Utrecht University

The influence of temperature and humidity on the development of *Dermanyssus gallinae* populations in Dutch laying hen houses

A master thesis at the faculty of Veterinary Medicine at Utrecht University

Written by: M.K. de Wit

Student number: 4250249

Supervisor: Dr. M.G.R. Matthijs & Dr. R. Bronneberg

Faculty: Veterinary Medicine

Department: Department Population Health Sciences

Date: July 2021

M.K. de Wit
July 2021

Abstract

Dermanyssus gallinae (*D. gallinae*), also called the poultry red mite (PRM), is a blood-sucking ectoparasite known for their significant threat to egg-laying hens worldwide. Ninety-four percent of Dutch laying hen farms are infested with *D. gallinae*. Experimental studies have shown that the environmental temperature influences the development time of *D. gallinae* and that the mites in all their stages are very sensitive to dehydration. Field data are lacking.
The objective of this field study was to quantify the influence of temperature and humidity during a 28-day period on the development of *D. gallinae* in Dutch laying hen houses.
During two years, the infestation of *D. gallinae* was determined monthly on in total 17 flocks originating from 14 farms. The mean temperature and humidity per house were determined per week for the four weeks before the count of each determination of the *D. gallinae* infestation. Using linear mixed models, the influence of temperature and humidity inside the laying hen houses on the amount of *D. gallinae* (mg) and the growth rate in amount of *D. gallinae* was examined using two data sets. The first data set contained 84 *D. gallinae* determinations. The second data set is a subset of the first containing 10 *D. gallinae* determinations.
The results showed that a rise in temperature of 1 ˚C inside the laying hen house during week 1 led to an increase of 20% in the amount of *D. gallinae*.

Key words: *Dermanyssus gallinae*, temperature, humidity, laying hens, field study

M.K. de Wit
July 2021

Table of contents

M.K. de Wit
July 2021

Introduction

The poultry red mite (PRM), *Dermanyssus gallinae* (*D. gallinae*), is a blood-sucking mite affecting poultry and other avian and mammal species. This makes them a significant threat to egg-laying hens worldwide (Kilpinen, 2001; Sparagano *et al.*, 2014). Infestation of *D. gallinae* causes health and welfare issues in chickens (Sigognault Flochlay, Thomas and Sparagano, 2017). Adult *D. gallinae* feed every two to four days on their hosts for periods up to an hour. The two nymphal stages of *D. gallinae* also feed on their hosts. Feeding happens typically during periods of darkness (Sparagano *et al.*, 2014). While not feeding, the mites spend their time in hiding places, like cracks and crevices, and therefore, are not present on their hosts. These cracks and crevices are frequently present in domestic poultry systems (Kilpinen, 2001). Feeding of the mites, and thus biting of the chickens during night time, prevents the chickens from sleeping well and creates a source of irritation (Sparagano *et al.*, 2014; Sleeckx *et al.*, 2019). Feather pecking or even cannibalism may be developed due to agitation in hens that are infested (Sparagano *et al.*, 2014; Tomley and Sparagano, 2018; Sleeckx *et al.*, 2019). Also, *D. gallinae* compromises chicken health due to blood loss, which can lead to anaemia and weakening of the birds (Sigognault Flochlay, Thomas and Sparagano, 2017; Tomley and Sparagano, 2018; Sleeckx *et al.*, 2019). This can make them more susceptible to infections. Besides, *D. gallinae* may act as a vector for specific chicken pathogens (Sigognault Flochlay, Thomas and Sparagano, 2017; Sleeckx *et al.*, 2019; Lima-Barbero *et al.*, 2020). Infestation of *D. gallinae* causes reduced egg production and egg size (Tomley and Sparagano, 2018). *D. gallinae* is capable of surviving transport of pullet, egg and manure (Decru *et al.*, 2020).

Life cycle

Female mites, who lay their eggs in hiding places, produce around 30 eggs in a lifetime (Sparagano *et al.*, 2014). Eggs, in clutches from 4 to 8 eggs, hatch into larvae that transform into two nymphal stages (protonymph and deutonymph) before becoming adults (see figure 1) (Sparagano *et al.*, 2014). The length of time required to complete this life cycle depends on the temperature in the habitat of the mites, completion usually occurs within two weeks (Axtell, 1998; Sparagano *et al.*, 2014). If conditions are optimal, the life cycle can take place in less than a week (Maurer and Baumgärtner, 1992; Tucci, Prado and Araújo, 2008; Sparagano *et al.*, 2014). Under experimental conditions, juvenile development is most successful at temperatures between 25 and 37 ˚C (Maurer and Baumgärtner, 1992). Tucci *et al.* (2008) showed that the development time of the various stages of *D. gallinae* under experimental conditions varied at different temperatures and was optimal at 30˚C (6 days) with also the highest viability of the eggs. Eggs seem to be very sensitive for dehydration (Tucci, Prado and Araújo, 2008). A different study, performed by Nordenfors *et al.* (1999), showed that all stages of *D. gallinae* were sensitive to dehydration. Larvae moulting success and juvenile development rates increased with an increase in humidity. At 5˚C female adults laid viable eggs, but these did not hatch. At 45˚C a few eggs were laid and died within two days. Adult mites died within 24 hours at this temperature (Nordenfors, Höglund and Uggla, 1999).

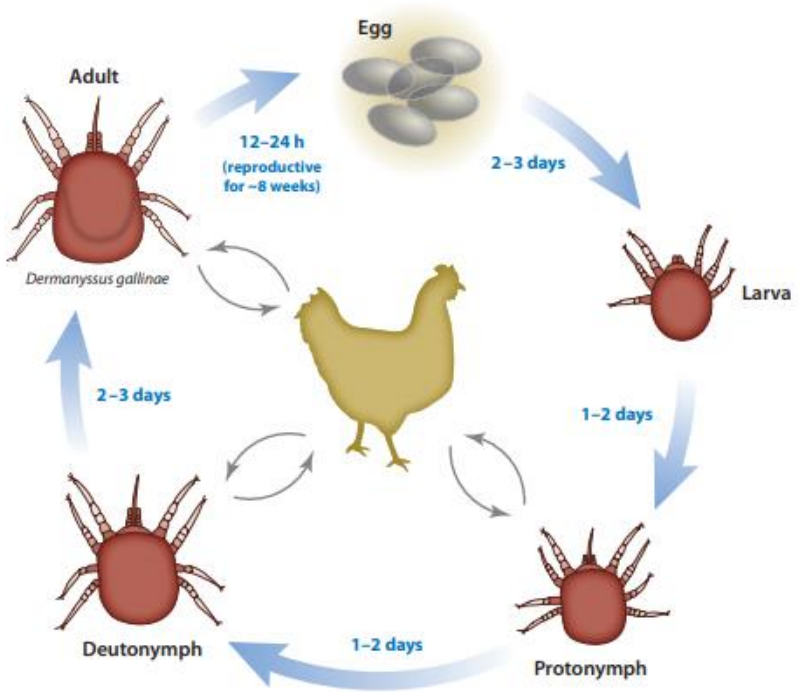

Figure 1: Life cycle of *D. gallinae* under favourable conditions (Sparagano *et al.*, 2014)

Prevalence

The prevalence of *D. gallinae* is high and is increasing in Europe, with 83% of European farms infested (Sigognault Flochlay, Thomas and Sparagano, 2017). In the Netherlands, Belgium and Germany the prevalence is even higher, reaching 94% (Mul, 2013; Sigognault Flochlay, Thomas and Sparagano, 2017). All production types form organic farms to cage or barn systems, can be affected (Sparagano *et al.*, 2014; Sigognault Flochlay, Thomas and Sparagano, 2017). Annually, the costs of *D. gallinae* infestations in the Netherlands are estimated at around 11 million euros (Sparagano *et al.*, 2009).

Options for treatment

Synthetic acaricides used to be the main treatment option for *D. gallinae*. Currently, however, many of them have been withdrawn from the European market. Therefore, only a few chemical acaricides are available for use nowadays (Sparagano *et al.*, 2014; Decru *et al.*, 2020). Resistance against commonly used acaricides for *D. galinae* has been reported (Marangi *et al.*, 2012; Decru *et al.*, 2020). During the periods between production rounds, when laying hen houses are empty (downtime), the houses can be cleaned thoroughly. Especially the cracks and crevices, in which *D. gallinae* spend their time hidden, can be cleaned more easily without the hens being present. Using hot water and soap instead of dry-cleaning are strongly advised (Decru *et al.*, 2020). A different method that can be used during downtime is Thermokill (Decru *et al.*, 2020). As mentioned before, temperatures above 45˚C are lethal to mites (Nordenfors, Höglund and Uggla, 1999). With Thermokill, the layer house is gradually heated up to at least 45˚C for a minimum of two days (van Emous, Fiks-van Niekerk and Mul, 2005).

During the production round, some other treatment options are available that do not interfere with the hens. First of all, it is essential to prevent pests form entering the layer house since *D. gallinae* can be dispersed by other vertebrates. Cracks and crevices need to be closed as much as possible, making it harder for *D. gallinae* to hide. Natural predators of *D. gallinae*, such as *Cheyletus eruditus* (*C. eruditus*)

and *Androlaelaps casalis* (*A. casalis*), are introduced in Europe and can function as biological control agents (Decru *et al.*, 2020). *A. casalis* feeds mostly on the juvenile stages of *D. gallinae* (Decru *et al.*, 2020; Zriki, Blatrix and Roy, 2020) while *C. eruditus* feeds on all stages (Decru *et al.*, 2020). A different way of controlling *D. gallinae* can be done by using Q-perch. This is a perch that contains an electrified wire, integrated just underneath it, that kills the mites but does not harm the hens sitting on it (Decru *et al.*, 2020; Lima-Barbero *et al.*, 2020). Products that are based on silica, like diatomaceous earth, function as a mechanically acting agent against *D. gallinae*. The protective layer of the mites epicuticle is damaged by silica, and subsequently, the mites will desiccate (Maurer and Perler, 2006). Other (chemical) treatment options include ByeMite® (Bayer Animal Health, Leverkusen), Elector® (Elanco, Utrecht, the Netherlands) and Exzolt® (Intervet International B.V., Boxmeer, the Netherlands) (Decru *et al.*, 2020). ByeMite® is an organophosphate, also known as phoxim (Decru *et al.*, 2020) that functions as an inhibitor of the enzyme acetylcholinesterase in the nervous system of mites, which leads to paralysis and death. It is known that some mite populations develop resistance against phoxim resulting in less efficacy (Pugliese *et al.*, 2019). Elector® is a natural acaricide based on Spinosad. It's production is a result of the fermentation of *Saccharopolyspra spinose* (Koziatek and Sokół, 2015). It is effective against all mobile stages of *D. gallinae* with good results in field evaluations (Liebisch, Hack and Smid, 2011). Exzolt®, a fluralaner and also an isoxazoline compound, is administered through the drinking water. It inhibits the GABA-gated chloride channels and L-glutamate-gated chloride channels in the central nervous and peripheral neuromuscular systems of the mites, which leads to paralysis and death (Gassel *et al.*, 2014; Thomas *et al.*, 2017). Exzolt® is highly effective in naturally infested chickens with a nearly 100% efficacy (Thomas *et al.*, 2017).

Research question

The objective of this field study is to quantify the influence of temperature and humidity on the development of *D. gallinae* in laying hen houses in the Netherlands. The following hypothesis will be analysed: the change in the amount of *D. gallinae* (in mg) and the growth rate in the amount of *D. gallinae* detected in laying hen houses during a 28-day period is not influenced by the temperature and/or humidity inside the laying hen houses.

M.K. de Wit
July 2021

Methods

Study design

A cohort study was performed in which the infestation of *D. gallinae* was followed in 27 layer flocks, located on 22 farms. All farms were affiliated with veterinary poultry practice AviVet B.V. To test the hypothesis, the level of *D. gallinae* infestation was determined once a month using the AviVet® Red Mite Trap (Lammers *et al.*, 2017). The temperature and humidity inside the houses were measured daily using a climate data logger (Extech RHT-10, Atal, Purmerend, the Netherlands). The farms were located in different areas of the Netherlands. They included flocks of brown and white layers housed in different housing systems: barn, organic or free range. The period during which the flocks of the farms were sampled varied from eight to sixteen months. The complete survey lasted 2 years starting in the end of 2018. The first sampling was performed early after arrival of the hens in the laying hen houses. Information about treatments that were used for *D. gallinae* control was collected during the entire analysed period.

Study population

From the 27 layer flocks initially participating in this study, the data of 17 flocks belonging to 14 farms were analysed. Ten of the 27 flocks were excluded from the analysis for various reasons: 7 out of 10 were excluded because the climate data logger had not been used in the same time period as the AviVet® Red Mite traps; 2 out of 10 flocks were excluded because there was no *D. gallinae* infestation during the analysed period, and 1 out of 10 showed an extraordinary fluctuation in the amount of *D. gallinae*, which raised doubts about the use of treatments without notification during the entire production period.

Of the 17 flocks included in the analyses, only from 1 flock all *D. gallinae* determinations could be used. From the other 16 flocks only part of the determinations could be used. This was due to one or two of the following reasons:

1. The data climate logger was not used simultaneously with the mite traps during part of the observation period, leading to *D. gallinae* determinations without the corresponding temperature and humidity data.
2. During the analysed period, either the farmer reported that a treatment was administered or a sudden strong reduction in the amount of *D. gallinae* was observed. This strongly suggested that a treatment might have been administered. Therefore, *D. gallinae* determinations of a (presupposed) treatment period were excluded. When the amounts of *D. gallinae* started to increase again, usually between 2 to 3 months after the treatment, the *D. gallinae* determinations were included again.

D. gallinae determinations with missing values in temperature or humidity in the four weeks previous to this determination were deleted. In total these 17 flocks resulted in 24 observation periods: 1 with a complete production period, 9 flocks with a single part of the production period and 7 flocks with two observation periods (n=14), see figure 2.

The data set used in the analyses was carefully selected to exclude contaminating effects of treatment. This was also checked with a subset of the data. This small dataset contained 10 *D. gallinae* determinations from flocks a, g and l (see figure 2) for which it was guaranteed that there had not been any treatment during the downtime and only data in the period until the (presupposed) treatment (i.e., not data of the period after the amount of *D. gallinae* started rising again after treatment). The development in amounts of *D. gallinae* of these flocks were compared with the results of the 84 *D. gallinae* determinations.

Data collection

Outcome variable

Amount of *D. gallinae* and growth rate in amount of *D. gallinae*

Every four weeks the level of *D. gallinae* infestation was determined by measuring the amount of *D. gallinae* (in mg) per AviVet® Red Mite Trap, as described in Lammers *et al*. (2017). The growth rate in the amount of *D. gallinae* has been determined by the ratio of the present amount of *D. gallinae* divided by the previous amount of *D. gallinae*.

A total of ten traps were distributed over the laying hen house and fixed under the perches. For houses with a Q-perch system, the AviVet® Red Mite Traps were hung on the upper side of the perch instead of under the perch. The traps were collected after two days, and frozen at -18 to -20 ˚C for 48h pending analysis i.e. until the moment the mites (and their stages) were weighed as described in Lammers *et al.* (2017). For the weighing process, a scale 0.1 mg precise (KERN AEJ 100-4CM, Kern & Sohn GmbH, Balingen, Germany) was used. Of all ten traps, an average of *D. gallinae* was determined.

Predictors

Temperature and humidity

Every hour of the day, temperature and humidity inside the laying hen houses were measured using the climate data logger during the same period as when the traps were present. To download the data from the climate data logger to the computer, RHT10 software from the company Extech™ was used. The data were converted to Excel-files.

Using Excel, the mean temperature and humidity per house were determined per week for the four weeks before the count of each determination of the *D. gallinae* infestation.

Week 1 *= average of days 1 to 7 prior to D. gallinae determination*
(day 1 is day of D. gallinae determination)
Week 2 *= average of days 8 to 14 prior to D. gallinae determination*
Week 3 *= average of days 15 to 21 prior to D. gallinae determination*
Week 4 *= average of days 22 to 28 prior to D. gallinae determination*

Confounders

Age, season and expdays

The age of the hens was expressed in number of days and was categorised for statistical analysis from 160-250, 251-350, 351-450 and 451-540 days. The season was expressed as spring, summer, autumn and winter. The number of days between two consecutive determinations of the amount of *D. gallinae* was expressed as expdays.

Statistical methods

Statistical analysis were performed using RStudio (R-version 1.3.1, R Foundation for Statistical Computing, Vienna, Austria). The main packages used were:

- *'readxl'*

(Hadley Wickham and Jennifer Bryan (2019). readxl: Read Excel Files. R package version 1.3.1. https://CRAN.R-project.org/package=readxl),

- *'ggplot2'*

(H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016)

- *'lmerTest'*

(Kuznetsova A, Brockhoff PB, Christensen RHB (2017). "lmerTest Package: Tests in Linear Mixed Effects Models." _Journal of Statistical Software_, *82*(13), 1-26.doi: 10.18637/jss.v082.i13 (URL: https://doi.org/10.18637/jss.v082.i13).

For the amount of *D. gallinae* and the growth rate in amount of *D. gallinae*, a log transformation has been done to achieve a normal distribution. The data involved multiple within-farm observations. Therefore, linear mixed models were used to determine the potential association of the predictors with the amount of *D. gallinae* and the growth rate in amount *of D. gallinae*. In this way within-farm correlation was accounted for. Random-intercepts for "flocks" and "farms" were also added to the models to account for between farm and flocks-variability of *D. gallinae*. In both data sets all associations were examined.

First, using univariable models, it was examined which variables (predictors and confounders) predicted either the amount of *D. gallinae* and/or the growth rate in amount of *D. gallinae*.

Second, correlations were calculated between all predictors. Thus, between the four mean week temperatures, the four mean week humidities, but also between the week temperatures and the different week humidities. R>0.6 or <-0.6 were considered to be strong correlations.

Third, a multivariable linear mixed model was performed in the case that multiple predictors and confounders using the same data set and outcome proved significant in the univariable linear mixed model. If two predictors had a correlation that was considered as strong (R>0,6 or <-0.6), no further multivariable linear mixed model between these two predictors was performed.

In all models, p-values, estimates and 95% confidence intervals were calculated using either Least-Squares Means or drop1 function and are shown in the results. P-values <0.05 were considered as significant.

Results

Descriptive data

As can be seen in figures 2 & 3, all 17 flocks, originating from 14 farms had layers infested with *D. gallinae* varying in average from 0.1 to 645 mg of mites per trap. The mean week temperature and humidity in the houses in which the flocks were located varied from 15 to 28 ˚C and 49 to 86% respectively. Figure 2 shows the range in the amount of *D. gallinae* (per trap), mean week temperature and humidity per flock. The age varied from 170 to 540 days of age. Table 1 & 2 shows the frequency and proportion of the four different age categories and the seasons. Ten farms were organic, one farm was free range and three farms used the indoor barn system (see figure 2).

		2019				2020						
Farm	Flock	*Winter*	*Spring*	*Summer*	*Autumn*	*Winter*	*Spring*	*Summer*	*Autumn*	Housing	Range PRM (mg)	Range T (˚C)	Range H (%)
a	1									Barn	6.5 - 13.4	16.7 - 26.4	60.2 - 72.7
b	2									Barn	0.1 - 4.2	18.9 - 25.1	53.0 - 79.2
b	3									Barn	3.3 - 31.4	19.4 - 21.6	57.2 - 72.9
c	4									Organic	15.8 - 143.1	21.7 - 28.3	50.1 - 86.4
d	5									Organic	62.5 - 174	23.2 - 27.1	54.4 - 72.7
e	6									Organic	26.5 - 60.3	21.5 - 23.5	62.2 - 68.3
f	7									Barn	0.9 - 378.5	19.0 - 26.4	51.2 - 70.7
g	8									Organic	0.2 - 6.7	20.4 - 25.2	51.9 - 70.2
h	9									Organic	6.4 - 11.1	20.0 - 20.5	65.2 - 68.3
i	10									Organic	1.5 - 63	15.1 - 20.0	54.3 - 78.5
j	11									Organic	0.6 - 15.9	16.8 - 24.1	63.1 - 75.0
k	12									Organic	131.2 - 237.5	20.6 - 26.8	61.7 - 72.4
k	13									Organic	46.7 - 186.5	20.4 - 24.8	60.0 - 66.9
l	14									Organic	145.5 - 645.3	20.9 - 27.3	53.9 - 72.4
m	15									Organic	8.3 - 21.6	17.4 - 20.9	57.8 - 76.1
m	16									Organic	45.1 - 164	20.4 - 23.8	48.5 - 63.8
n	17									Free Range	0.3 - 463.1	17.1 - 24.3	59.4 - 75.1

Figure 2 : Yellow shows the total period of analysis for each flock. Orange shows the periods for each flock that are included in de analyses. The black vertical lines in the orange periods indicates that there are two separate intervals for analysis in that particular flock. PRM = Poultry Red Mite, T = Temperature, H = Humidity

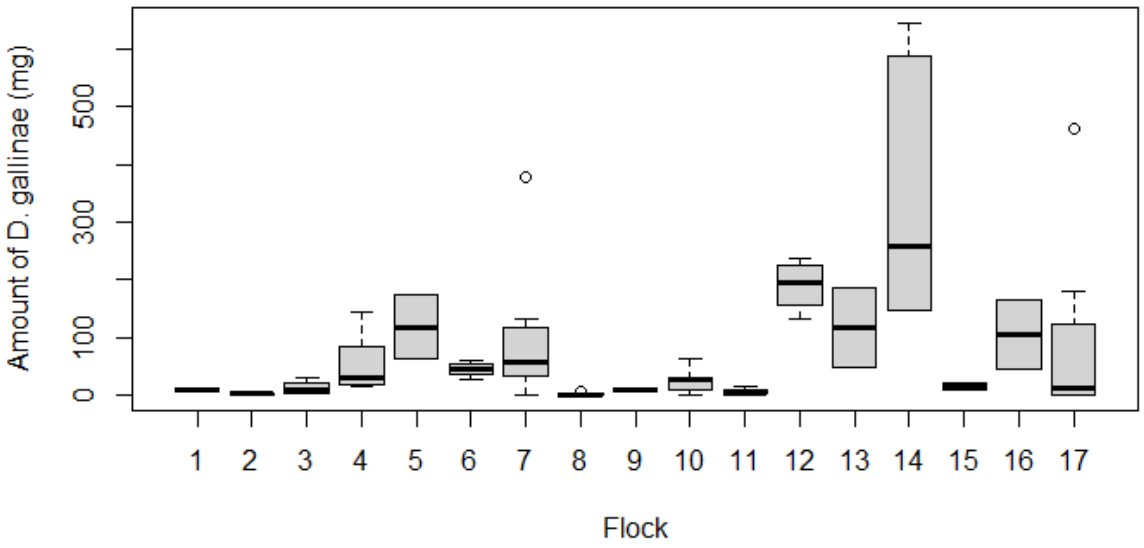

Figure 3: The range in amount of D. gallinae (mg per trap) per flock

D. gallinae determinations n=84	
Outcome	
***D. gallinae*, mean (SD)**	69.7 (124.2)
Growth rate in *D. gallinae*, mean (SD)	3.3 (3.8)
Predictors	
Temperature (˚C), mean (SD)	21.1 (2,2)
Humidity (%), mean (SD)	66.0 (6.0)
Confounder	
Age, frequency (prop)	
160-250	13 (0.16)
251-350	28 (0.33)
351-450	30 (0.36)
451-540	13 (0.16)
Confounder	
Season, frequency (prop)	
Summer	16 (0.19)
Autumn	20 (0.24)
Winter	26 (0.31)
Spring	22 (0.26)
Confounder	
Expdays, mean (SD)	29.4 (14.4)

Table 1: Overview of descriptive statistics of outcome, predictors and confounders for the big data set (n=84). SD = standard deviation, prop = proportion

D. gallinae determinations n=10	
Outcome	
***D. gallinae*, mean (SD)**	127.3 (258.4)
Growth rate in *D. gallinae*, mean (SD)	2.9 (3.7)
Predictors	
Temperature (˚C), mean (SD)	21.4 (2,2)
Humidity (%), mean (SD)	65.9 (4.2)
Confounder	
Age, frequency (prop)	
160-250	2 (0.2)
251-350	7 (0.7)
351-450	1 (0.1)
451-540	0 (0.0)
Confounder	
Season, frequency (prop)	
Summer	1 (0.1)
Autumn	5 (0.5)
Winter	4 (0.4)
Spring	0 (0.0)
Confounder	
Expdays, mean (SD)	28.2 (1.2)

Table 2: Overview of descriptive statistics of outcome, predictors and confounders for the small data set (n=10). SD = standard deviation, prop = proportion

The mean humidity in the houses was the highest during winter with a median of 70.5% (95% confidence interval (CI) 63.1 – 79.2), and the lowest during spring with a median of 59.1% (95% CI 53.2 – 73.4), see figure 4. During summer the median was 65.3% (95% CI 57.6 – 86.4), and during autumn the humidity had a median of 67.3% (95% CI 55.9 – 75.6).

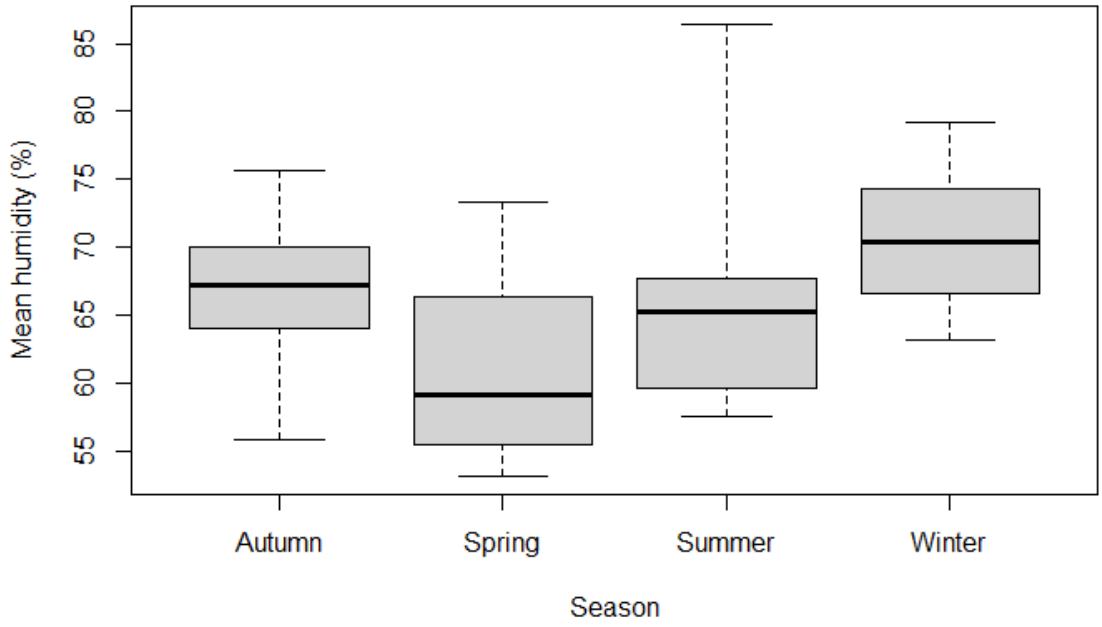

Figure 4: Boxplot of the mean humidity (%) during the four seasons

The mean temperature in the houses was the highest during summer with a median of 24.1 ˚C (95% CI 20.5 – 27.3), and the lowest during winter with a median of 19.8 ˚C (95% CI 15.6 – 22.3), see figure 5. During spring the average temperature had a median of 20.4 ˚C (95% CI 18.5 – 23.1), and during autumn the average temperature had a median of 21.6 ˚C (95% CI 18.2 – 26.1).

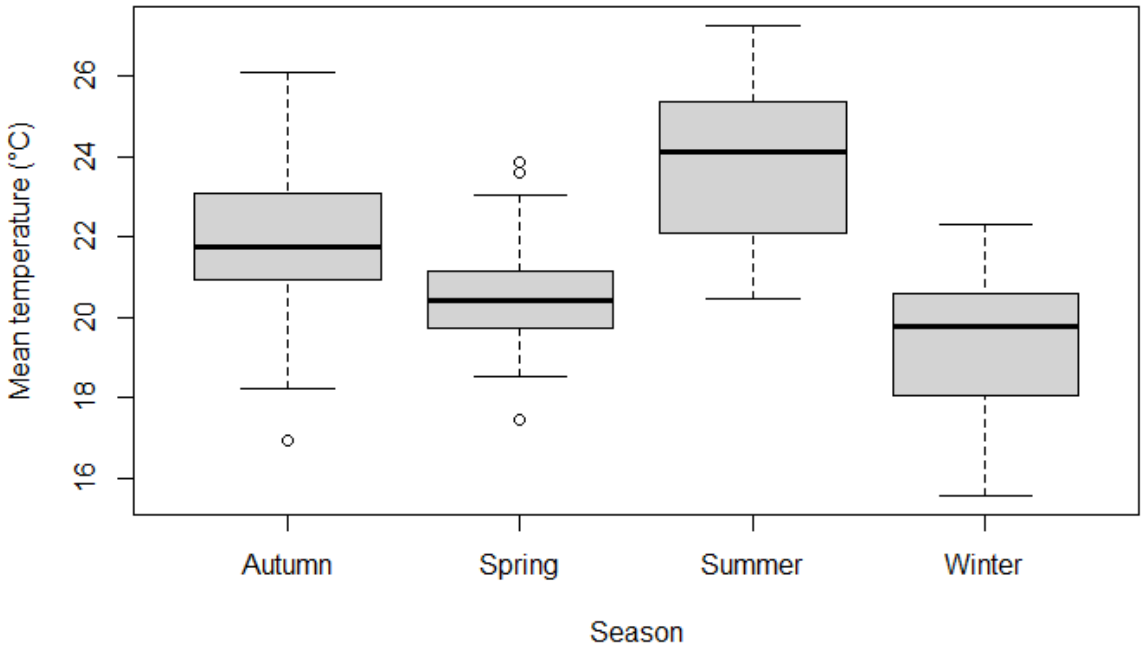

Figure 5: Boxplot of the mean temperature (˚C) during the four seasons

Statistical analyses

N=84, amount of *D. gallinae*

The influence of temperature and humidity in a 28-day period on the amount of *D. gallinae* (n=84) in laying hen houses was tested. The temperature in week 1 showed an effect (p=0.034) in a univariable analysis on the amount of *D. gallinae* (see table 3). With an increase of temperature by 1 ˚C inside the laying hen house, the amount of *D. gallinae* will increase with 20%, leading to an exponential increase with rising temperatures. Figures 6 & 7 show that overall, the amount of *D. gallinae* increases when the temperature inside the laying hen houses during week 1 increase.

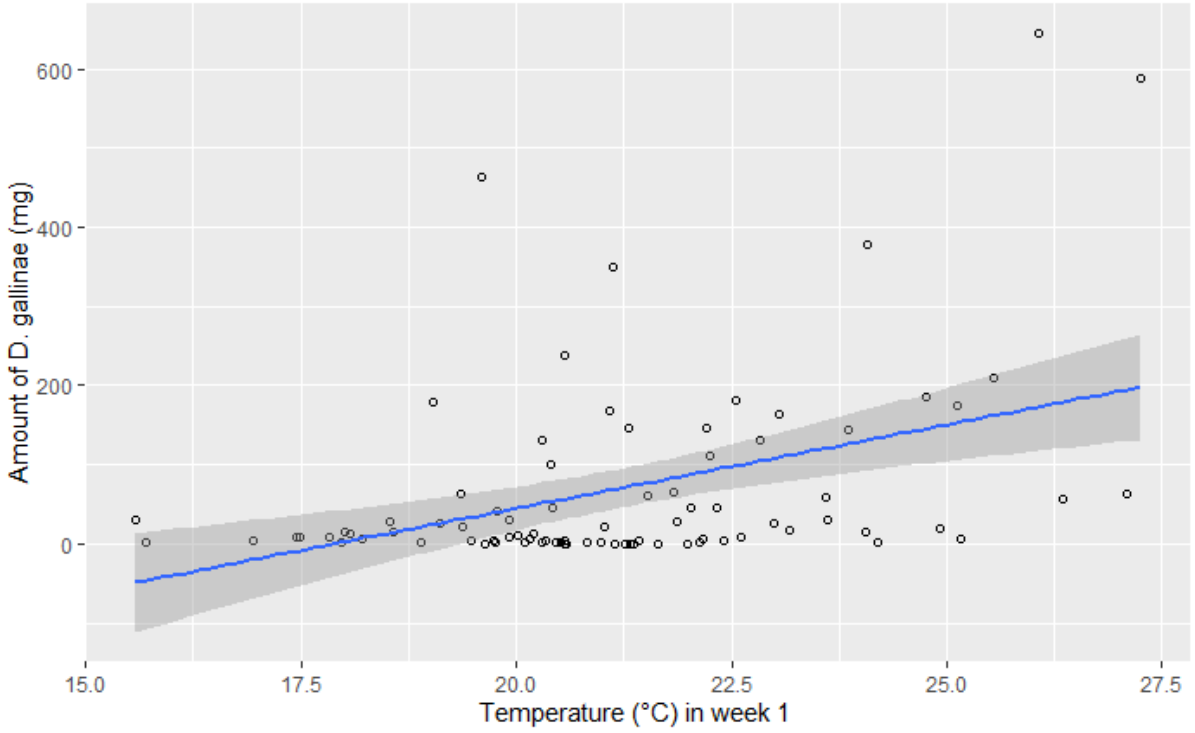

Figure 6: Relationship between the temperature (˚C) in week 1 and the amount of *D. gallinae* (mg) found. In blue the regression line is shown; an increase in temperature in week 1 leads to an increase in amount of *D. gallinae* (mg).

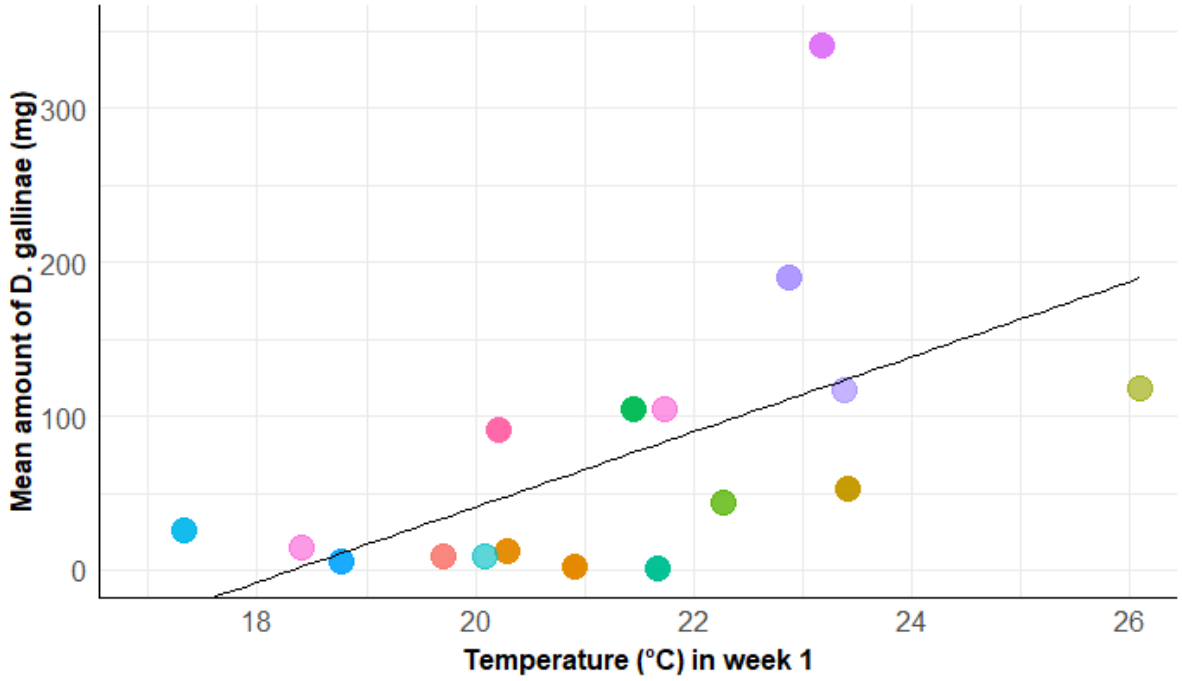

Figure 7: Relationship between temperature (˚C) in week 1 and the amount of *D. gallinae* (mg) found. Each dot represents 1 out of 17 flocks, with dots of the same colour being flocks originating from the same farm. The regression line is shown in black.

As shown in table 3, the temperature in weeks 2, 3 and 4 had no effect on the amount of *D. gallinae* (p-values varies from 0.102 to 0.184). The humidity had no influence on the amount of *D. gallinae* (p-values varied from 0.153 to 0.342) in any of the weeks.

	Amount of D. gallinae **(n=84)**		
	Univariable		
	β	95%CI	p
T_week1	**1.200**	**1.01 - 1.43**	**0.034**
T_week2	1.13	0.94 - 1.37	0.184
T_week3	1.20	0.96 - 1.50	0.102
T_week4	1.14	0.94 - 1.40	0.178
H_week1	0.96	0.91 - 1.01	0.117
H_week2	0.97	0.91 - 1.03	0.342
H_week3	0.96	0.90 - 1.02	0.153
H_week4	0.96	0.91 - 1.02	0.156

Table 3: Results of univariable analyses with all *D. gallinae* determinations used (n=84).β=estimate, 95% CI= 95% confidence interval and p=p-value. P-values <0.05 were considered significant.

No seasonal effect on the amount of *D. gallinae* was found (p-values varied from 0.305 to 0.941).
The number of days between two consecutive *D. gallinae* determinations (expdays) had no effect on the amount of *D. gallinae* (p=0.831).
The difference in amount of *D. gallinae* found between the youngest age category (160 -250) and the oldest age category (451 – 540) was significant (p=0.033). The medians in amount of *D. gallinae* in the youngest and oldest age categories were respectively 4.7 mg (95% CI: 0.3 – 31.4) and 58 mg (95% CI: 0.5 – 174), see figure 8.

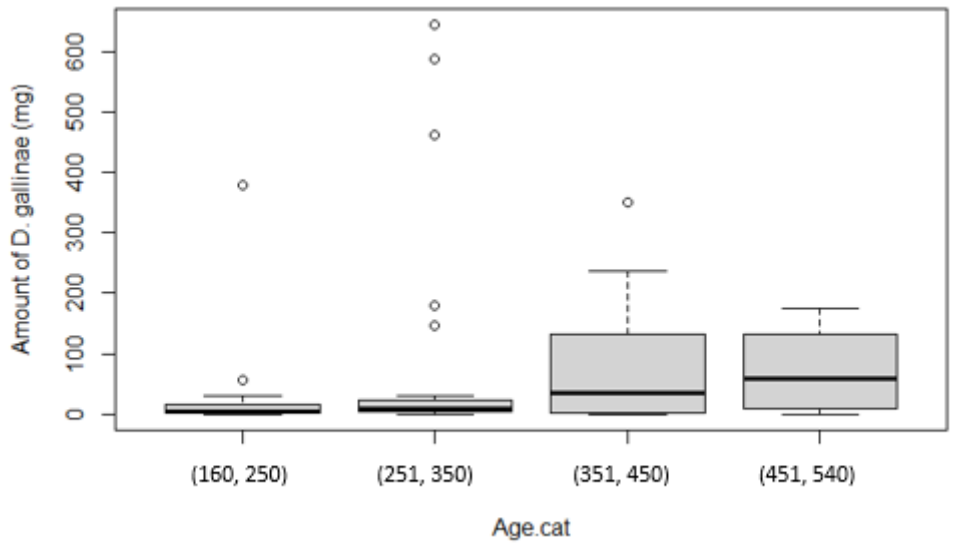

Figure 8: Boxplot with the amount of *D. gallinae* (mg) and the four different age categories. The difference in amount of *D. gallinae* between the youngest and oldest age categories was significant (p=0.033).

N=84, growth rate in amount of *D. gallinae*

None of the predictors showed an effect on the growth rate in amount of *D. gallinae* using an univariable analyses with all *D. gallinae* determinations (n=84) used, as can be seen in table 4.

Growth rate in amount of D. gallinae **(n=84)**			
	Univariable		
	β	95%CI	p
T_week1	1.049	0.97 - 1.14	0.231
T_week2	1.019	0.93 - 1.12	0.658
T_week3	1.014	0.92 - 1.13	0.775
T_week4	1.034	0.94 - 1.14	0.474
H_week1	1.014	0.99 - 1.04	0.318
H_week2	1.024	0.99 - 1.06	0.123
H_week3	1.023	0.99 - 1.06	0.133
H_week4	1.002	0.97 - 1.03	0.910

Table 4: Results of univariable analysis of the growth rate in amount of *D. gallinae* with all *D. gallinae* determinations used (n=84). β=estimate, 95% CI= 95% confidence interval and p=p-value. P-values <0.05 were considered significant.

None of the confounders showed an effect on the growth rate in amount of *D. gallinae*. The differences between the growth rate in amount of *D. gallinae* and the four age categories had p-values between 0.089 and 0.998. The difference between the growth rate in amount of *D. gallinae* and the four seasons had p-values between 0.067 and 0.939.
The number of days between two consecutive *D. gallinae* determinations (expdays) had a p-value of 0.235.

N=10, amount of *D. gallinae*

Table 5 shows the results of the univariable analyses on the amount *D. gallinae* using the small dataset (n=10). Both the temperature and the humidity in the four weeks did not have an effect on the amount of *D. gallinae*.

Amount of D. gallinae (n=10)			
	Univariable		
	β	95%CI	p
T_week1	0.94	0.66 - 1.55	0.726
T_week2	0.92	0.72 - 1.23	0.516
T_week3	0.74	0.43 - 1.50	0.307
T_week4	0.92	0.62 - 1.48	0.674
H_week1	1.00	0.82 - 1.18	0.998
H_week2	1.00	0.86 - 1.16	0.973
H_week3	0.92	0.77 - 1.08	0.292
H_week4	0.98	0.85 - 1.11	0.769

Table 5: Results of univariable analyses on the amount of *D. gallinae* using the small dataset (n=10). β=estimate, 95% CI= 95% confidence interval and p=p-value. P-values <0.05 were considered significant.

The number of days between two consecutive *D. gallinae* determinations had no influence on the amount of *D. gallinae* (p=0.357).
No statistical analyses could be performed on the influence of the four different seasons and age categories on the amount of *D. gallinae* since the proportion of the four seasons and age categories are very unevenly distributed (see table 2).

N=10, growth rate in amount of *D. gallinae*

The humidity in weeks 2 and 4 turned out to have an effect with p-values of 0.042 and 0.0007 respectively, as can be seen in table 6. An increase in humidity of 1% in the laying hen houses led to a decrease of the growth rate in amount of *D. gallinae* of 12% and 16% in weeks 2 and 4, respectively.
Since the humidity in weeks 2 and 4 are very strongly correlated (R=0.77), no further multivariable analyses between both predictors could be performed.
Expdays turned out to have an effect (p=0.012), meaning that an increase of 1 day between two consecutive *D. gallinae* determinations led to an increase of 59% in the growth rate in amount of *D. gallinae*.

Growth rate in amount of *D. gallinae* (n=10)			
	Univariable		
	β	95%CI	p
T_week1	1.10	0.92 - 1.55	0.282
T_week2	1.15	0.95 - 1.43	0.141
T_week3	1.17	0.83 - 1.74	0.346
T_week4	1.25	0.92 - 1.69	0.153
H_week1	0.92	0.78 - 1.02	0.121
H_week2	**0.88**	**0.79 - 0.99**	**0.042**
H_week3	0.90	0.77 - 1.05	0.171
H_week4	**0.84**	**0.79 - 0.95**	**0.000674**

Table 6: Results of univariable analyses of growth rate in amount of *D. gallinae* (n=10). β=estimate, 95% CI= 95% confidence interval and p=p-value. P-values <0.05 were considered significant

A multivariable analysis between the humidity in week 2 and expdays with the growth rate in the amount of *D. gallinae* as outcome showed very similar results as the univariable analyses. Both p-values are significant (see Tabel 7), with estimates of 0.89 and 1.57 for the humidity in week 2 and expdays respectively. An increase of humidity in the laying hen houses of 1% led to a decrease of 11% in the growth rate in amount of *D. gallinae*. An increase of 1 day between two consecutive *D. gallinae* determinations led to an increase of 57% in the growth rate in amount of *D. gallinae*.

Growth rate in amount of *D. gallinae* (n=10)			
	Multivariable		
	β	95%CI	p
H_week2	0.89	0.83 - 0.94	0.0016
Expdays	1.57	1.30 - 1.91	0.0005

Table 7: Results of multivariable analyses of humidity in week 2 and expdays as variates and the growth rate in amount of *D. gallinae* as outcome (n=10). β=estimate, 95% CI= 95% confidence interval and p=p-value. P-values <0.05 were considered significant.

Discussion

In this study we looked at the influence of temperature and humidity, for a 28-day period, on the amount of *D. gallinae* and the growth rate in amount of *D. gallinae* found in Dutch laying hen houses. Two datasets have been used to answer our hypotheses. The big data set (n=84) was carefully selected by excluding data from periods with (presupposed) treatments against *D. gallinae*. From this big data set, a smaller data set (n=10) was extracted. This data set contained only *D. gallinae* determinations with corresponding temperature and humidity data of flocks for which it was guaranteed that no treatments during downtime were administered and only data in the period until the (presupposed) treatment.

N=84

Key results

An increase of 1 ˚C in temperature in laying hen houses during week 1 led to an increase of 20% in the amount of *D. gallinae* (p=0.034). There was also a difference (p=0.033) in the amount of *D. gallinae* between hens in the youngest age category (160-250) and the oldest age category (451-540).

No effects of temperature and humidity have been found on the growth rate in amount of *D. gallinae*. Also, no effects of season, age of the hens or number of days between two consecutive *D. gallinae* determinations on the growth rate in amount of *D. gallinae* have been found.

Context of results

Field studies which quantified the influence of temperature and humidity on the amount of *D. gallinae* detected in laying hen houses over periods of time, like this study, have not been reported earlier. Therefore, the results found in this study cannot be compared directly with other comparative field studies.

There is one field study by Nordenfors and Höglund (2001) that measured temperature and humidity once a month in two with *D. gallinae* infested layer hen farms. A further unquantified seasonal pattern was found with increasing amounts of mites in early summer and a decrease in mites during autumn. This, in spite of multiple treatments that were administered during the observational period (Nordenfors and Höglund, 2001).

There are, however, multiple experimental studies focussing on the influence of temperature on the reproduction, juvenile development and survival of *D. gallinae*, like Nordenfors *et al.* (1999) and Tucci *et al.* (2008). A major difference between the experimental studies and this field study is the variance in temperature and humidity. Nordenfors *et al.* (1999) used temperatures varying from -20 ˚C to 65 ˚C and humidities varying from 30% to 90%. In this field study the range in temperature and humidity inside the laying hen houses was 15 to 28 ˚C and 49 to 86%, respectively. However, most measurements showed a temperature of around 20 to 22 ˚C and a humidity of around 60 to 70%. Of course, it is the farmer's intention to have a controlled temperature inside the laying hen houses of between 18-22 ˚C (Hoeve, 2004). Therefore, the extreme temperatures used in this experimental study are not comparable with the range in temperatures observed in this field study. Nevertheless, Nordenfors *et al.* (1999) showed that more eggs were laid by *D. gallinae* at a temperature of 25 ˚C than at a temperature of 5 ˚C. In addition, 98% of the eggs laid by 25 ˚C hatched into larvae, while at 5 ˚C this was 0%.

Tucci *et al.* (2008) found that the development time of different stages of *D. gallinae* was the shortest at 30 ˚C, i.e. 6 days, while at 20 ˚C and 15 ˚C it was 11 and 28 days, respectively, with a humidity ranging from 70 to 85% (Tucci, Prado and Araújo, 2008). This suggests a very clear influence of temperature on the development time of *D. gallinae*.

No effect of temperature on the growth rate in amount of *D. gallinae* was found. This suggests that the growth rate in amount of *D. gallinae* is constant over the whole range of temperatures measured inside the laying hen houses. For example, a rise in temperature from 20 to 21 ˚C will have the same growth rate in amount of *D. gallinae* as a rise in temperature from 25 to 26 ˚C, see table 8.

The effect found between hens in the youngest and oldest age category on the amount of *D. gallinae* can be explained by the fact that the youngest hens arrive in houses that have been cleaned during downtime. Although not all houses will be cleaned as thoroughly during downtime, during the production period the houses cannot be cleaned as good as during downtime. Also, during downtime some of the houses used a treatment against *D. gallinae* leading to very low levels of *D. gallinae* at the start of the production period. It is a matter of time until the mites start to develop and reproduce. Because hens of the oldest age category have lived in the houses for the longest time period, it is understandable that most mites will be found in these flocks. In addition, the rise in amount of *D. gallinae* in week 1 seems to be exponential. This could lead to a large rise in mites in a short period of time if the temperature inside the laying hen houses also increases rapidly. For a theoretical example, see figure 9. According to the expert opinions of serval poultry veterinarians *D. gallinae* increases rapidly during periods of hot days (Niekerk *et al.*, 2012).

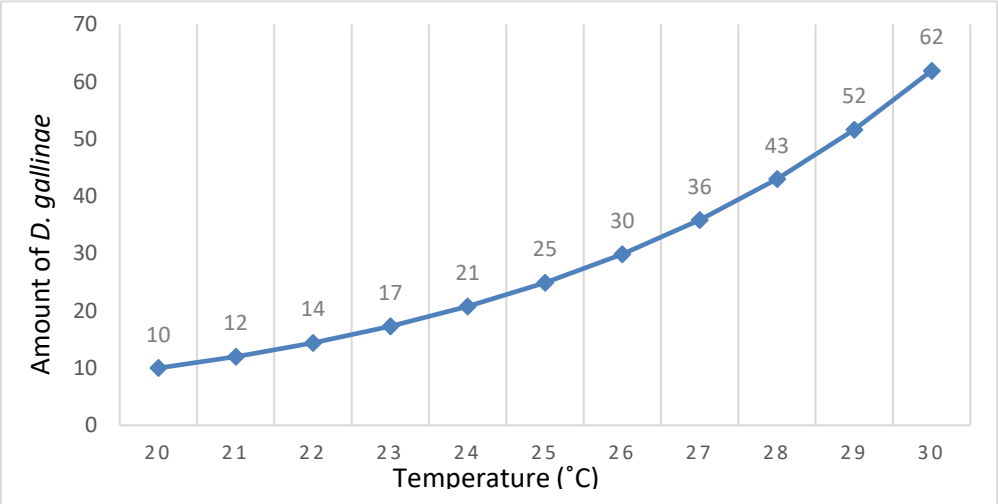

Figure 9: A theoretical example of exponential rise in amount (in integers) of *D. gallinae* with 20% more mites found with every rise in temperature by 1 ˚C. Starting with 10mg, the amount of *D. gallinae* will rise to 62mg.

Amount of *D. gallinae* (mg)	Temperature (˚C)	Growth rate in amount of *D. gallinae*
10	20	1.2
12	21	1.2
14	22	1.2
17	23	1.2
21	24	1.2
25	25	1.2
30	26	1,2
36	27	1.2
43	28	1.2
52	29	1.2
62	30	-

Table 9: The numbers of Figure 9 and the growth rate in amount of *D. gallinae*, which is a constant 20% increase over the entire temperature range.

N=10

Key results

No influence of temperature and humidity have been found on the amount of *D. gallinae*. Neither was there an effect of the number of days between two consecutive *D. gallinae* determinations.

The humidity in weeks 2 and 4 turned out to have an effect on the growth rate in the amount of *D. gallinae*. An increase of 1% in humidity inside the laying hen houses led to a decrease of respectively 11 and 16% in the growth rate in amount of *D. gallinae* in weeks 2 and 4. There was an effect of the number of days between two consecutive *D. gallinae* determinations on the growth rate in amount of *D. gallinae*. An increase of 1 day between two consecutive *D. gallinae* determinations led to an increase of 57% in the growth rate in amount of *D. gallinae*.

Context of results

There are only a small amount of studies on the direct influence of humidity on the amount of *D. gallinae*. Nordenfors *et al*. (1999) showed that all stages of *D. gallinae* are sensitive to dehydration and that larvae moulting success and juvenile development rates increased with an increase in humidity (Nordenfors, Höglund and Uggla, 1999). Pritchard *et al.* (2015) added to this that *D. gallinae* thrives under circumstances with high humidity (>70%) and does not thrive in dry conditions (Pritchard *et al.*, 2015). In this study, however, we found the contrary: our results showed that an increase of 1% in humidity in weeks 2 and 4 led to a decrease of 11 and 16% in mites, respectively, on the growth rate in amount of *D. gallinae*.

The effect found of the number of days between two consecutive *D. gallinae* determinations on the growth rate in amount of *D. gallinae* can be explained as more time between two consecutive *D. gallinae* determinations gives more mites the opportunity to develop.

Comparing both data sets

The data sets showed different results. The big data set showed a positive influence of temperature during week 1 on the amount of *D. gallinae* and the small data set showed a negative influence of humidity on the growth rate in amount of *D. gallinae*. The question is why both data sets did not show similar results.

In this study, a lot of treatments were used during the analysed period. Therefore, a lot of data could not be used due to interference of the treatment. More ideal would have been if no treatments during downtime and during the production period were administered. Since this is a field study, one cannot blame farmers for treating against *D. gallinae*. Data after a (presupposed) treatment were excluded from analysis in this study in an attempt to minimize the influence of any treatments. However, a small long-time influence of treatments during downtime cannot be excluded completely. Perhaps that could explain the absence of an effect of temperature in weeks 2-4. Week 1, during which an effect was found, is the week closest to a *D. gallinae* determination, and therefore the furthest away from a (presumed) treatment. More research is needed to quantify potential long-lasting effects of treatments during downtime on the amount of *D. gallinae* inside laying hen houses.

The small data set contained 10 *D. gallinae* determinations originating from three different flocks on three different farms. One of these three flocks only had 2 *D. gallinae* determinations. Therefore, it is very difficult to tell whether there is an influence of temperature and humidity on the amount or the growth rate in amount of *D. gallinae*. Cencek (2003) investigated the prevalence of *D. gallinae* on Polish poultry farms. In their field study, the number of farms they investigated was 12 (Cencek, 2003), which is comparable with the amount of determinations in our small data set. However, they only

investigated whether these farms were infested with *D. gallinae* and they did not follow these farms in time like the present study.
The results of our small data set are also in contradiction with the literature. The assumption can be made that this data set is too small to draw conclusions. The influence of humidity found in weeks 2 and 4 could be the result of coincidence. Also, this data set had a very uneven distribution for the four age categories and four seasons. With zero *D. gallinae* determinations in the oldest age category and during springtime, and only 1 determination during summer and in the age category of 351 – 450 days of age, no statistical analyses between differences in the amount of *D. gallinae* or the growth rate in amount of *D. gallinae* of these confounders could be performed.

Seasonal influence
Only numerical differences were found between the four seasons on the amount or growth rate in amount of *D. gallinae*. This, despite the major differences in temperature outside the laying hen houses during the four seasons. As described earlier, it is the intention of the farmer to keep the temperature inside the laying hen house constant at around 18-22 ˚C during the entire year (Hoeve, 2004). This makes it hard to demonstrate a seasonal effect of temperature outside the laying hen houses on the amount of *D. gallinae* found inside the laying hen houses.

Hotspots

D. gallinae is known to form clusters in poultry houses (Sleeckx *et al.*, 2019; Decru *et al.*, 2020). These so called hotspots (with more than 250 mites per trap) were also found in this study. In all the laying hen houses in this study, a total of ten mite traps per month were analysed. These mite traps were located at fixed locations throughout the house so that every month the same spots in the house were analysed. A different distribution and a higher number of mite traps could have possibly influenced the number of mites found, because other locations could have detected less, or even more, hotspots.

Generalizability

This field study used laying hen houses located throughout the Netherlands, using both white and brown layers of different ages and different types of housing. Thus, this study has a good external validity for laying hens that are housed in the Netherlands and in other countries with similar types of housing systems and climate conditions.

Conclusion

Based on the big data set, the change in amount of *D. gallinae* detected in Dutch laying hen houses during a 28-day period is positively influenced by the temperature inside the houses. The growth rate in amount of *D. gallinae* detected in Dutch laying hen houses during a 28-day period is not influenced by the temperature or humidity inside the houses.

M.K. de Wit
July 2021

Acknowledgements

First of all, I would like to thank both of my supervisors, dr. Mieke Matthijs and dr. Roland Bronneberg, for guiding me during this research project. I am also grateful for all employees at poultry practice AviVet B.V. for their practical work in gathering all data. Berrian Lammers, I would like to thank you for helping me getting started in this research project. Last but not least, I would like to thank dr. ing. Miel Hostens and Hans Vernooij for helping me with the statistical analysis of the data.

References

Axtell, R. C. (1998) © 1999 Kluwer Academic Publishers. Printed in the Netherlands. Poultry integrated pest management: Status and future.

'Bloedluizen (vogelmijten) op papier en in de praktijk' (2005), p. 52.

Cencek, T. (2003) 'PREVALENCE OF DERMANYSSUS GALLINAE IN POULTRY FARMS IN SILESIA REGION IN POLAND', p. 5.

Decru, E. et al. (2020) 'Possibilities for IPM Strategies in European Laying Hen Farms for Improved Control of the Poultry Red Mite (Dermanyssus gallinae): Details and State of Affairs', Frontiers in Veterinary Science, 7. doi: 10.3389/fvets.2020.565866.

Gassel, M. et al. (2014) 'The novel isoxazoline ectoparasiticide fluralaner: Selective inhibition of arthropod γ-aminobutyric acid- and l-glutamate-gated chloride channels and insecticidal/acaricidal activity', Insect Biochemistry and Molecular Biology, 45, pp. 111–124. doi: 10.1016/j.ibmb.2013.11.009.

Hoeve, W. (2004) Handboek pluimveehouderij. 36. Lelystad: Animal Sciences Group, p. Available at: https://library.wur.nl/WebQuery/wurpubs/357962 (Accessed: 9 June 2021).

Kilpinen, O. (2001) 'Activation of the poultry red mite, Dermanyssus gallinae (Acari: Dermanyssidae), by increasing temperatures', Experimental & applied acarology, 25, pp. 859–67. doi: 10.1023/A:1020409221348.

Koziatek, S. and Sokół, R. (2015) 'Dermanyssus gallinae still poses a serious threat for the rearing of laying hens', Polish Journal of Natural Science, 30, pp. 451–463.

Lammers, G. A. et al. (2017) 'Experimental validation of the AVIVET trap, a tool to quantitatively monitor the dynamics of Dermanyssus gallinae populations in laying hens', Poultry Science, 96(6), pp. 1563–1572. doi: 10.3382/ps/pew428.

Liebisch, G., Hack, R. and Smid, G. (2011) 'Efficacy of spinosad against the poultry red mite, Dermanyssus gallinae (Mesostigmata: Dermanyssidae), in laboratory and field trials*', Zoosymposia, 6(1), pp. 282–287. doi: 10.11646/zoosymposia.6.1.38.

Lima-Barbero, J. F. et al. (2020) 'Challenges for the Control of Poultry Red Mite (Dermanyssus gallinae)', Parasitology and Microbiology Research. doi: 10.5772/intechopen.90439.

Marangi, M. et al. (2012) 'Acaricide Residues in Laying Hens Naturally Infested by Red Mite Dermanyssus gallinae', PLOS ONE, 7(2), p. e31795. doi: 10.1371/journal.pone.0031795.

Maurer, V. and Baumgärtner, J. (1992) 'Temperature influence on life table statistics of the chicken miteDermanyssus gallinae (Acari: Dermanyssidae)', Experimental & Applied Acarology, 15(1), pp. 27–40. doi: 10.1007/BF01193965.

Maurer, V. and Perler, E. (no date) 'Silicas for control of the poultry red mite', p. 2.

Mul, M. (2013) 'Fact sheet Poultry Red Mite in Europe'.

Niekerk, T. G. C. M. van et al. (2012) Verlagen van uitval bij leghennen. Lelystad: Wageningen UR Livestock Research, p. Available at: https://library.wur.nl/WebQuery/wurpubs/429355 (Accessed: 10 July 2021).

Nordenfors, H. and Höglund, J. (2001) 'Long term dynamics of Dermanyssus gallinae in relation to mite control measures in aviary systems for layers', British poultry science, 41, pp. 533–40. doi: 10.1080/713654991.

Nordenfors, H., Höglund, J. and Uggla, A. (1999) 'Effects of Temperature and Humidity on Oviposition, Molting, and Longevity of Dermanyssus gallinae (Acari: Dermanyssidae)', Journal of Medical Entomology, 36(1), pp. 68–72. doi: 10.1093/jmedent/36.1.68.

Pritchard, J. et al. (2015) 'Understanding the biology and control of the poultry red mite Dermanyssus gallinae: a review', Avian Pathology, 44(3), pp. 143–153. doi: 10.1080/03079457.2015.1030589.

Pugliese, N. et al. (2019) 'Efficacy of λ -cyhalothrin, amitraz, and phoxim against the poultry red mite Dermanyssus gallinae De Geer, 1778 (Mesostigmata: Dermanyssidae): an eight-year survey', Avian Pathology, 48(sup1), pp. S35–S43. doi: 10.1080/03079457.2019.1645295.

Sigognault Flochlay, A., Thomas, E. and Sparagano, O. (2017) 'Poultry red mite (Dermanyssus gallinae) infestation: a broad impact parasitological disease that still remains a significant challenge for the egg-laying industry in Europe', Parasites & Vectors, 10(1), p. 357. doi: 10.1186/s13071-017-2292-4.

Sleeckx, N. et al. (2019) 'Production losses in laying hens during infestation with the poultry red mite Dermanyssus gallinae', Avian Pathology, 48(sup1), pp. S17–S21. doi: 10.1080/03079457.2019.1641179.

Sparagano, O. et al. (2009) 'Prevalence and key figures for the poultry red mite Dermanyssus gallinae infections in poultry farm systems', Experimental and Applied Acarology, 48(1–2), pp. 3–10. doi: 10.1007/s10493-008-9233-z.

Sparagano, O. A. E. et al. (2014) 'Significance and Control of the Poultry Red Mite, Dermanyssus gallinae', Annual Review of Entomology, 59(1), pp. 447–466. doi: 10.1146/annurev-ento-011613-162101.

Thomas, E. et al. (2017) 'Field efficacy and safety of fluralaner solution for administration in drinking water for the treatment of poultry red mite (Dermanyssus gallinae) infestations in commercial flocks in Europe', Parasites & Vectors, 10(1), p. 457. doi: 10.1186/s13071-017-2390-3.

Tomley, F. M. and Sparagano, O. (2018) 'Spotlight on avian pathology: red mite, a serious emergent problem in layer hens', Avian Pathology, 47(6), pp. 533–535. doi: 10.1080/03079457.2018.1490493.

Tucci, E. C., Prado, A. P. and Araújo, R. P. (2008) 'Development of Dermanyssus gallinae (Acari: Dermanyssidae) at different temperatures', Veterinary Parasitology, 155(1), pp. 127–132. doi: 10.1016/j.vetpar.2008.04.005.

Zriki, G., Blatrix, R. and Roy, L. (2020) 'Predation interactions among henhouse-dwelling arthropods, with a focus on the poultry red mite Dermanyssus gallinae Running title: Predation interactions involving Dermanyssus gallinae in poultry farms', Pest Management Science, 76(11), pp. 3711–3719. doi: 10.1002/ps.5920.