Sveriges lantbruksuniversitet
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Evaluation of Ectopar for the control of the poultry red mite *Dermanyssus gallinae*

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Uppsala 2013

Degree project / Swedish University of Agricultural Sciences,
Department of Animal Nutrition and Management, **418**

Examensarbete, 30 hp
Masterarbete
Husdjursvetenskap
Degree project, 30 hp
Master Thesis
Animal Science

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Utvärdering av Ectopar som bekämpningsmedel mot det röda hönskvalstret *Dermanyssus gallinae*

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Omfattning:
Extent: 30 hp
Kurstitel:
Course title: Degree project in Animal Science
Kurskod:
Course code: EX0552
Program: Agronomprogrammet – Husdjur
Programme:
Nivå:
Level: Advanced A2E
Utgivningsort:
Place of publication: Uppsala
Utgivningsår:
Year of publication: 2013
Serienamn, delnr: Examensarbete / Sveriges lantbruksuniversitet, Institutionen för husdjurens utfodring och vård, 418
Series name, part No:
On-line publicering:
On-line published: http://epsilon.slu.se
Nyckelord:
Key words: Poultry red mite, *Dermanyssus gallinae*, ectoparasite, control method, laying hen

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Abstract

The poultry red mite, *Dermanyssus gallinae*, is a common problem in poultry facilities, causing great discomfort for the hens and effecting the production economically. Few efficient acaricides are available and there is a need for new control methods since resistance has become a problem in some cases. The aim of this study was to evaluate the efficiency of the new liquid silica control agent Ectopar, on the poultry red mite. An initial *in vitro* test of Ectopar and its two components individually was performed, where live mites were sealed in together with filter paper impregnated with one of the three solutions prepared. The survival of the mites was thereafter recorded repeatedly during a period of 120 hours and compared to a control. For the field study, two poultry farms with different housing systems were used, one with furnished cages (farm 1) and one with a single tier floor system (farm 2). Plastic cardboard traps were placed out where mites were known to cluster in both farms for weekly monitoring of the mite infestation level. The traps were collected every 7th day and replaced with new ones. The number of mites in each trap were counted and recorded in a laboratory. When the mites over time had doubled in number, ensuring a population growth prior to treatment, Ectopar was administrated in the two farms respectively. Monitoring of mite numbers continued for the following 6 weeks on farm 1 and 5 weeks on farm 2. The results from the *in vitro* test showed no differences between treatments and the control, indicating no controlling effect of Ectopar on the poultry red mite. In the field study, Ectopar treatment merely accomplished a reducing effect of up to 37 % on farm 1 which were non-significant (NS). On farm 2, initially the reduction reached a maximum of 24 % and was also NS. After the manure trays on this farm had been removed, however, there was a significant reduction obtained of 86 %, compared to before treatment. This was because a major site of mite aggregation was removed and therefore this reduction cannot be assigned Ectopar. In conclusion, Ectopar alone is not an effective control agent against *D. gallinae* under the presumptions of this study.

Sammanfattning

Det röda hönskvalstret, *Dermanyssus gallinae*, är ett vanligt förekommande problem i fjäderfäanläggningar, och orsakar stor t obehag för hönsen och påverkar produktionen ekonomiskt. Få effektiva akaricider finns tillgängliga och det finns ett behov av nya kontrollmetoder då utveckling av resistens har blivit ett problem i vissa fall. Syftet med denna studie var att utvärdera effektiviteten av det nya flytande kiselpreparatet Ectopar på röda hönskvalster. En inledande *in vitro*-test av Ectopar och dess två komponenter individuellt utfördes, där levande kvalster förseglades in tillsammans med filterpapper impregnerat med en av de tre tillredda lösningarna. Överlevnaden av kvalstren registrerades därefter upprepade gånger under en period av 120 timmar och jämfördes mot en kontroll. För fältstudien har två äggproducenter med olika inhysningssystem som används, en med inredda burar (gård 1) och en med envånings golvsystem (gård 2). Plastkartongfällor placerades ut där kvalstergömmor hade identifierats på båda gårdarna för veckovis övervakning av kvalsternivåerna. Fällorna samlades in var 7: e dag och ersattes med nya. Antalet kvalster i varje fälla räknades och registrerades på laboratorium. När kvalster över tid hade fördubblats i antal, vilket garanterar en populationstillväxt före behandling, administrerades Ectopar på de två gårdarna. Övervakning av kvalsterantalen fortsatte sedan i ytterligare 6 veckor på gård 1 och 5 veckor på gård 2. Resultatet från *in vitro*-testet påvisade inga skillnader mellan behandlingarna och kontrollen, vilket indikerar att Ectopar inte har någon kontrollerande effekt på röda hönskvalster. I fältstudien, åstadkom Ectopar enbart en reducerande effekt på upp till 37 % på gård 1 vilken var icke-signifikant (NS). På gård 2 uppnåddes initialt en reducerande effekt på upp till 24 % vilken även denna var NS. Efter det att gödsellådorna under redena på denna gård hade tagits bort, erhölls dock en signifikant minskning på 86 % jämfört med före behandling. Detta beror främst på att en stor kvalstergömma tagits bort och därför kan denna minskning inte tillskrivas Ectopar. Som slutsats, kan det fastställas att Ectopar inte ensam är en effektiv kontroll mot *D. gallinae* under de förhållanden som rådde under denna studie.

1 Introduction

1.1 Background

The production of eggs in Sweden has increased continuously for the last 4 years. In 2011, 116 000 tons shelled eggs were produced, the same year 121 000 tons shelled eggs were consumed. Consumption, however, has decreased for the last two years, while a larger portion of the production is exported. In 2011, there were 6.4 million hens in Sweden (Lannhard-Öberg & Lukkarinen, 2012). The trend is going towards more hens, fewer flocks and larger flock sizes. Further, the proportion of hens held in loose housing systems and organic production systems are increasing while caged systems are decreasing (Lannhard-Öberg & Lukkarinen, 2012). About 35 % of the hens are held in furnished cages and 65 % in loose housing systems (Hermansson & Odelros, 2011).

The poultry red mite, (PRM), *Dermanyssus gallinae*, is an external ectoparasite and a poultry pest. It is a common problem in poultry houses and it is the most economically important ectoparasite in laying hen production (Chauve, 1998). In Sweden, *D. gallinae* is the only hematophagous mite found in poultry facilities causing problems both in deep litter system as well as in battery cage systems (Höglund *et al*., 1995). Red spots on the eggs, red rashes on the keeper, reduced production and anxious hens are all indications of severe infestations levels, but once you discover the mites the problem is already pretty pronounced (Jordbruksverket, 2009).

In an empty poultry house, PRM can survive up to 9 months (Nordenfors *et al*., 1999). It is this characteristic, that it can survive long periods without feeding, which makes it difficult to entirely eliminate them. Once you have got mites in a facility, they will often come back even after treatment. Today there is only one acaricide approved to be used against mites in poultry houses when you have birds in the facility, Baymite (Jordbruksverket, 2009). The availability of approved acaricides against PRM is diminishing continuously (Thind & Ford, 2007) and it is therefore a need to find new control agents effective against mites.

1.2 Aim

The aim of this study was to determine the efficiency of the silica based liquid pest control “Ectopar” controlling *D. gallinae* infestations in Swedish egg producing farms. The liquid formula was tested in two common types of housing systems for laying hens, one with furnished cages and one with barn type housing.

2 Literature review

2.1 The lifecycle and prevalence of the red mite

D. gallinae is approximately 0.3 to 1 mm in size (Sikes & Chamberlain, 1954) and is grey in colour, but turns red when it has fed (Jordbruksverket, 2009). It feeds by sucking blood from its host, which they do mostly at night when the birds are less active and lights are dimmed. An adult female engorge on average about 0.2 mg blood per meal from its host (Sikes & Chamberlain, 1954) and the mite usually stays on birds for 0.5-1.5 h to feed (Chauve, 1998). After feeding they withdraw in clusters to cracks and cavities in the interior of the stable, where they go to hide, mate and lay their eggs. The eggs normally hatch into larvae after 1.5-2 days after being laid and the larvae thereafter turn into protonymphs in less than a day, without feeding. The protonymphs, however, turns into deutonymphs about a day after it has fed on a bird and after a second meal the deutonymphs within two days subsequently turn into sexually mature adults. The adult PRM, mate soon after moulting and the female usually lay her eggs within three days after she had a blood meal (Sikes & Chamberlain, 1954). A female can lay about 30 eggs during her lifetime (Chauve, 1998). Under favourable conditions, it takes about 8-9 days for the mite to develop from an egg to an adult (Sikes & Chamberlain, 1954). The life cycle of the PRM is illustrated in figure 1.

Optimal temperatures for reproduction and juvenile development are between 25-37 ^{o}C. At lower temperatures, reproduction is restricted. For instance, at 10 ^{o}C the egg stage of the life cycle take a minimum of 12 days and at 5 ^{o}C it takes 50 days or more (Maurer & Baumgärtner, 1992). However, during low temperatures (5 ^{o}C) when reproduction is low, *D. gallinae* has been known to survive up to 9 months without feeding. Temperatures above 45 ^{o}C and below -20 ^{o}C have been found to be lethal. Further, at 20 ^{o}C PRM's seemed to have the maximum longevity when relative humidity was high, 70-90%, conditions which are common in Swedish poultry houses (Nordenfors *et al.*, 1999).

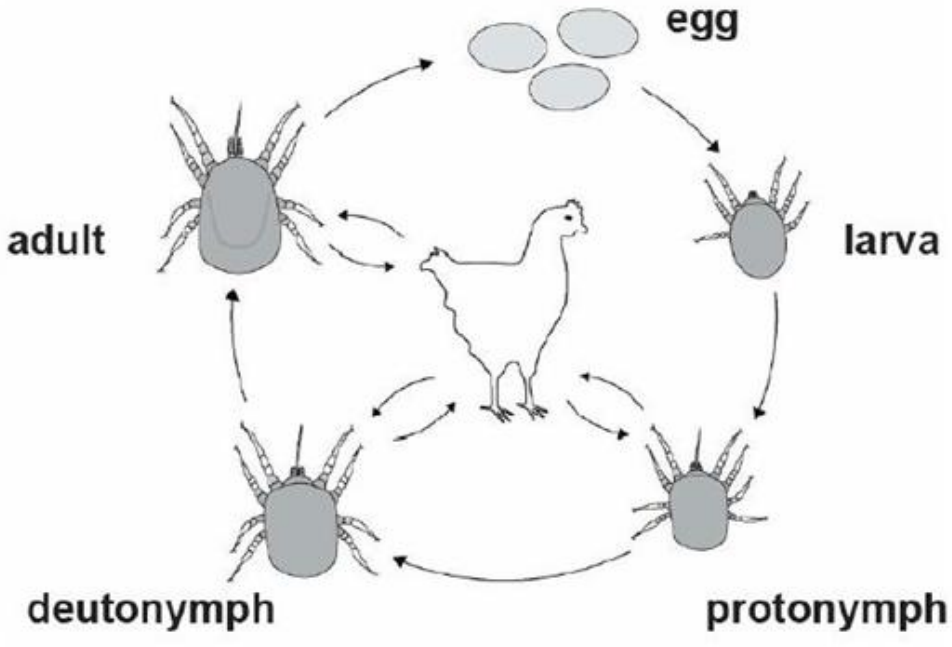

Figure 1. Life Cycle of the Poultry Red Mite, *Dermanyssus Gallinae* (Mul *et al*., 2009: © Maurer).

In Sweden, *D. gallinae* is present in flocks of all system types; however the problem with mite infestation is more common in systems which provide more hiding places for the mites. Also the mites are more difficult to get rid of in these systems (Höglund *et al*., 1995).

It is not entirely clear how the poultry red mites are spreading between herds in Sweden. Likely pathways are purchase of new poultry, from old equipment, egg trays or by vehicles. The mites can also be thought to come from wild birds found in association with poultry houses (Jordbruksverket, 2009). However, studies have shown that the mites found in Swedish herds differ genetically from those found in wild birds (Brännström et al., 2008; Øines & Brännström, 2011). Thus, it is unlikely that wild birds are a source of spreading mites to the herds. Most likely, the pathways are within the production line.

The problems with PRM infestation are somewhat seasonal. In the study by Nordenfors and Höglund (2000), they could see that there were significantly more mites in the summer than winter in facilities in Sweden. This is because mites prefer warm, humid environment and under these conditions, they multiply significantly. Finally, after mites are detected in a laying hen house, it takes 4-6 months for the numbers to reach an equilibrium, around which it fluctuates (Nordenfors & Höglund, 2000).

2.2 Feeding Behaviour and Life Habit

The main host of *D. gallinae* is birds, though they are also known to feed on rodents, man and other mammals. Mites that have fed on man, however, lay eggs that do not hatch and the percentage of hatched eggs are lower if they have fed on rodents (Sikes & Chamberlain, 1954).

Several different factors seem to be important for mites locating its host. Increased temperatures is one such factor (Kilpinen, 2001), as well as odor (Koenraadt & Dicke, 2010) and carbon dioxide (Kilpinen, 2005; Koenraadt & Dicke, 2010). Further, the recognition of bird skin seems to be linked to a diesters produced by the uropygial gland of the bird (Zeman, 1988). The PRM will seek out the birds both at night as well as in daylight. In daylight, however, the laying hen can see approaching mites on the perch and consequently peck and kill them (Kilpinen, 2005).

Odor is also important for mites to locate each other. For instance, PRM's that have fed have shown to respond strongly to volatiles from aggregates of conspecific mites (pheromones). This is when the mite naturally wants to find a hiding place and procreate (Koenraadt & Dicke, 2010).

2.3 Effects on animal welfare and production

The PRM is the most serious ectoparasite affecting laying hens. This is because layers are kept for long periods of time, allowing mites to increase to great numbers. The mite parasitize on the hens by sucking their blood, causing the birds discomfort and skin irritation (Chauve, 1998). Hence, PRM infestations can affect the welfare of the layers greatly, which is ultimately shown in the increased mortality caused by severe anaemia (Chauve, 1998; Kilpinen *et al*., 2005). Fossum *et al*. (2009) have found that the PRM caused an increased mortality in the Swedish loose housing systems; however, no signs of mite infestation could be seen in the cage systems included in the study. Even with low infestation levels the weight gain of young birds may be reduced, which can be due to higher activity amongst infected animals. During mite infestations birds have shown to be more active during all hours of the day and consequently getting less rest and sleep. Increased feather pecking and increased self-grooming are other indications that the welfare of the birds are affected negatively (Kilpinen *et al*., 2005).

In addition to the mites themselves causing discomfort, they can also carry pathogens and as a result possibly spread diseases. Chirico *et al*. (2003) have shown that the bacterium *Erysipelothrix rhusiopathiae* which causes erysipelas, are found inside mites that have fed on infected animals. Consequently, *D. gallinae* is likely to function as a vector for *E. rhusiopathiae* by spreading erysipelas amongst laying hens. Since mites are difficult to eliminate in between flock cycles, there is a risk that infected mites could act as a reservoir and transmit the disease to replacement hens as well (Chirico *et al*., 2003). Additional studies have found mites to carry other pathogens, for instance; *Salmonella Enteritidis* (Valiente Moro *et al*., 2007a; Valiente Moro *et al*., 2007b), St. Louis encephalitis virus (Smith *et al*., 1948), and possibly *Mycobacterium* spp. (De Luna *et al*., 2008).

Reduced egg quality, in the form of blood stained eggs, is another concern with mite problems. Blood stains appear when the eggs roll across blood-filled mites that consequently crush, leading to

degradation of the eggs. This, in turn, affects the farmer economically since payment is lower (Chauve, 1998).

Finally, the workers themselves can be attacked by mites, possibly causing skin irritation as well as dermatitis on those affected (Rosen *et al*., 2002).

2.4 Control methods

Management tools are essential to keep PRM infestations under control. For instance, to avoid transmission of mites, poultry houses should have a hygiene zone where personnel change clothes and shoes before entering the poultry facilities. Between flock cycles, the poultry house should be thoroughly washed and disinfected. All possible hiding/resting places for the mites should be sealed and those who cannot be sealed must be carefully rinsed and vacuumed meticulously. Furthermore, there should be an "all-in-all out" principle between flock cycles, and to avoid rodents in the poultry house a zone free from vegetation closest to the house should be established (Jordbruksverket, 2009).

Because temperatures above 45 ^{o}C are lethal to *D. gallinae*, heat can effectively be used in the poultry houses as a control method (Lovén Persson, 2009). When using heat treatment, the temperature must be at least 55 ^{o}C to reach high enough temperatures everywhere. Heat is mainly used between rounds, when hens are present it can only be used selectively in locations which are particularly exposed (Jordbruksverket, 2009). According to Mul *et al*. (2009) heating, in combination with chemical treatment, is the most promising control method against *D. gallinae*.

Focus should primarily be to clean the house between rounds, when the house is empty of animals (Lovén Persson, 2009). However, if there are problems during on-going production cycle, treatment of the facilities is necessary to ensure animal welfare (Jordbruksverket, 2009).

2.4.1 Silica Preparations

A common method to control *D. gallinae* infestations in poultry facilities is with silica dusts (silicon dioxide) and it is the most widely used control measure in Swedish poultry herds (Hermansson & Odelros, 2011). It is administrated into the nests, on equipment and in the entire house where mites are present. This may be done between flock cycles, but can also be done during an on-going batch to keep the number of mites down (Lovén Persson, 2009). Silica dusts have been shown to be effective against mites (Kirkwood, 1974), even under conditions where the mites have recently fed and are particularly viable (Maurer *et al*., 2009). Actually, birds instinctively use dust as protection against ectoparasites when they are dust bathing (Ebeling, 1971).

Silica is thought to damage the protecting chitin-layer of the mite (Lovén Persson, 2009). Its large surface area in relation to body volume makes insects and mites vulnerable to dehydration if their protective water barrier is impaired (Ebeling, 1971). Furthermore, the mites are not likely to develop resistance to these dusts since the action is physical in mechanism (Kirkwood, 1974).

According to Melichar & Willomitzer (1967) the advantage of sorptive dusts are their selective action against ectoparasites and that they are not harmful to birds and mammals (cited in Ebeling, 1971). Silica dust/SiO_2 passes through the digestive tract of the hen without being absorbed. When used properly silica dusts are considered not to be harmful, neither to layers or workers. It should, neverthe-

less, be used with caution in the vicinity of laying hens since it is not advisable to inhale the dust (Jordbruksverket, 2009). Heavy exposure to silica dusts can lead to silicosis and decreased pulmonary function in humans (Wang *et al*., 1997), and can possibly be harmful to hens as well.

In a study by Maurer *et al*. (2009) a liquid form of synthetic silica was tested in vitro against *D. gallinae* and was found to have less effect compared to silica earth. Previous studies however have shown a longer residual effect when these substances were tested in heavily infested farms in Switzerland (Maurer & Perler, 2006).

Finally, silica dust can be used when hens are present without there being a withdrawal period on the eggs (Jordbruksverket, 2009).

2.4.2 Other control methods

Baymite® is the only approved acaricide against PRM in Sweden today (Jordbruksverket, 2009). It contains an organophosphorus (OP) compound, commercially called phoxim, as the lethal active ingredient (AI) (FASS, 2012). Phoxim is the AI in a variety of mite control agents which are used internationally. The effect of phoxim on *D. gallinae* was investigated by Abdel-Ghaffar *et al*. (2009). They found that when mites were exposed to phoxim for 24 hours, 96.2% was killed, although it did not give full effect against red mites. Studies have shown that residues of phoxim in eggs are within permissible levels even directly after treatment (Hamscher *et al*., 2007). However, in Sweden, when using BayMite ® there is a 12 hours withdrawal period on the egg and 24 hours in organic farms (Jordbruksverket, 2009).

Internationally other chemical acaricides are available; however, there are indications of mites developing resistance to some of these (Mul *et al*., 2009). For instance permethrin is a compound which is widely used to control PRM, but resistance to this acaricide among mites have been documented (Nordenfors *et al*., 2001). Also, exposure to residues of some acaricides may not be completely safe for hens and humans (Mul *et al*., 2009).

A variety of oils and plant extracts have been tested against mites with varying results. Birkett *et al*. (2011) have studied the effect of substances in catmint on *D. gallinae* which seems to have promising properties. Also, some effect of thyme oil has been found (George *et al*., 2010) and even orange oil (Maurer *et al*., 2009). Essential oils and plant extracts may be used in poultry houses without withdrawal period (Jordbruksverket, 2009).

Other control methods that are promising for the future are vaccination, use of predatory mites and entomopathogenic fungi (Mul *et al*., 2009). Tavassoli *et al*. (2011) have studied the effect of fungi on PRM. Different concentrations of the fungus *Metarhizium anisopliae* dissolved in sunflower oil was used to treat livestock with mite problems. At high concentrations (1 × 109 konidier / ml), the fungus gave a reduction in the number of mites, seen already one week after treatment. This was followed by the number of mites down for 3 weeks (Tavassoli *et al*., 2011). However, there is a need for further studies on the longer-term effects of the fungus before it can be used in practice to control mites (Tavassoli *et al*., 2011).

2.5 Control agent - Ectopar

Ectopar is a liquid for PRM control containing a patented combination of silica compounds, siloxanes. The product consists of two parts, A and B. Part A contains the active ingredient of a dimeticone silicon emulsion, while part B is a non-ionic surface-active agent which serves as a cleaner and spreading vehicle for part A. The two parts are diluted with water and then applied by a sprayer to surfaces in poultry houses. Since Ectopar is a silica-based control measure, there is no immediate concern of *D. gallinae* developing resistance to the compound.

Ectopar has formerly been evaluated by the manufacturer in a small trial in the UK (Anonymous, 2010). It was tested on a farm with severe mite problems and high mortality amongst birds due to mite inflicted anaemia. Two poultry houses with two units in each, making it a total of four units, were included in the trial. Each unit comprised of 15, 360 hens in three sections of cages, 4 tiers high. After spraying, the farmer could see an almost immediate improvement in one of the treated houses with effective control for 12-14 weeks. In the other house the mite numbers were reduced for a period of 10 weeks after spraying.

For Ectopar to be thought to have full effect against mites, it has been decided that a 92 % reduction of mites is desirable in this study.

3 Materials and methods

3.1 Experimental design

The experimental of design of this study was as followed; 1. An initial screening test where of the efficiency of Ectopar were tested in a laboratory environment, looking at the individual effect of its components. 2. Pre-monitoring of the mite infestation level at the test facilities used in the field study to document a population growth. 3. A field study, where the effect of Ectopar was investigated at the two poultry facilities.

3.2 *In vitro* screening test

To investigate the activity of Ectopar *in vitro*, live mites were collected from an infested egg producing facility. Transparent plastic mite traps (see figure 3) were placed out in areas of the poultry house where mites were known to aggregate. These were collected 7 days later by the personnel and sent in to the laboratory in sealed plastic bags. The poultry house had not been treated with any acaricide during the last 12 month.

Ectopar solution was prepared according to instructions; 10 % part A, 10 % part B and 80 % water to a volume of 1 litre. In order to evaluate the different components of Ectopar, the AI (A) and the emulsifier (B) were also prepared in two separate solutions of 1 litre each with 90 % water and 10 % of component A and B respectively.

Cardboard paper was then impregnated with one of these three solutions. As a control, cardboard paper impregnated with water was used. The card board was left to dry, then 48 small pieces was cut out from each of the four treatments. These were then placed separately in the cells of an ELISA-plate together with one live mite. The cell was sealed with a lid.

Using a stereo microscope, mite survival was checked in regular intervals of; 3, 6, 9, 12, 24, 72, 96 and 120 hours of exposure to the different components A, B, A+B and the control.

3.3 Test facilities

The study was performed in two egg producing facilities with a history of red mite infestations, one farm with furnished cages (Farm 1) and one with free range hens in a barn type system (Farm 2). The specifics of the two farms are summarized in table 1.

At farm 1, two groups of birds of different age were housed in two sections in one building. This study was performed in one of the sections, the one with a significant mite problem. The two groups of birds were separated by a wall, but workers had to pass through the first group were the trial was performed, to get to the second group. This section had four rows of furnished Victorsson T8 cages, three floors high. In total 372 cages housed 2950 Lohmann Selected Leghorn hens (LSL).

Table 1. Facts of the two farms, Farm 1 and Farm 2

	Farm 1	Farm 2
Type	Furnished cages	Single tier floor system, two sections (Jansen nests)
Number of hens	2950	6500 (5500 + 1000)
Age of hens		
- Start of trial	69 weeks	55 weeks
- Treatment	72 weeks	57 weeks
Race	Lohmann Selected Leghorn	Hy-line (5500 White and 1000 brown)
Number of cages	372	-
Number of nest boxesNumber of traps	- 40	102 (68 + 36) 22 (14 + 8)
Location	Södermanland, Sweden	Södermanland, Sweden
Administration method	Knapsack sprayer	Knapsack sprayer
Dosage Ectopar administrated	40 L	45 L
Day of spraying	1 dec 2011	8 dec 2011
Barn size, m	6 x 42	12 x 67

Farm 2 had several bird houses, but only one of the houses was studied in this trial. All houses, however, were separated by hygiene zones, with no direct contact between them. The house studied contained two groups of hens, one with 5500 white Hy-line hens and the other with 1000 brown Hy-line hens. The two groups were separated by chicken wire. The larger group had 68 Janssen nest boxes and the smaller group had 36. At the start of the trial period, manure trays were located directly under the nest boxes to collect manure. However, these were later removed in the middle of the trial period. At this farm, mites are known to aggregate in connection to these manure trays.

No control measures had been used in the facilities for the last 12 months.

3.4 Collecting of data /Sampling

Since there was no room for treatment-control groups in this study, the mite population was monitored prior to the treatment in order to note a population increase before any actions were taken. Hence, the first weeks of the trial, the current mite infestation level was monitored in test facilities of the two farms. After observing a doubling in the number of mites retrieved from the traps compared to the first week of monitoring, Ectopar was administrated in each facility. The administration was done immediately after replacing the traps with new ones. Monitoring of infestation level was then continued for the following 4-6 weeks at one week intervals.
In order to monitor the mite population, semi-transparent plastic traps (figure 2) (100 mm x 70 mm 3 mm) were placed in the nest boxes under the Astro turf mats. These were distributed evenly throughout the whole facilities.

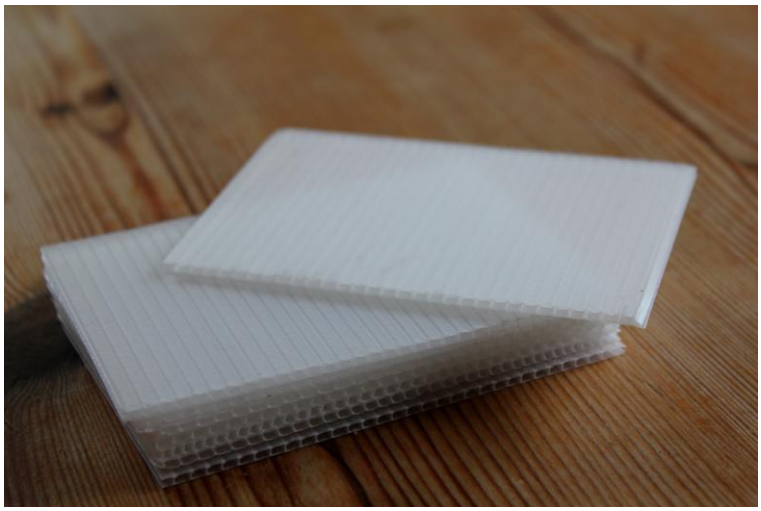

Figure 2. Plastic mite traps. Photo: Sophie Santesson.

In farm 1, a trap was placed in every 10th cage, alternating between the three floors. In total 40 traps were placed out. In farm 2, every 5th nest box was provided with a mite trap. In total 22 traps were placed out in farm 2. Every week, with a 7 day interval, the traps were collected and replaced with new traps. The traps were placed in the same position each week. When time for treatment, Ectopar was administrated directly after a new set of traps were positioned out.
The traps collected were individually placed in plastic zip-lock bags and sent to the laboratory facility where they were killed by freezing. Thereafter, the mites in each trap were counted and the numbers recorded. If the number of mites in one trap was under 500 mites, they were poured onto a Petri dish and counted by the aid of a stereo microscope. If the number of mites in a trap were abundant (>500), they were estimated by volume in a calibrated measuring cylinder.

3.5 Treatment

After a doubling in the number of mites over time was recorded, Ectopar was mixed and directly thereafter applied in the poultry houses by the farmer according to the manufacturer's instructions. The two components of Ectopar were mixed thoroughly with water according instructions from the manufacturer; 125 ml part A, 125 ml part B per every 1 litre of water. A 15 l knapsack sprayer was filled with the mixture and sprayed in the poultry houses at normal pressure at a distance of approximately 75 cm. All areas were mites aggregated and immediate surrounding areas, was sprayed thoroughly from the top areas of the house and then downwards. The farmer was instructed to use about 1 litre of Ectopar per 85 birds in the caged system and 1 litre per $20m^2$ in the free range system. All directions were given according to instructions from the manufacturer.

3.6 Statistical Analysis of Data

All data from the mite counts were analysed using Excel software statistical package (Microsoft Corporation, 2007). The mean values, standard deviation and standard errors were calculated for each farm and week. Using a two sided t-test, p-values were calculated to see if the mite population was affected by the treatment of Ectopar. The mean number of mites per trap after treatment was compared in a t-test with the mean number of mites the last week before treatment. To find out if the population of mites was increasing significantly prior to treatment the first and last week of monitoring before treatment was also compared with a two sided t-test. The significance level was set to $p<0.05$.

Because of different housing systems, data from the two farms were analyzed separately.

4 Results

4.1 *In vitro* screening test

Table 2. The percentage of mites surviving treatment with Ectopar and its two components (Part A and Part B) separately, compared to a control

Exposure, h	Control, %	Ectopar (A+B), %	Component A, %	Component B, %
0	100	100	100	100
3	100	91,67	93,75	97,92
6	93,75	87,5	91,67	97,92
12	93,75	87,5	91,67	97,92
24	87,5	79,17	87,5	95,83
72	85,42	75	79,17	93,75
96	79,17	68,75	75	87,5
120	60,42	52,08	62,5	83,33

4.2 Mite-count

The results from the statistical analysis are shown in table 3 and 4 for farm 1 and 2 respectively. Further, the detailed mite counts from each trap each week are shown in Appendix 1.

4.2.1 Farm 1

Table 3. Mean number of mites per trap collected from week 1-9 at farm 1 and the results from the statistical analyses where the mean numbers of mites from different weeks are compared. Weeks 1-3 are pre-treatment and week 4-10 are post-treatment. P-values and significance level are presented

Week	Mean, Number of mites	SD	SE	n	t-test with	p-value	Significance level
1	652	2459	389	40	-	-	-
2	932	1577	249	40	-	-	-
3	1345	3062	484	40	Week 1	0.13	NS
4	1148	2277	360	40	Week 3	0.74	NS
5	878	1377	218	40	Week 3	0.38	NS
6	843	1735	274	40	Week 3	0.37	NS
7	1316	2419	383	40	Week 3	0.96	NS
8	927	2637	417	40	Week 3	0.51	NS
9	1050	1754	277	40	Week 3	0.60	NS

There was a reduction in the number of mites after spraying Ectopar, however the mites did not go down to levels low enough to be significant when compared to the last week before treatment (week 3). The lowest number could be observed in week 6, three weeks after treatment, where a 37% reduction was obtained. The growth in mites population before Ectopar was administrated, from week 1 to 3, was non-significant (NS) (p=0.13).

4.2.2 Farm 2

*Table 4. Mean number of mites per trap collected from week 1-7 at farm 2 and the results from the statistical analyses where the mean numbers of mites from different weeks are compared. Week 1-2 are before treatment and week 3-7 after. Data from week 4 is missing. ** The manure trays under the nest boxes were removed between week 5 and 6, affecting the result of week 6-7*

Week	Mean, Number of mites	SD	SE	n	t-test with	p-value	Significance level
1	694	1604	350	21	-	-	-
2	1330	2247	490	21	Week 1	0.21	NS
3	1014	2885	626	21	Week 2	0.58	NS
4	-	-	-	-	-	-	-
5	1013	3807	831	21	Week 2	0.65	NS
6**	183	651	142	21	Week 2	0.02	*
7**	214	575	125	21	Week 2	0.02	*

At this farm, a significant difference in the number of mites was obtained by week 6 (p=0.02), when the manure trays had been removed. The reduction was 86 % compared to the last week before treatment. Though, the substantial reduction obtained before the manure trays were removed was about 24 % and was NS (p=0.58).

The growth in mites population before Ectopar was administrated, from week 1 to 2, was NS (p=0.21).

5 Discussion

5.1 *In vitro* screening test

In the *in vitro* test, the effect of Ectopar was compared to a control. Also, the individual effect of the two components of Ectopar, the active substance and the emulsifier, were tested. In this test, mites exposed to Ectopar were not affected as expected. After 24 hours there was a mortality of 21 % and after 120 hours the mortality was 48 % of mites exposed to Ectopar. This can be compared to the control where the mortality was 13.5 % and 40% after 24 and 120 hours respectively. The reduction of mites observed in the control, are most likely due to dehydration, which occurs after a while in the sealed ELISA wells. Because the difference between the control and Ectopar was not that great, it cannot be excluded that the mite reduction in Ectopar treatment also was caused by dehydration. In addition, the fact that survival was greatest amongst mites exposed to component B (see table 2), indicates that the differences between treatments were only random, since the AI was present in component A. Hence, no conclusions on the efficiency of Ectopar as a control agent can be done from this *in vitro* test alone. Preliminary results from this test however, indicate no control effect of Ectopar.

5.2 Field trials

The two farms included in this study had immense problems with mite infestations, both in the past as well as during these current batches. In a situation analysis of Swedish egg production by Hermansson & Odelros (2011) it was recorded that farms with caged hens had more problems with mites. However, in this study the mean number of mites in the traps before treatment was almost the same for both farms, indicating similar infestation levels. The increase in mite population preceding the treatments was NS in both farms. Still, the doubling in the number of mites indicates there is a population growth when no control measures are applied. It can then be assumed that the population would continue to grow until reaching equilibrium, if no control measures are used.

On farm 1, the mite population did decrease successively the first 3 weeks after treatment, but seemed to start to increase again thereafter. This indicates that Ectopar had a slight initial reducing effect during 3 weeks post treatment in this facility. The reduction post Ectopar treatment reached levels of up to 37 %, but was NS.

Similar to the results from the first weeks after treatment on farm 1, mite numbers on farm 2 decreased slightly, up to 24 %, directly after treatment followed by the same level during W 3 and W 5 (no data from W 4). Still, this drop was NS when compared to the week before treatment (W 2). During W 6 in farm 2, 4 weeks post-treatment, the number of mites did reach levels significantly lower than before treatment when a reduction of 86 % was obtained ($p=0.02$). However, the fact that this coincided with the removal of the manure trays under the nest boxes, instead indicates the importance of cleaning and management practices as alternatives to keep the mites under control. The farmers usually remove these when the hens turned 60 weeks as a way of keeping the mite infestation under control, as mites are known to cluster near the manure trays on this farm. It is not very likely that Ectopar alone would affect the mite population to reach significantly lower level the fourth week after treatment. The effect of an effective mite controlling agent would instead be expected to be seen immediately after administration.

It would have been interesting to follow the development of the mite infestation a couple of more weeks on farm 2 to study whether the numbers of mites started to increase again. This might show that the decrease in mites was only temporary due to removal of the manure bins and also confirming the identification of an important hiding place for the mites. However, the farmer wanted to terminate the trial and take other actions against the mite problem, since Ectopar had not met his expectations. On farm 1 on the other hand, monitoring ceased after W 9 as the birds were going to be slaughtered.

The traps in one of the nest boxes were not collected weekly on farm 2; consequently the number of nest boxes in the result is 21 (see table 4). Also all the traps from week 4 for the same farm, were lost before reaching the laboratory and the mites count from this week is therefore missing.

In whole, the decline in mite numbers never reached a 92 % decrease in either farm, as desired, showing that Ectopar was not an effective control agent under the presumptions of this study. Also, because the primary statistical analysis clearly showed that Ectopar had not achieved the wanted effect on these two farms, consequently, it was pointless to analyze the data further by more advanced statistical methods.

According to the manufacturer's instructions, the proper amount of Ectopar is 1 l per 80 hens or per every $20m^2$, i.e. at farm 1 containing 2950 hens, would require 37 l; however, the amount used was 40 l. On farm 2, 45 l Ectopar was used; a volume which should be sufficient for an area of 900 m^2. The barn size was about 800 m^2 but the accessible area of the interior was estimated to be 550 m^2. The farmer restricted the treatment to the area of the interior, as this is where the hens are residing and where mites were identified. This practice is supported by Kirkwood (1974) who has found that treatment of the litter with silica is useless since mites are normally not present there. With this is mind, following given instructions, enough Ectopar was used in both facilities.

With several of the PRM control agents, the application of the compound needs to be repeated after 7 days to be efficient, for instance when using the acaricide BayMite (Svenska ägg, 2010: FASS, 2012) or silica dusts (Maurer & Perler, 2006). This is because the compound does not persist in the environment long enough for all eggs to be given time to hatch enabling all mites to be exposed to the substance (Svenska ägg, 2010). Further, it is important that the temperature is over 18 ^oC, otherwise the eggs might not hatch (Svenska ägg, 2010). In the previous trial of Ectopar, performed by the manufacturer (Anonymous, 2010), it was found that one administration was sufficient to provide effective

control against red mites for 14 weeks. Since this was not the case in this trial, it is possible that Ectopar is not that long lived on surfaces, making a second treatment necessary. However, the fact that the Ectopar did not show any significant effect in the *in vitro* test makes this unlikely. Hence, a second treatment would probably not make an immense difference.

As shown in appendix 1 for farm 1, there are persistently more mites in trap 1-20 compared to 21-40; these were the two rows of cages closest to the other group of birds in the adjacent room. This strongly indicates that mites are transmitted between these two groups. Therefore, both groups should be treated at the same time, to get a good result after treatment with any effective mite control agent. Otherwise new mites will continuously migrate from the untreated room. Ideally, different groups of birds should be separated completely with hygienic zones to minimize transmission of mites (Jordbruksverket, 2009).

In addition to the small difference between the mean values, the large variation in mite numbers between the different traps will also contribute to the high p-values obtained. To get a more reliable result, it would therefore have been beneficial to have used more traps each week.

In a study by Maurer & Perler (2006) a liquid formulation of silica was found to have longer residual effect than silica dusts (diatomaceous earth). Other possible advantages of liquid silica preparations compared to silica dusts are that it is easier to administrate and it does not dust. Advantages which the liquid compounds share with silica dusts are that they are not thought to be harmful to the hens and workers and it is not likely that the mites develop resistance against them. However, the control effect of Ectopar in particular, is too low to have a future as a control agent against *D. gallinae*.

The supplier was offered to do further tests on the batches of Ectopar used in this study. However, they did not see any need for it.

5.3 Conclusions

From both the *in vitro* screening test and the farm testing of Ectopar, it is evident that the formula does not meet the standards for an efficient mite control under the presumption performed in this study. After treatment the reduction in mite counts did not reach low enough levels. However, adjustments in treatment dosage, frequency or cleaning in connection to spraying could be beneficial to obtain a more satisfying result.

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Acknowledgements

Thanks to my supervisor Jan Chirico for all the help with everything regarding this thesis and also to everyone at the parasitology laboratory on SVA for sharing their work place with me. Also a special thank you to everyone at Svenska Ägg, particularly Alexandra Hermansson, Magnus Göransson, and Claes Björck, for their help with finding suitable farms to work with and for trusting in me to do this project. Finally, I would like to thank the personnel at all three farms included in study for all the time they put in to the collection of traps each week and also for letting me do this trail at their farm.

Appendix 1

Farm 1

Trap	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
1	500	2500	1500	413	500	270	500	178	329
2	179	1000	1000	9000	3500	9000	9500	16000	5000
3	5000	7000	2000	4000	5000	1500	4000	1500	1500
4	92	2000	1500	2000	4000	3000	3000	1500	2000
5	15000	2500	4000	3000	2000	3500	5500	4000	5500
6	426	294	318	192	500	500	500	1000	1000
7	500	2000	2000	1500	2000	1500	3000	1500	1500
8	150	500	230	271	383	267	1500	253	480
9	1000	78	397	78	500	500	245	4500	1000
10	111	2000	2000	2000	2000	500	1500	500	157
11	500	2000	5500	5000	1000	1000	1000	69	500
12	327	500	500	222	357	313	500	236	107
13	140	1500	6000	10000	1500	5500	11000	1000	3500
14	500	500	1000	500	1000	1000	1000	500	500
15	45	220	1000	245	500	351	500	65	500
16	73	244	1000	500	1000	1000	2000	500	2000
17	179	2000	2500	2000	5500	2000	2000	500	2500
18	29	177	432	173	500	382	1000	1000	3000
19	400	6000	18000	2500	1000	215	2000	169	251
20	366	3000	2000	1500	1000	217	500	201	500
21	2	2	5	6	7	12	17	13	24
22	2	0	5	9	1	1	6	6	138
23	21	29	22	12	159	62	101	20	1000
24	1	2	33	2	6	7	20	29	26
25	4	4	1	2	10	10	7	1	4
26	0	4	0	0	3	0	2	0	44
27	115	113	500	500	500	500	500	132	500
28	1	5	5	0	0	0	0	0	0
29	0	7	3	3	0	0	2	11	1
30	11	1000	163	77	500	500	1000	1500	8000

31	394	39	126	186	83	75	92	129	273
32	1	4	4	5	0	5	12	4	0
33	2	4	7	2	1	0	2	4	9
34	2	7	0	0	1	0	0	1	0
35	2	2	10	5	2	1	8	0	0
36	9	12	43	8	75	30	98	11	99
37	0	4	2	2	1	2	6	9	1
38	0	1	0	1	1	0	0	0	0
39	5	10	8	10	21	4	36	25	36
40	0	1	2	1	0	0	0	0	1
Total	**26089**	**37263**	**53816**	**45925**	**35111**	**33724**	**52654**	**37066**	**41980**

Farm 2

Trap	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**
1	3000	3500	500	-	0	19	7
2	3	52	30	-	1	0	5
3	234	2500	500	-	154	53	153
4	27	25	8	-	2	3	0
5	2	274	12	-	52	2	4
6	373	13	1000	-	27	228	500
7	12	28	10	-	0	2	8
8	6	1	1	-	1	0	1
9	0	2	3	-	0	0	2
10	34	5500	1000	-	1000	45	65
11	5	17	18	-	4	1	10
12	6000	6500	13000	-	17500	3000	2500
13	294	500	500	-	191	317	1
14	500	10	500	-	20	0	194
15	1	6000	4000	-	2000	119	1000
16	-	-	-	-	-	-	-
17	9	2	0	-	0	0	0
18	21	51	0	-	98	8	8
19	0	1500	61	-	205	16	2
20	13	88	1	-	1	3	0
21	33	35	0	-	4	1	0
22	4000	4000	153	-	23	21	24
Total	**14567**	**30598**	**21297**	**-**	**21283**	**3838**	**4484**

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