaning (in combination with other measures), 61% used silica based preparations (silicon dioxide) together with other measures, 55% used Baymite® (in combination with others measures), and 21% other preparations such as detergents, disinfectants and garlic powder. Most commonly applied control measures and number of producers using the different measures are shown in Figure 6.

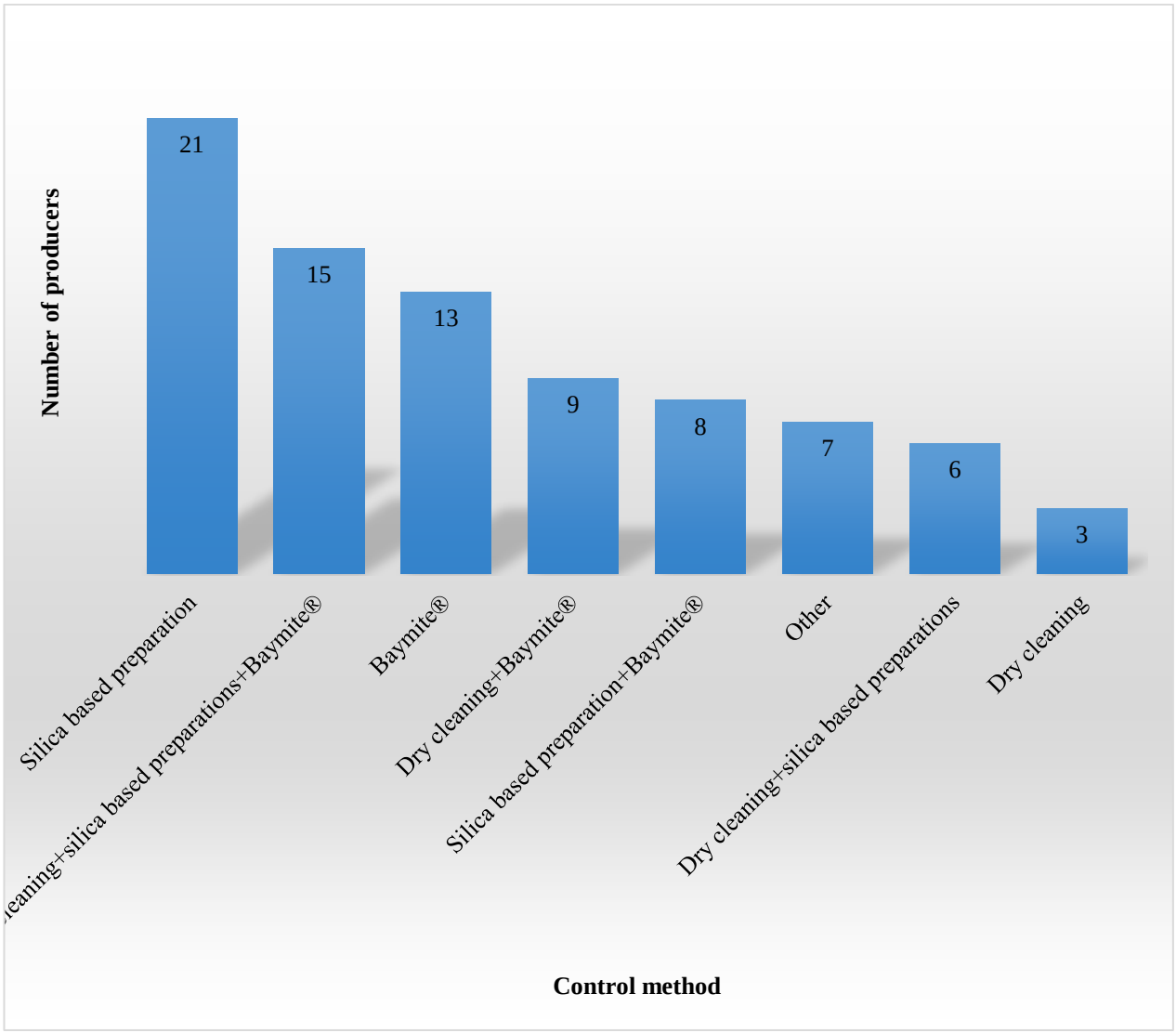

Figure 6. Number of producers applying certain control methods against mites during production reported by 82 producers. Other control measures included detergents, disinfectants and garlic powder. Two of the producers that applied dry cleaning, one of the producers that used silica based preparation, four of the producers that used Baymite® and three of the producers that dry cleaned, used silica and Baymite® also combined this with other measures.

4.1.5 Control between production cycles

On the question regarding which control measures the producers had used against mites between production cycles during the last 5 years, 103 producers responded. Of these 103 producers, 57% answered that they had used control measures between production cycles, 41% had used control measures in all flocks and 16% for some but not all flocks. The remaining 43% of producers had not used control measures at all against mites between production cycles. The 59 producers who used measures between production

cycles, had used dry cleaning, wet cleaning, combined dry- and wet cleaning, silica based preparations, Baymite® and various combinations of these, see Figure 7. Thirteen producers had used other preparations and 7 of these specified the preparations used. The preparations mentioned were Interkokask and Gimra para des, both disinfectants with active agent p-chloro-m-kresol. Among the producers who answered that they had used other preparations two answered 'Anticimex', three Interkokask, one Gimra para des and one "the new agent". Three producers answered that they used a different method than the alternatives given in the questionnaire and methods mentioned were chemical pesticides, oil and steam cleaner. During treatment between flocks most of the producers used a combination of measures and most common was to use both dry and wet cleaning, in combination with Baymite® (n=12), see Figure 7. In total, 55 producers used control measures both during production cycles and during ongoing production (not in Figure).

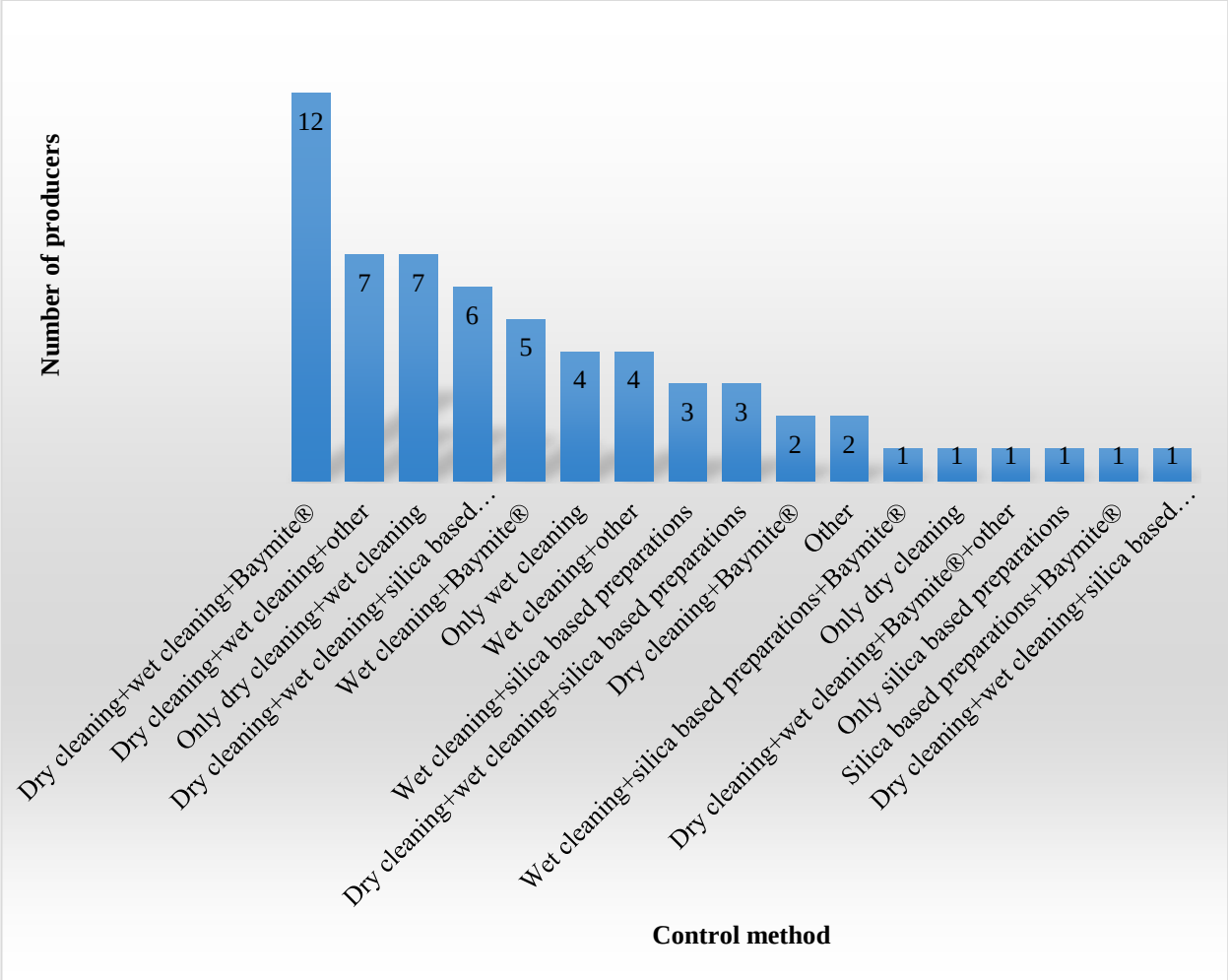

Figure 7. Number of producers applying certain control methods against mites between flocks reported by 59 producers. Other control measures including Interkokask, chemical pesticides, oil and steam cleaner.

4.1.6 Experienced effect of applied mite control measures

A statistical evaluation of the connection between control measures used during ongoing production or between production cycles and how the producers' experienced the effect of the applied measures were not possible due to too few observations in each category.

In total 86 producers answered the question about what effect the control measures have had on their farms. Of these 62 (72%) thought that the mite problem had decreased, 13 (15%) experienced that the mites had disappeared and 10 (12%) stated that the treatment had poor or no effect and 1 (1%) did not know what effect the applied treatment had had.

4.2 Mite count study

4.2.1 Mite abundancy

Mite traps from 54 different units at 30 egg producing farms were examined and mites were recovered from traps placed in 46 different units and 25 farms. The discrepancy between the number of traps sent out and collected traps varied between the farms. Sixteen of the 30 farms (34 units) returned all traps. Twelve farms (16 units) returned more than 80% but not all traps. Two farms with 3 and 1 units respectively returned 60% and 50% of the traps. The number of farms with occurrence of mites is summarised in Table 4 and the amount of mites recovered from the traps is summarised in Table 5. In Table 5 the number of mites are graded as "No mites detected" when 0 mites/trap were recovered, "Low abundance" when there was between 1-1000 mites/trap recovered, "Moderate" when there were 1001-2500 mites/trap recovered and "Abundant" when there was >2500 mites/trap recovered.

Table 4. Number of farms with occurrence of mites, recorded by traps placed in 54 different units at 30 egg producing farms.

	No of farms	**% of farms**
Mites detected	25	83
No mites detected	5	17

Table 5*f*. Number of producer's units with no mites detected, low abundance, moderate abundance or abundant number of mites recorded by traps placed in 54 different units at 30 egg producing farms.

	No of units	**% of units**
Abundant	6	11
Moderate abundant	5	9
Low abundance	35	65
No mites detected	8	15

4.2.2 Abundancy of mites in different production system

The number of units with no mites detected, low abundance, moderate abundance and abundant number of mites in the different production systems are given in Table 6. According to Fisher's exact test and Ordinal logistic regression there were no differences in occurrence of mites related to production system.

Table 6. Number of units with no mites detected, low abundance, moderate abundance and abundant number of mites in different production systems.

	Grading				
Production system	No mites detected	Low abundance	Moderate abundant	Abundant	Total number of units
Organic aviary system	1	3	2	0	6
Barn traditional floor system	1	12	0	0	13
Barn aviary system	6	12	3	3	24
Free range Traditional floor system	0	1	0	0	1
Free range aviary system	0	0	0	2	2
Furnished cages	0	7	0	1	8
Total number of units	8	35	5	6	54

4.2.3 Impact of number of years of egg production on the premise and in the current unit

According to Fisher's exact test there was no significant difference in the abundance of mites between farms producing eggs for >10 years, 6-10 years and 1-5 years (Table 7). Regardless of how long eggs had been produced at the farms 60-67% of the flocks had a low abundance of mites. None of the farms with egg production for 1-5 years had abundant amount of mites.

Table 7. Number of units with no mites detected, low abundance, moderate abundance and abundant number of mites between farms producing eggs for >10 years, 6-10 years and 1-5 years recorded by traps placed in 54 different units at 30 egg producing farms.

	Grading				
Year	No mites detected	Low abundance	Moderate abundant	Abundant	**Total**
>10 years	7	24	6	4	**37**
6-10 years	0	6	2	2	**10**
1-5 years	1	4	1	0	**6**
Unknown	-	-	-	-	**1**
Total	8	34	5	6	**54**

Ordinal logistic regression model showed no significant difference in the distribution between total number of years with egg production in the sampled unit and the mite occurrence. It is only in units where egg production have been conducted in 9-14 years, compared to units were egg production have been conducted for <9 years or for ≥15 years, where there is abundant amount of mites. However, it is also in these units where there are no mites detected.

4.2.4 Impact of age, flock size and hybrid

Fisher's exact test showed a significant difference between older flocks and the occurrence of mites (p=0.02). Older flocks (>52w) had more often moderate to abundant occurrence of mites and flocks <36w had more often low abundance of mites (see Table 8). Ordinal logistic regression model showed no significant difference in the distribution between age group <36w and 36-52w but older (>52w) had a higher risk of greater amounts of mites compared to the age group 36-52w. Fisher's exact test showed no significant difference between hybrids and the degree of mites (Table 9). Kruskal-Wallis equality-of-populations rank test showed no significant difference in the distribution regarding flock size and the degree of mites (not in Figure).

Table 8. Number of units with no mites detected, low abundance, moderate abundant and abundant amount of mites in flocks <36 weeks of age, 36-52 weeks of age and >52 weeks of age. The degree of mites was recorded by traps placed in 49 different units at 30 egg producing farms.

	Grading				
Age of the flocks	No mites detected	Low abundance	Moderate abundant	Abundant	**Total**
<36 weeks	2	14	1	0	**17**
36-52 weeks	4	10	1	1	**16**

>52 weeks	0	8	3	5	**16**
Unknown	-	-	-	-	**5**
Total	6	32	5	6	**54**

Table 9. Number of units, per hybrid, with no mites detected, low abundance, moderate abundant and abundant amount of mites recorded by traps placed in 54 different units at 30 egg producing farms.

	Grading			
Hybrid	No mites detected	Low abundance	Moderate abundant	Total
Bovans	2	11	4	17
Lohmann	5	22	7	34
Other/Unknown breed	0	1	0	1
Unknown	-	-	-	2
Total	7	34	11	54

4.3. Efficacy test

Mites from 18 different layer farms were tested for efficacy against the two acaricides phoxim and cypermethrin, With the method using a 96- welled flat-bottomed high absorbency ELISA-plate (method 1) it became evident that mites exposed to phoxim and cypermethrin were not affected as expected as all mites remained active after 48h. Therefore, only results from the method using petri dishes (method 2) are presented in Table 10.

Table 10. Percentage of petri dishes pre-treated with water (control), phoxim or cypermethrin, with different degree of mite activity after 48 hours. The mites tested were recovered from 18 layer farms and the number of mites per petri dish was 40

	Control 48h n=18	**Phoxim 48h n=18**	**Cypermethrin 48h n=18**
All mites active	72%	0%	0%
≤10 mites inactive	22%	0%	5%
>10 mites inactive	6%	17%	56%
All mites inactive	0%	83%	39%

In the efficacy test all mites from 15 of 18 flocks (83%) tested for phoxim were inactive. In the remaining 3 flocks more than 25% of the mites were inactive. In the test with cypermethrin all of the mites from 7 of 18 flocks (39%) were inactive. Of the remaining 11 flocks there was 1 flock where less than 25% was inactive and 10 flocks with more than 25% inactive mites. Of these 11 flocks with active mites despite treatment with cypermethrin, 3 flocks distinguished from the rest. In 2 flocks only 50% of the mites were inactive and in 1 flock only 10% of the mites was inactive.

5 Discussion

5.1 Questionnaire as a study method

Online survey research is useful when the population of interest is large or separated by large geographic distances. It provides access to groups and individuals who would be difficult to reach though other channels and is both time and cost effective (Garton *et al*., 1997). However, when conducting online survey research, little may be known about the characteristics of the population and respondents will not always share sensitive information. Online survey research also tends to have low response rate and with self-reported data, there is no guarantee that the respondents provides accurate demographic or characteristics information (Stanton, 1998; Dillman, 2000).

This survey may serve as an indication of the red mite situation in Sweden however, with a response rate of 38%, the results of the questionnaire study may not be entirely representative for the Swedish laying hen industry. The data from the survey indicates that *D. gallinae* is likely widespread throughout Sweden in all types of housing system, causing varying degrees of problems for Swedish layers and egg producers. According to the results from this limited study, Baymite® appears to be an effective compound in the control of *D. gallinae* while Intermitox® on the other hand seems to be less effective.

5.2 Occurrence of *D. gallinae*

In the study by Höglund *et al*. (1995) the occurrence of *D. gallinae* was examined in different types of production system for egg layers in Sweden. In their study the occurrence of mites was found in all production systems examined (floor housing systems indoors, backyard flocks and caged systems). Our present study indicates that *D. gallinae* is still present in commercial layer flocks in Sweden, some which despite production system. Occurrence of mites in different types of production systems has also been observed in several countries worldwide (Sparagano *et al*., 2014). In the present study, 82 out of 106 producers took measures (dry cleaning, silica based preparations, Baymite®) against mites during ongoing production, and only 59 out of 103 producers took measures (dry cleaning, wet cleaning, dry/wet cleaning, silica based preparations, Baymite®) in between production

cycles showing that regular measures are taken by the majority of the producers. However, in the questionnaire, the question was not designed optimally. In fact, many of the producers may have thought that control measures were equal to the use of preparations and that cleaning was not meant as a control measure. Cleaning (and disinfection) shall always be done between production cycles and was therefore probably done regardless of whether there were mites in the flocks or not.

The reason to the higher frequency of mite infestation at farms with more than 10 years in egg production may be due to several factors. Suggested factors contributing to this circumstance are that *D. gallinae* is very hard to eradicate once established in poultry facilities, has a rapid life cycle and spends most of if its time off the host hiding in cracks and crevices (Marangi *et al*., 2012; George *et al*., 2015). Older facilities and older equipment provides more hiding places for mites as well as being more difficult to clean. Many years in production also contribute to more opportunities for transmission of mites. This may also be an explanation to why it is more common in flocks older than 52 weeks to have a higher amount of mites compared to younger flocks. The rather high percentage of producers with mites in their flocks (63% in the questionnaire study) is of major concern due to that *D. gallinae* can have serious impact on the health and welfare of hens as well as a negative impact on egg production, egg quality and on human health (Kilpinen *et al*., 2005; George *et al*., 2015; Rosen *et al*., 2002).

With the definition in the current study of unit as a space delimited by solid walls follows that several units might have been located in the same building implying that mites could easily be transmitted between units. *Dermanyssus gallinae* have a tendency to multiply again after treatment and are easily spread indirectly through workers and equipment (Marangi *et al*., 2012; Brännström *et al*., 2008; Oines & Brännström, 2011). This makes it likely to assume that it is difficult for a producer to completely eradicate all mites from their poultry facilities. However, good management and cleaning procedures may contribute to keeping the infestations at low rates. In this study good management and cleaning procedures could be a likely cause to that the majority, 65% of the producers had low amounts of mites in their flocks. Renovation of premises, new housing equipment or new facilities may also contribute to a lower rate of infestation. It is also more time consuming to regularly clean and control several units rather than one unit.

There is an ongoing shift from furnished cages to other housing systems in Sweden and other countries in northern Europe. The number of layers in aviaries indoor (barn eggs) and in organic production have increased rapidly during the last years (Lannhard-Öberg, 2016). Most of the organic farmers in this study had recently started their egg production which may have contributed to the lower frequency of mite infested flocks in organic production.

5.3 Occurrence of mites - questionnaire study vs mite count study

The 30 producers, selected by e-mail and mail, who volunteered to be part of the mite count study cannot be considered as a representative sample of the 120 producers answering the questionnaire. It is possible that the motivation to place traps in the housing system, implying extra work, was likely higher among producers experiencing problems with mites. However, despite this there were a rather good agreement between the producers' answers in the questionnaire study and the actual findings of mites in the units investigated in the mite count study.

The 30 producers that took part in the mite count study answered additional questions in a referral. Several of the housing and management factors examined in the referral did not show any significant differences. This is most likely a consequence of too few observations for each of the different factors. However, in order to conclude that too few observations alone are the only cause of outcome, more studies are needed.

5.4 Problems caused by *D. gallinae*

Data from the questionnaire indicated that *D. gallinae* are perceived to cause a series of problems in layers in Sweden. The reason to that as many as 73% of the producers experienced some sort of problem related to mites may be due to several factors. *Dermanyssus gallinae* have various negative effects on layers both due to their presence on the hens and through their blood meals. In the survey the producers considered bloodspots on eggshells, hens becoming stressed, personal being attacked by mites and increased feather pecking to be the major problems caused by *D. gallinae*. These problems are not only affecting the poultry welfare but may also affect the productivity of the layers as well as the workers working environment. According to Arkle (2007) there is a direct effect of the size of the mite population on bird mortality, indicating a lower flock productivity. In addition, eggs that roll over fed mites get bloodspots on their shells and would therefore be downgraded. This in turn have a negative impact on the economy for the producers. Moreover, *D. gallinae* can have a serious impact upon human health. It does not only cause itching and skin irritation but can also cause allergic skin reactions on personal working in infested poultry facilities (De Luna *et al*., 2008; Rosen *et al*., 2002).

5.5 Control measures during ongoing production and between production cycles

Silica based preparations and Baymite® were likely used to a large extent due to the restricted availability of other preparations allowed for use during ongoing production in Sweden. Silica based preparations are attractive because of their efficiency and low toxicity to hens and humans (Mul *et al*., 2009; Schulz *et al*., 2014). The high use of Baymite® may partly be due to

it being the only acaricide allowed for use on laying hens in Sweden. Dry cleaning in combination with other measures was another very common, (40% of the producers) method used during ongoing production. This method needs to be applied thoroughly and may be both time consuming and laborious. Garlic powder was used to a very limited extent during ongoing production however, garlic have been shown to be an effective acaricide against *D. gallinae*. Ranjbar-Bahadori *et al*. (2014) and Faghihzadeh Gorji *et al*. (2014) showed that administration of garlic extract had an efficacy rate of 92% and 96% respectively. Garlic essential oil has been shown to be toxic against mites in studies by George *et al*. (2010) and garlic based products are available in several countries for use against ectoparasites on poultry (George *et al*., 2010).

According to data from the questionnaire as many as 77% of the 106 producers responded to have used control measures during ongoing production. There may be several factors contributing to the use of control measures, such as that mites have major negative effects on the production, welfare and health of the hens and the working environment (Kilpinen *et al*., 2005; Marangi *et al*., 2012). It can also be a routine manner in order to help suppress mites during ongoing production. Only 57% of the 103 producers responded that they used control measures (dry cleaning, wet cleaning, dry/wet cleaning, silica based preparations, Baymite®) between production cycles during the last five years. However, the question was not designed optimally and the survey should have been tested more in advance to avoid misunderstandings. It is likely that producers with mites in their premises answered that no measure against mites were applied between production cycles although dry and/or wet cleaning were actually routinely done regardless of presence of mites or not. Another explanation may well be that producers who answered that they don't know if they have presence of mites, don't have mites or experience a small problem with mites, don't use any measures during ongoing production or between production cycles. The empty period between production cycles in Sweden is recommended to be at least three weeks, however in many cases this is often longer (personal message. A. Jeremiasson 2016-10-24). For sanitary clearance (starving the mite population for a given duration, e.g. between production cycles) to have a negative effect on *D. gallinae* it is recommended to extend the empty periods as long as possible (Chauve, 1998). However, a few weeks are of minor importance in the mite control due to that mites are able to survive for up to 9 months without its host (Nordenfors *et al*., 1999).

5.6 Experienced effect of applied mite control measures

It is noteworthy that of the 55 producers answering to have used control measures both during ongoing production and between production cycles, 73% experienced that the problems with mites had decreased or disappeared. This indicates that the effect of management factors such as good cleaning routines and control measures against mites at farms should not be

underestimated. However, due to the short life cycle of *D. gallinae*, high reproductive rate and the large number of eggs in cracks and crevices that are hard to target (Sparagano *et al*., 2009; Huber *et al*., 2011), mechanical cleaning and sanitary clearance alone are not enough to eradicate *D. gallinae*, but cleaning can reduce the population to a level where it doesn´t cause problems. Nordenfors *et al*., 1996 investigated the control methods used for *D. gallinae* and found that mechanical cleaning of poultry houses was as efficient as spraying with metriphonate in controlling mites.

5.7 Efficacy test

The test comparing mite's efficacy against the acaricides phoxim and cypermethrin showed that, under the conditions prevailing in the experiment, the most effective acaricide against Swedish populations of *D. gallinae* was phoxim. Unfortunately, live mites for examination were only available from flocks from 18 different producers. There was large variability in the number of mites in the traps between flocks due that the occurrence of mites varied between producers. Unfortunately, the low amount of mites collected from some producers only allowed for one control when testing was conducted according to "Method 2", instead of one for each of the two acaricides. The lack of control therefore makes the test results not fully reliable. More traps should therefore have been sampled from each farm. It should also be mentioned that the mites in the efficacy test where stored in the fridge for a relatively long time (1-19 weeks) but should likely not have affected the outcome as mites can survive for up to 9 months without feed and are tolerant to low temperatures (Nordenfors *et al*., 1999; Maurer & Baumgärtner, 1992).

With the first method modified by Nordenfors *et al*. (2001), where the effect of the corrugated cardboard impregnated with either phoxim or cypermethrin were examined using ELISA-plates, there was an evident lack of response in the mites. This method is however precise and makes it possible to study each individual mite in detail, compared to method 2 were mites were studied as a population. Impregnated corrugated cardboard have previously been successfully used in unpublished studies (personal message Chirico 2017-01-27).

When using the second method, where the two acaricides were sprayed on filter papers in petri dishes, the results showed that phoxim inactivated all mites from 15 out of 18 flocks after 48 hours. This indicates that Baymite®, with the acaricide phoxim, seems to be a relatively efficient measure against mites. The fact that not all mites were inactivated in 3 mite populations of 18 when testing phoxim may be due methodical error such as an uneven distribution of the preparations or an indication of tolerance towards the acaricide. It should also be noted that this is a limited study and the environment in the petri dish differs from the environment in poultry facilities. However, most of the mites (including the 3 farms) was inactivated by phoxim. Phoxim has also been shown to have good efficacy on *D. gallinae*

in several studies. For example, Keita *et al*. (2006) and Meyer-Kühling *et al*. (2007) applied a spray solution of 2,000 ppm phoxim onto surfaces in close environment of the birds in commercial layer houses with cage system in France and Germany. The treatment was applied twice within a seven-day interval. The efficacy of phoxim was >97% (from day 10 to 49 of treatment) according to Keita *et al*., (2006) and >99% (from day 7 to 49 of treatment) in a study by Meyer-Kühling *et al*., (2007). Zdybel *et al*. (2011) used plates made according to own design (veneer disc with a diameter of 90 mm placed in the centre field of the plate). Phoxim in a concentration of 0.4% was distributed on the surface of the disc, the disc was dried for 24h and 80-100 mites from commercial layer flocks in Poland were added per plate. The efficacy was 89-100% after 24 h (Zdybel *et al*., 2011). Abdel-Ghaffar *et al*. (2009) used a method were mites collected from two commercial layer houses in France and Germany were transferred into 8.5 cm Petri dishes with filter paper dosed with 440 µl of 2,000 ppm phoxim. The efficacy of phoxim was 96.2% after 24 hours (Abdel-Ghaffar *et al*., 2009).

In our study, petri dishes where mites were exposed to cypermethrin showed that Intermitox® with the acaricide cypermethrin inactivated all mites from only 7 out of 18 flocks after 48 hours. Mite strains resistant to pyreothroid-based formulations (cypermethrin and α-cypermethrin) have been detected in red mite populations in several studies. For example, Fiddes *et al*. (2005) exposed mites *in vitro* for two hours to acaricide (bendiocarb, cypermethrin, malathion, permethrin) impregnated Whatman filter paper sealed in 3.5 x 5.5 cm "tea bags". Mites was sampled from poultry units in England and was compared with laboratory-reared susceptible mites from Hannover (Germany) The in vitro test showed that resistance to cypermethrin was detected among mites from all farms. Kim *et al*. (2007) used a contact filter paper bioassay to examine the toxicity of plant preparations and insecticides on adult *D. gallinae*. Mites were exposed to the acaricide α-cypermethrin for 24h and the treatment was found to be ineffective. Zdybel *et al*. (2011) studied the efficacy of α-cypermethrin *in vitro* against *D. gallinae* collected from Polish commercial layer houses with cage system. The test used plates made according to own design (veneer disc with a diameter of 90 mm placed in the centre field of the plate) and a concentration of α-cypermethrin was distributed on the surface of the disc. The test demonstrated a low efficacy, only 7%, of α-cypermethrin. Intermitox®, with the acaricide cypermethrin, is not approved for use in Sweden but was included as a comparison in the study because the preparation is used in other European countries. However, based on the outcome in the present limited study it seems to be quite inefficient in the control of mites.

This was most apparent in the flocks that were exposed to cypermethrin. Hence, the effect of cypermethrin seemed to be insufficient in several of the flocks tested in this study. This may indicate that mites from some of the

populations used in the present study had developed resistance for cypermethrin.

The global acaricide distribution and expansion together with an intense use as well as a potential misuse of acaricides is a contributing factor to the increased mite resistance to acaricides (Marangi, 2012; Sparagano *et al*., 2009; Mul & Koenraadt, 2009). In Sweden Nordenfors *et al*. (2001) reported mite resistance to permethrin and cypermethrin, both belonging to the same group of acaricides, pyrethroids.

Management procedures suggested to prevent further development of resistance to acaricides includes regular drug monitoring tests of resistance, use of different combinations of acaricides and rotational use of effective acaricidic groups with different actions of mechanism (Chauve, 1998; Abbas *et al*., 2014). In Sweden however, these options are extremely limited since there is only one acaricide allowed for use, but despite this the situation looks good so far. It is also important to combine these control methods with other management procedures such as good cleaning routines and to use biosecurity measures to minimize the spreading of mites (Abbas *et al*., 2014) which are already applied in Sweden.

Conclusion

The main conclusion that can be drawn from the questionnaire study is that there is occurrence of *D. gallinae* in Swedish laying hen flocks. It occurs in all types of housing systems examined in the study and is causing varying degrees of problems for Swedish egg producers and their layers. Applying effective control methods and correct management are necessary to maintain the health and welfare of laying hens as well as to avoid development of resistant mite populations. The results from the efficacy test indicate that phoxim seems to be relatively effective in the control of *D. gallinae* while cypermethrin seems to be less effective. Future studies should include sampling of more traps in the efficacy test for more reliable results, methods used should be tested on beforehand to avoid methodical errors and the survey should be supplemented with interviews to get more accurate data. The situation in Sweden looks good so far however, this needs to be further studied in a context more similar to conditions prevailing in practice before firm conclusions can be drawn.

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Acknowledgements

This project was conducted in collaboration between the National Veterinary Institute (SVA), Swedish University of Agricultural Sciences (SLU) and the Swedish Egg Association. Financial support was provided by the Swedish Board of Agriculture. Specially thanks to my two supervisors Helena Wall and Helena Eriksson for your strong commitment throughout the entire project. Jan Chirico for the great help with all the laboratory work, Ann Nyman for the valuable assistance with the statistics, Alexandra Jeremiasson and Magnus Jeremiasson for always answering my questions and providing me with information. Last but not least many thanks to all the egg producers who participated in this project!

Appendix 1

Questions answered during August 25th- November 30th 2015 by egg producers participating in the questionnaire survey. Available alternatives are shown below each question. Producers were able to skip questions (no question was mandatory). In addition, there were conditions posted on certain questions so that these only appeared for the producers responded according to certain alternatives previously.

Questionnaire
1. In what region do you operate your egg production? **a) Götaland (Southern part of Sweden)** **b) Svealand (Middle part of Sweden)** **c) Nedre Norrland (Southern part of the northern part of Sweden)** **d) Övre Norrland (Northern part of the northern part of Sweden)**
2. For how long has egg been produced on your premise **a) Less than one year** **b) 1-5 years** **c) 6-10 years** **d) More than ten years**
3. For each unit that you have please state production system and the number of layers per unit. **Here, in this question a unit means a room enclosed by solid walls. Groups of hens only separated by nets are thus housed in the same unit** **a) Furnished cages** **b) Barn egg production in traditional floor system** **c) Barn egg production in aviary system** **d) Traditional floor system with free range** **e) Aviary system with free range** **f) Organic egg production in traditional floor system** **g) Organic egg production in aviary system**
4. What is the presence of mites on your farm like? **a) I have never had mites on my farm** **b) I presently have mites on my farm** **c) I previously had mites on my farm but not anymore** **d) I do not know**
5. Do you consider mites being a problem today (autumn 2015)? **a) No problem** **b) Small problem** **c) Problem now and then** **d) Major problem**

6. What kind of problems do you consider to be associated to the presence of mites (at present or past)?

a) **I have no problems with mites**
b) **Bloodspots on eggshells**
c) **Personal being attacked**
d) **Increased mortality in hens**
e) **Hens become stressed**
f) **Feather pecking**
g) **Pecking/ cannibalism**
h) **Increased feed consumption**
i) **Other**

7. Have you, during the last five years used control measures against mites during ongoing production?

a) **No**
b) **Yes, but not in all flocks**
c) **Yes, in all flocks**

8. If yes to the question above, which control measures against mites have you used during ongoing production during the last five years?

a) **Dry cleaning (sweeping, vacuuming, etc.)**
b) **Control measures with silica based preparation**
c) **Control measures with Baymite®**
d) **Control measures with other products, specify product**
e) **Others**

9. Have you, during the last five years used control measures against mites in between production cycles (in an empty house)?

a) **No**
b) **Yes, but not in all flocks**
c) **Yes, in all flocks**

10. If yes to the question above, which control measures against mites have you used in between production cycles (in an empty house) during the last five years?

a) **Dry cleaning (sweeping, vacuuming, etc.)**
b) **Wet cleaning**
c) **Combined dry and wet cleaning**
d) **Control measures with silica based preparations**
e) **Control measures with Baymite®**
f) **Control measures with other products, specify product**
g) **Others**

11. What effect do you consider that the control measures have had on your farm?

a) **The mites have disappeared**
b) **The mite problem has decreased**

c) **The control measures have had poor or no effect**
d) **I do not know**