Experimental validation of the AVIVET trap, a tool to quantitatively monitor the dynamics of *Dermanyssus gallinae* populations in laying hens

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ABSTRACT *Dermanyssus gallinae (**D.gallinae**)* infestation causes economic losses due to impaired health and production of hens and costs of parasite control across the world. Moreover, infestations are associated with reduced welfare of hens and may cause itching in humans. To effectively implement control methods it is crucially important to have high quality information about the *D.*gallinae populations in poultry houses in space and time. At present no validated tool is available to quantitatively monitor the dynamics of all four stages of *D.gallinae* (i.e., eggs, larvae, nymphs, and adults) in poultry houses.

This article describes the experimental validation of the AVIVET trap, a device to quantitatively monitor dynamics of *D.gallinae* infestations. We used the device to study *D.gallinae* in fully equipped cages with two white specific pathogen free Leghorn laying hens experimentally exposed to three different infestation levels of *D.gallinae* (low to high).

The AVIVET trap was successfully able to detect *D.gallinae* at high (5,000 *D.gallinae*), medium (2,500 *D.gallinae*), and low (50 *D.gallinae*) level of *D.gallinae* infestation. The linear equation Y = 10^10^(0.47 + 1.21X) with Y = log10 (Total number of *D.gallinae* nymphs and adults) in the cage and X = log10 (Total number of *D.*gallinae nymphs and adults) in the AVIVET trap explained 93.8% of the variation.

The weight of *D.gallinae* in the AVIVET trap also appears to be a reliable parameter for quantifying *D.gallinae* infestation in a poultry house. The weight of *D.gallinae* in the AVIVET trap correlates 99.6% ($P < 0.000$) to the counted number of all stages of *D.gallinae* in the trap (i.e., eggs, larvae, nymphs, and adults) indicating that the trap is highly specific.

From this experiment it can be concluded that the AVIVET trap is promising as quantitative tool for monitoring *D.gallinae* dynamics in a poultry house.

Key words: monitoring tool, AVIVET trap, *Dermanyssus gallinae;* validation, laying hen

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INTRODUCTION

The Poultry Red Mite, *Dermanyssus gallinae* ***(D.gallinae)***, is a serious problem in the poultry industry worldwide, in laying hens in particular. Firstly, *D.gallinae* infestations have a high negative impact on animal welfare and health (Sparagano, 2009; Huber et al., 2011). *D.gallinae* feeds during the night on hens by sucking their blood, causing restlessness, fatigue, reduced egg production and growth, reduced egg quality, loss of condition, increased mortality, and anaemia (Schnieder, 2005; Van Emous et al., 2005a; Taylor, 2007; Lesna et al., 2009; Huber et al., 2011). Furthermore, *D.gallinae* can also affect the health of the hens indirectly through its role as within and between flock vector for pathogens such as *Salmonella* spp. and *E.coli* spp. (Hoffmann, 1988; Schnieder, 2005; Van Emous et al., 2005a; Marangi et al., 2009; Valiente Moro et al., 2009; George et al., 2015). Secondly, depending on the severity of a *D.gallinae* infestation, financial losses range from €0.18 to €2.25 per hen, caused by production losses and control costs. (Van Emous et al., 2005a;b; Marangi et al., 2009; Sparagano, 2009). Finally, the presence of *D.gallinae* may also affect the health and wellbeing of persons working with the poultry, since *D.gallinae* can also suck blood from humans, causing itching, allergic reactions, and inflammation of the ears or skin (Van Emous et al., 2005a; Taylor, 2007). According to George et al (2015), *D.gallinae* is becoming of increasing concern in humans and a pest in other non-avian animals, due to its genetic plasticity and its ability to suck blood through non-avian skins and membranes (George et al., 2015).

Controlling *D.gallinae* infestations should consist of two steps. First, between production rounds, the poultry house needs to be mechanically cleaned well with hot water followed by proper disinfection. Subsequently, in current practice, several chemical, mechanical, physiological, and biological control methods are used, such as silica, Elector (Spinosad), heating the poultry

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Table 1. Strengths and limitations of the different described monitoring methods for *D.gallinae.*

Method	Strengths	Limitations
Mite Monitoring Score (Cox et al., 2009)	-Quick impression on infestation -Cheap	-Subjective -Qualitative -Not sensitive for low numbers -Not validated, only tested -Not all stages of *D.gallinae* are analyzed
Trap of cardboard (Nordenfors and Chirico, 2001)	-Quantitative -Fixed locations	-Sensitive to moisture, manure and picking of hens -Large size of cardboard: difficult to hang -Not continuously counting -Costs of counting: Labor intensive -Expensive -Not validated, only tested
Examining feathers and dust and other impurities (Pavlicevic et al., 2007)	-Semi-quantitative Cheap	-Cost of counting: Labor intensive -Not continuously counting -Not all stages of *D.*gallinae are detected -No fixed locations -Not validated, only tested
ADAS Mite monitor Trap of cardboard with holder (Mul et al., 2015)	-Quick impression -Semi-quantitative -Fixed locations	-Subjective -Not sensitive for low numbers -Not continuously counting -Not validated, only tested.
Examining dried droppings (Zenner et al., 2009)	-Semi-quantitative -Cheap	-Cost of counting: Labor intensive -Not continuously counting -Not sensitive for low numbers -Not all stages of *D.*gallinae are detected -No fixed locations -Not validated, only tested
Tube trap (Emous van, R.A; Fiks van, T.G.C.M; Mul, 2005a)	-Semi-quantitative (weight) -Fixed locations	-Large trap: difficult to hang. -Influenced by moisture (weight of trap) -Time in poultry house (one week) -Not continuously counting -Not validated, only tested.
Automatic Counter (Mul et al., 2015)	-Quantitative -Fixed locations -Continuous counting -Validated -Not labor intensive	-Not all stages of *D.gallinae* are detected -No discrimination of different mite species -Investment in automatic counting devices

house for at least two days at 45°C, adding predatory mites, applying biodiesel, or using attractants to catch *D.gallinae* (Chauve, 1998; Nordenfors and Höglund, 2000; Schnieder, 2005; Van Emous et al., 2005a). However, to effectively control a *D.gallinae* infestation in a poultry house, quantitative knowledge regarding its severity in space and time is crucial to detect the problem at an early stage, determine the correct moment of treatment and evaluate its effectiveness.

Although several methods are available to detect a *D.gallinae* infestation, almost all methods are either qualitative in nature or are unassessed for their ability to reliably quantify infestations. An overview of the described methods and their strengths and limitations is shown in Table 1 (Nordenfors and Chirico, 2001; Van Emous et al., 2005a; Pavlicevic et al., 2007; Cox et al., 2009; Zenner et al., 2009; Mul et al., 2015).

In this paper, we describe and validate a new method to quantitatively assess *D.gallinae* dynamics in poultry houses. The method uses tube traps with corrugated cardboard and is robust and easy to perform. It was validated by experimentally infesting poultry cages under controlled conditions, allowing test result to be compared against a known infestation. Before conducting the two main experiments, we set up a pilot study in four cages to determine if adjustments were necessary for proper growth of *D.gallinae* populations.

Additionally, this paper provides practical guidelines showing how this method can be used under field conditions.

MATERIAL AND METHODS

Description of the Monitoring Method

The AVIVET Trap The AVIVET trap was a black Tylene tube with six blue stripes. (ZPE Kiwa PE40 SDR 9, Wildkamp, Roden, Netherlands). The tube has an inner diameter of 12 mm, an outer diameter of 16 mm and an exact length of 50 mm (Figure 1).

This tube contained a rolled corrugated cardboard 50 mm by 60 mm and 1 mm thick (Golfkarton 0,75 m x 25 m, Hornbach, Groningen, Netherlands).

The AVIVET trap was labeled with a sticker which contains a barcode with identification number.

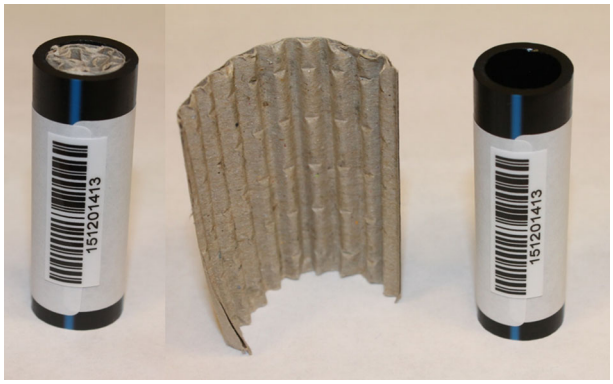

Figure 1. A photo with a complete AVIVET trap with cardboard with a length of 50 mm and an outer diameter of 16 mm and an inner diameter of 12 mm, and an AVIVET trap and its corrugated cardboard with a length of 50 mm and a width of 60 mm and a thickness of 1 mm, separated from each other.

Its appearance was based upon the Arends tube trap (1984) used to catch litter beetles (Stafford et al., 1988). This trap was modified by Bronneberg (AviVet B.V., Austerlitz, the Netherlands). The mechanism of action of the AVIVET trap exploits the biological behavior of *D.gallinae*. It creates favourite *D.gallinae* hiding spots, like cracks and crevices in wood. Specifically, in the AVIVET trap these spaces were created by the rolled corrugated cardboard (processed wood) (Nordenfors and Höglund, 2000; Bruneau et al., 2001).

Processing of the AVIVET Trap in a Poultry House The AVIVET trap was hung for two days in a cage, a period which, from a range of five tested sampling periods, appears to be the most optimal (Nordenfors and Chirico, 2001). However according to Meyer-Kühling et al., one day might also be sufficient (Meyer-Kühling et al., 2007). After the two day period each trap was individually collected and sealed in a small plastic bag. All individually sealed traps were put in a large plastic bag and stored in a freezer at −18 to −20°C for at least 48 hours. According to literature, 48 hours is sufficient to kill most *D.gallinae* and stop further reproduction and development (Nordenfors et al., 1999; Nordenfors and Höglund, 2000; Van Emous et al., 2005a)

Processing and Analyzing the AVIVET Trap in the Laboratory The envelope with AVIVET traps was opened and checked for the presence of all traps and the quality of the material to be analyzed. Peculiarities were noted on the datasheet with the results of the counting.

Furthermore, the identification number and location number in the poultry house or cage was noted on the data sheet for each trap. Per trap, all adults, nymphs, larvae, and eggs were collected in a small glass dish with a diameter of 90 mm (Xenos, Ede, Netherlands). Before the collection of *D.gallinae* was started the scale was tared for the weight of the dish. A precision scale with a range from 1 mg to 25.000 mg and a precision of 1 mg was used (Myweigh type GEMPRO-250 Precision; Bradford, UK).

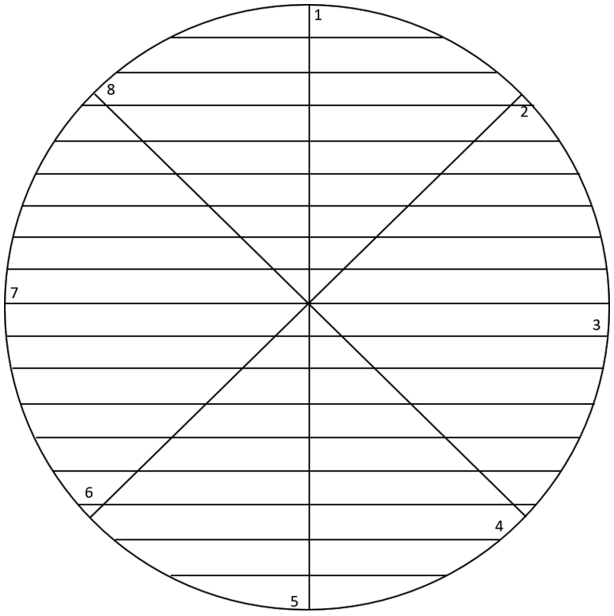

Figure 2. Petri dish with a diameter of 14 cm, divided into eight equal parts and with horizontal lines with a spacing of 8 mm.

The AVIVET trap was removed from the plastic bag, and all *D.gallinae* contained within the plastic bag and trap were collected into a small dish using a brush.

The corrugated cardboard was then removed from the inside of the trap and the trap was wiped internally with a brush to add any remaining *D.gallinae* to the small dish. The same was done with the corrugated cardboard: it was first wiped on both sides, then each corrugated rim was opened carefully, wiped with a brush, and any *D.gallinae* were added to the collection dish. The weight of *D.gallinae* was noted on the data sheet.

To count *D.gallinae*, a glass Petri dish 14 cm in diameter was used. This dish was divided into eight equal, numbered parts and further divided by horizontal lines spaced 8 mm apart to facilitate the counting (Figure 2).

A stereomicroscope (Carl Zeiss, Jena, Germany) with left prism 475022 and right prism 475265 was used to determine all stages of *D.gallinae* and possible other mites. *D.gallinae* were determined and counted at a magnification of 1.2 × 10 times. With this magnification it was possible to identify all four stages of *D.gallinae*, i.e., eggs, larvae, nymphs, and adults and to distinguish them from other mite species. Mite species were distinguished on the basis of following morphological characteristics: overall size, positioning of the legs, presence of hairs on the legs, and morphology of the gnathosoma. A laboratory counter with 5 keys (Clay Adams, serial Lot#0885, Parsippany, NJ) was used to count the various stages of *D.gallinae*.

The following protocol was used to determine the numbers of adults, nymphs, larvae and eggs. If the weight of *D.gallinae* was <250 mg, the small dish was emptied onto the large Petri dish and the entire sample differentiated (adults, nymphs, larvae, and eggs) and counted. The numbers were noted on the data sheet. If

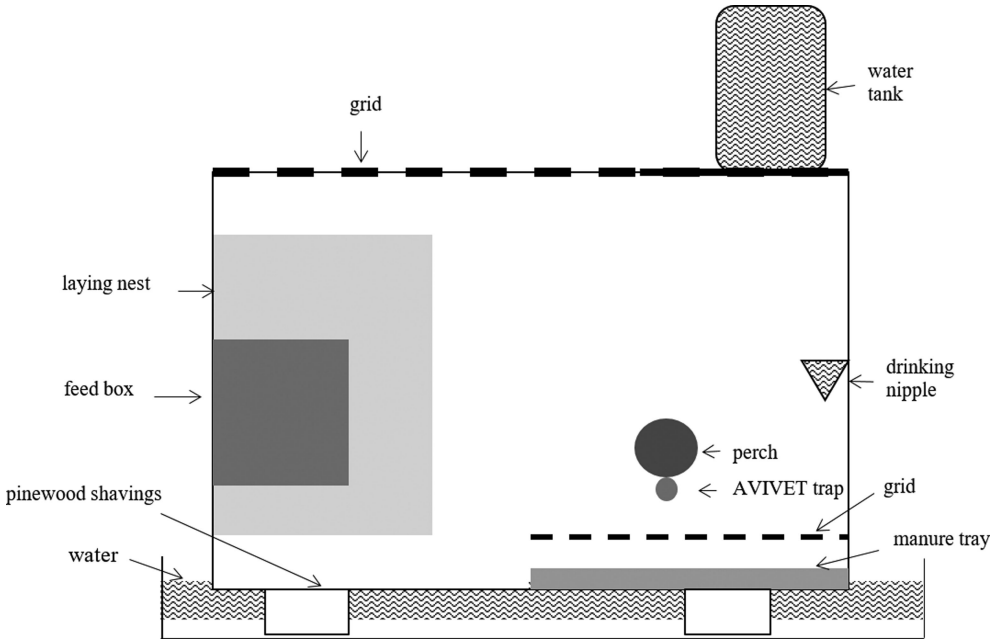

Figure 3. A cross-section of the cage. Open access: Copyright Mul, 2015 (Mul et al., 2015).

the weight of *D.gallinae* was >250 mg, the content of the small dish was homogeneously mixed and an aliquot of approximately 100 mg was taken. This aliquot was emptied on the large Petri dish and differentiated and counted, then extrapolated to the initial weight to estimate *D.gallinae* load.

The entire analytical process required about 7 to 10 minutes per AVIVET trap, depending on the degree of infestation.

Description of the Validation Study

The setup of the infestation experiment was previously described by Mul et al. (2015) for the validation of their automatic counter (Mul et al., 2015). The experiment complied with all legal requirements set by Dutch law (Approved Experimental Number 2013145). The essential points of the study design are briefly outlined below.

Animals and Housing The cages (120 cm long, 60 cm high, 60 cm width) that were used, were each equipped with a round metal perch, a water tank with nipple drinker and drip tray, a feeding box, a laying nest (30 cm long, 50 cm high, 40 cm width) with an opening (20 cm width, 30 cm high), a grate above a manure tray, and an area with dust as "free space". The front of the cage was a transparent Perspex plate. The remainder of the cage was metal. The periphery of the cage was equipped with a glue layer, to capture escaping *D.gallinae*. The cage was placed, using four bricks, above a water-filled tray, in which *D.gallinae* would drown if escaping. For a schematic cross-section of the cages, see Figure 3.

The cages were sampled with the AVIVET trap on day -7 without hens, and on day −2 with hens. Each individual cage was also visually inspected and no *D.gallinae* observed. For a detailed timeline of the study, see Table 2.

In each cage, two White specific pathogen free Leghorn laying hens (GD Animal Health, Deventer, The Netherlands) were housed. Before the hens were put in the cages, each hen was checked for the presence of *D.gallinae* in the feathers. Birds were provided feed and water ad libitum. Feed has been frozen to inactivate possible (feed) mites. The health and welfare of the hens were monitored daily. Manure was removed every 14 days and examined for *D.gallinae*.

Experimental Study Design

Dermanyssus Gallinae The *D.gallinae* mites that were used to infest the cages originated from two heavily infested layer farms in the Netherlands (year 2013). Only viable, moving mites were used. The mites were visually discriminated for the stage (nymph or adult) and then collected with a paintbrush and put in a plastic vial with a screw cap (10.2 cm high with a diameter of 5.2 cm, VWR International BV). The vials were placed, in the afternoon, in the dust of the cages, under the laying nests. The caps of the vials were removed from the vials to release the mites, but both vials and caps remained in the cages to give *D.gallinae* time to explore the cage.

Pilot Study In the pilot study, in two cages 50 mites (25 adults and 25 nymphs) and in the other two cages 500 mites (250 adults and 250 nymphs) were released. The pilot study was performed in order to find out how populations develop and whether adaptations had to be made in the study design, before the actual experiments.

Table 2. Timeline of the study with the three different experiments and activities performed.

Experiment	Day	Activity
Prior to study	−7	Measurement and inspection of cages without hens, to state cages free of *D.gallinae* with AVIVET trap
	−2	Measurement and inspection of cages with hens, to state cages free of *D.gallinae* with AVIVET trap
Pilot study	0	Release of 50 vs. 500 mites
	5	Four AVIVET traps placed in cages
	7	Four AVIVET traps removed from cages
	12	Four AVIVET traps placed in cages
	14	Four AVIVET traps removed from cages Manure tray emptied
	19	Four AVIVET traps placed in cages
	21	Four AVIVET traps removed from cages
	26	Four AVIVET traps placed for final measurement
	28	Four AVIVET traps removed from cages Four cages removed from trial (two low infested and two high infested cages) Mites in cage and on hens were collected and counted.
1	33	All four cages are emptied, cleaned and disinfected Six cages are used in this experiment
	34	Release of 250 vs. 2,500 mites
	38	Release of 250 vs. 2,500 mites
	44	Release of 250 vs. 2,500 mites
	47	Manure tray emptied
	50	Release of 250 vs. 2,500 mites
	52	Six AVIVET traps placed for final measurement
	54	Six AVIVET traps removed from cages Collecting and counting mites in the cages. All six cages are emptied, cleaned and disinfected and used in experiment two
2	55	Release of 500 vs. 5,000 mites
	58	Release of 500 vs. 5,000 mites
	68	Manure tray emptied Release of 500 vs. 5,000 mites
	72	Release of 500 vs. 5,000 mites
	73	Six AVIVET traps placed for final measurement
	75	Six AVIVET traps removed from cages Collecting and counting mites in cages

Temperature in the pilot study was set at 27°C and relative humidity at 70%.

The cages were monitored with the AVIVET trap weekly. The trap was hung each Monday and removed on Wednesday i.e., remained for 48 hours in the cage. At the end of the pilot study, traps were placed on day 26 for the final measurement and removed on day 28.

The four cages were sampled and counted at day 28, an hour after the AVIVET trap was removed from the cages.

Improvements for Experiment One and Two In order to restrict fungal growth in the actual experiments, possibly causing poor growth of *D.gallinae* populations in the pilot study (Mul et al., 2015), we made two improvements. At first, temperature and relative humidity were lowered and set at 25°C and 60% RH, respectively. Secondly, the experiments were shortened from 28 days to twenty days.

To obtain higher numbers of *D.gallinae* than were obtained in the pilot study we increased the size of the *D.gallinae* populations in three ways. At first, by releasing higher numbers of *D.gallinae* in each release. Secondly, by releasing mites at four time points in each experiment. At third, place the traps only for one final measurement. This to exclude possible inhibitory influence of the AVIVET trap on the growth and development of small *D.gallinae* populations.

At day 33, the four used cages were thoroughly cleaned and disinfected to ensure that the cages would be completely free of *D.gallinae* population and remove fungi (*Aspergillus* spp., *Penecilium* spp., *and Mucor* spp.) that had grown in the cages in the pilot study.

Experiment One In the first experiment, starting on day 34 of the study, six cages were used. In two cages, 250 mites (125 adults and 125 nymphs) and in the other four cages 2,500 mites (1,250 adults and 1,250 nymphs) were released on day 34. During this experiment, the same numbers of *D.gallinae* were released an additional three times in these cages (days 38, 44, and 50). The six cages were sampled and analyzed on day 54.

Experiment Two At the start of the second experiment, starting on day 55 of the study, the six cages used in the first experiment were again thoroughly cleaned and disinfected, before releasing 500 mites (250 adults and 250 nymphs) in two cages and 5,000 mites (2,500 adults and 2,500 nymphs) in the other four cages. During this second experiment, the same numbers of *D.gallinae* were released an additional three times in these cages (days 58, 68, 72).

Following the same procedure as in experiment one, six cages were sampled and analyzed on day 75.

AVIVET Trap Placement Procedure In each cage, one AVIVET trap was hung at the perch with black cable ties with a length of 200 mm and a thickness of 2 mm (Fa.W. Hesse GmbH, Werl, Germany).

In the pilot study the traps were hung on each Monday and removed on each Wednesday i.e., a period of 48 hours. At the end of the pilot study the trap was hung for a final measurement i.e., the trap was placed two days before the end of the experiment and removed one hour before the cage was completely sampled and removed. In experiments one and two, only final measurements were performed.

The traps were hung to measure the *D.gallinae* population at the end of each experiment in order to find out how many *D.gallinae* would be caught with the AVIVET trap from the population *D.gallinae* in the cage.

Samples

Collection of Samples At the end of each experiment, for 'the final measurement', the AVIVET trap was removed one hour before the cages were removed from the experiment and each trap was individually sealed in a small plastic bag.

When the cages were removed from the experiment, the whole cage was sampled and the hens checked for the presence of *D.gallinae*. The whole cage was emptied and swept clean. Samples were taken from: (1) the wet manure, (2) the dry manure and litter, (3) the dry manure on the grid above the manure tray, and (4) the dust in the laying nest. Those four samples were collected in large plastic bags. A mixed sample (5) was taken from the water tank, all walls of the cages, material on the perch, material on the grate and plate above the cage, the outside of the feeding box and laying nest, and the wooden supporting beams, and combined in a large Petri dish with 70% alcohol. A last sample (6) were combined *D.gallinae* remaining on the plastic vials, screw caps and cable ties in the cages.

Analyzing Samples Samples 1 to 4 were analyzed in two steps. *D.gallinae* that climbed out of the sample to the top of the plastic bag in 24 hours (for dust) and 48 hours (for manure) were counted and removed. In the next step the rest of the sample was weighed and thoroughly mixed, and four subsamples of 1 to 2 g from each bag were separately analyzed and mites counted. The counted numbers from the subsamples were extrapolated to the total initial weight of the original sample.

Sample 5 was collected in a glass dish with a diameter of 20 cm and a height of 9 cm, which contained 70% alcohol (to kill *D.gallinae*). The sample on the dish was visually observed. If allowed by the density of *D.gallinae,* the whole sample was counted under the microscope. If *D.gallinae* density did not allow full counting, the dish was subsampled (10% of the whole volume) four times, after thoroughly and homogenously mixing and put on a glass, 14 cm diameter Petri dish. The total number of mites in the four subsamples were extrapolated to the initial whole sample.

The AVIVET traps were analyzed as described above.

The mites in sample 6 were analyzed in the same way as described for the AVIVET trap i.e., by carefully brushing *D.gallinae* from all the different objects (plastic vials, screw caps and cable ties). All *D.gallinae* were collected and weighed in the small dish and then further analyzed as described earlier.

To obtain the total number of mites in the cages, all samples were summed: samples 1 to 6, the mites on the hens and total number of mites in the trap.

Statistical Analysis

In this report, "Total number A" refers to all blood sucking stages of *D.gallinae*, the nymphs and adults. "Total number B" refers to all stages of *D.gallinae* (i.e., eggs, larvae, nymphs and adults). **Tot.N-cage** is the "total number A" in the cage. **Tot.N-trap** is the "total number A" in the AVIVET trap.

For statistical analysis, we used the statistical software R(R Core Team, 2014). Below detailed information is given on the used statistics.

Data Since our main objective of the study was not to follow the specific development of the *D.gallinae* over time, but especially to validate the AVIVET trap at one defined moment (number of mites in cage versus number of mites in trap, the final measurement) it seems justified for us to take all data of the three test (pilot study and experiment one and two) into account. Taking the pilot study into account has the advantage that the AVIVET trap is exposed to three, instead of two levels of *D.gallinae* infestations (low, moderate, and high). This makes the validation of the trap even more valuable.

D.gallinae in AVIVET Trap in Relation to D.gallinae in Cage To analyze the relationship between Tot.N-trap and Tot.N-cage, we used package "nlme" of R to create a model (Pinheiro et al., 2015). Several models were tested with and without cage and/or experiment effects, because we have multiple observations per experiment and different moments of releasing *D.gallinae* and two different climates.

The best fitting model, with the lowest AIC-value (**AIC** = akaike information criterion) was chosen. The best fitting model for the relationship between Tot.N-trap and Tot.N-cage was a model with experiment and cage effects to take the correlation between observations and experiments into account.

The regression line fitting the model for the relation between Tot.N-trap and Tot.N-cage was

$$Y = 10\text{^}10\text{^}(b + aX)$$

with: Y = log10 ("total number A" in cage); X = log 10 ("total number A" in AVIVET trap), and "b" the intercept with the y-axis and "a" the regression coefficient.

Table 3. An overview on the number of *D.gallinae* released per experiment per cage, and the total numbers counted at the end of each experiment in each cage (including AVIVET trap) and the numbers caught with the AVIVET trap.

Experiment	Cage	Number *D.gallinae* released	Total number *D.gallinae* in cage	Number *D.gallinae* in AVIVET trap
Pilot study	2	500	63	12
	3	500	45	6
	8	50	12	4
	11	50	14	6
1	1	250	868	225
	4	2,500	3,019	363
	5	2,500	17,992	259
	6	2,500	4,999	589
	10	250	4,796	704
	12	250	834	65
2	1	500	1,585	321
	4	5,000	18,207	1,136
	5	5,000	43,826	3,351
	6	5,000	21,253	947
	10	500	17,842	1,272
	12	500	1,677	190

The R-square of this model was calculated with package "MuMIn" of R (Barton, 2015).

D.gallinae in AVIVET trap in Relation to Weight of D.gallinae in AVIVET Trap To analyze the relationship between "Total number A" and the weight of *D.gallinae* in the AVIVET trap, and between "Total number B" and the weight of *D.gallinae* in the AVIVET trap, we determined regression lines and correlation coefficients. The regression lines fitting the relations: Y = b + aX, with Y = "Total number A" or "Total Number B" in the AVIVET trap; X: weight of *D.gallinae* in the AVIVET trap; "b" intercept with y-axis; "a" regression coefficient.

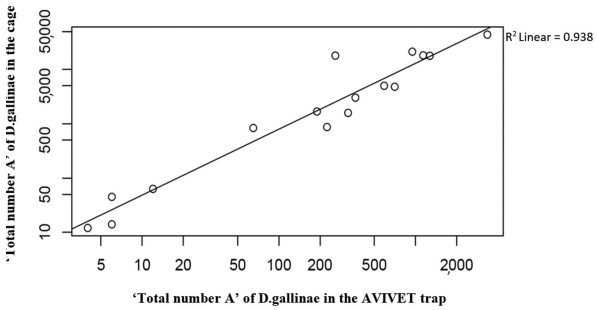

Figure 4. The "Total number A" of D.gallinae in the cage related to the "Total number A" of D.gallinae in the AVIVET trap (on logarithmic scale).

RESULTS

The raw data of the counts of the AVIVET trap and the total number of mites in the cages are listed in Table 3.

In the pilot study, only low *D.gallinae* infestations were present. In experiments one and two, medium and high *D.gallinae* infestations were also observed. In total three ranges of *D.gallinae* infestation were tested with the AVIVET trap.

Regression and Correlation of D.gallinae in AVIVET Trap in Relation to D.gallinae in Cage

The regression line for the relationship between total number A in AVIVET trap and total number A in the cage is:

$$Y = 10\hat{}10\hat{}(0.47 + 1.21X)$$

with factor b = 0.47(95% CI 0.049, 0.8914)

($P = 0.042$) and factor a = 1.21 (95% CI 1.038, 1.390)

($P < 0.000$). Y = log 10 (Tot.N − cage),

X = log 10 (Tot.N − trap).

See Figure 4.

The R-square of this model is 93.8%, indicating that 93.8% of the variation in Tot. N-trap is explained by Tot.N-cage.

With log 10-transformed data, the regression line is straight. However, the absolute numbers of *D.gallinae* in the cage and the AVIVET trap show a line that slightly flattens at increasing Tot.N-trap (Figure 5).

Regression and Correlation of D.gallinae in AVIVET Trap in Relation to Weight of D.gallinae in AVIVET Trap

The relationship was determined for the weight of all *D.gallinae* (i.e., eggs, larvae, nymphs, and adults) in the AVIVET trap relative to "total number B"' (i.e., eggs, larvae, nymphs, and adults). The regression line for this relationship is:

$Y = 58.50 + 9.56x$ with factor 'b′ = 58.50

(95% CI 10.845, 106.149)

and factor 'a′ = 9.56 (95% CI 9.202, 9.925),

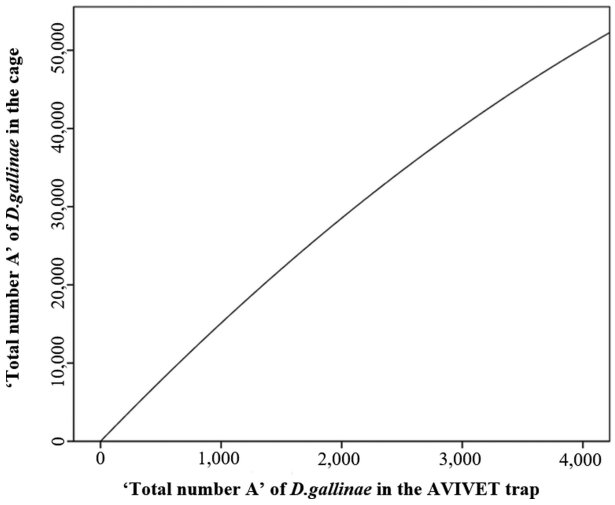

Figure 5. Line on real-scale, showing the regression between "Total number A" of D.gallinae in AVIVET trap related to the "Total number A" of D.gallinae in cage.

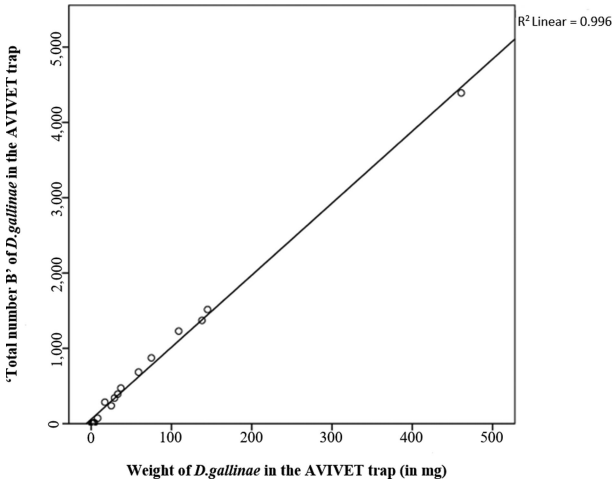

Figure 6. "Total number B" of D.gallinae (i.e., eggs, larvae, nymphs, and adults) in the AVIVET trap related to the weight of D.gallinae (in mg) in the AVIVET trap.

where Y = 'total number B' of *D.gallinae* in AVIVET trap and X = Weight of *D.gallinae* in AVIVET trap (where x must be > 0).

For the regression line, see Figure 6. The R-square of this relation is 0.996 ($P < 0.000$), indicating that 99.6% of the variation in weight of all *D.gallinae* in the AVIVET trap is explained by the "total number B" in the AVIVET trap.

DISCUSSION

The aim of this study was to describe and validate the AVIVET trap as a monitoring method for *D.gallinae*. In this study, a wide range of *D.gallinae* population sizes were monitored with the AVIVET trap.

Total Numbers and Distribution of D.gallinae Found in this Study Compared with Literature and Field Circumstances

High numbers of *D.gallinae* were found in this study. However, in practice, using similar protocols, even larger numbers of *D.gallinae* have been caught with the AVIVET trap (up to almost 20,000 nymphs and adults) (personal observation G.A. Lammers, 2012–2015). *D.gallinae* populations in poultry houses can vary considerably due to variable climate in commercial poultry houses (temperature and relative humidity). A logical next step would be to evaluate and/or validate the AVIVET trap under field conditions. However, the most important reason to first conduct this experimental study is that it is impossible to know and to measure a complete *D.gallinae*-population in a poultry house.

In our experiment, we did not find any other insects in our traps. In practice we sometimes find, however very rarely, some predators of *D.gallinae,* like predatory mites, the beetle "Alphitobius diaperinus" and occasionally a feather lice is found.

Total Number of D.gallinae in the AVIVET Trap in Relation to Total Number of D.gallinae in the Cage

The results show that it is possible to quantify small and large populations of *D.gallinae* using the AVIVET trap, indicating that the trap is able to detect and follow the growth or decline of *D.gallinae* populations in time. However, when quantifying small populations one has to keep in mind that the AVIVET trap might have an inhibitory effect on the growth of *D.gallinae* populations.

The amount of variation of Tot.N-cage explained by Tot.N-trap is very high (93.8%). For comparison, the automatic counter of Mul et al., has a correlation coefficient of 90.3% (Mul et al., 2015). Papers describing other monitoring methods of *D.gallinae* have not reported these parameters.

The trend of the absolute numbers of *D.gallinae* shown in Figure 5 suggests that the AVIVET trap is an even more attractive hiding place at higher Tot. N-cage. This might be explained by communication between *D.gallinae* (e.g., pheromones) (Koenraadt and Dicke, 2010), however another explanation could be that at higher *D.gallinae* densities the cracks in the AVIVET trap become more convenient than those in the cages and offer more protection. This observation differs from results of the automatic counter of Mul et al. (Mul et al., 2015), which might be explained by the fact that the automatic counter is a closed system (once in the counter, forever trapped) and, consequently, communication between *D.gallinae* is hindered.

Moreover, while a maximum of 150 *D.gallinae* (moving stages) were found by Mul et al. (Mul et al., 2015) with their automatic counter, the AVIVET trap

showed a maximum of 3,351 mites. At all low levels of *D.gallinae* the AVIVET trap managed to trap mites, whilst the automatic counter sometimes did not. The latter suggests that the automatic counter might be less sensitive at low densities of *D.gallinae.*

Both methods find high numbers of *D.*gallinae. However, finding especially low numbers is crucial in the control of *D.gallinae* (Mul and Koenraadt, 2009). At low infestations, *D.gallinae* populations are easier to control and treat than large populations. Further, using pesticides against smaller populations will reduce the likelihood that resistance to such agents develops.

Weight of D.gallinae in the AVIVET Trap

The correlation between the weight of *D.gallinae* and "Total number B" of *D.gallinae* (all stages: i.e., eggs, larvae, nymphs, and adults) showed a very high R-square (0.996). Consequently, almost the whole weight of *D.gallinae* in the AVIVET trap is explained by the four stages of *D.gallinae* in it. Indicating that in this study the AVIVET trap has a very high specificity for *D.gallinae.*

These findings confirm our assumption that in addition to the *D.gallinae* count, the weight of *D.gallinae* in the AVIVET trap is also a very reliable parameter for determining the infestation of *D.gallinae* in a cage. Weighing the *D.gallinae* in the AVIVET trap is a faster and cheaper way to obtain results of *D.gallinae* infestations in poultry houses. However, it is a less informative parameter for those interested in the specific distribution of the four stages of *D.gallinae.*

Moreover, under field conditions this correlation coefficient might be different, because there might be more influence of dust and other insect and acarid species that possibly would hide in the AVIVET trap. To investigate this correlation coefficient for AVIVET traps under field conditions, we intend to obtain and analyze data in future.

Use of AVIVET Traps in Practice

For routine monitoring in poultry houses we advise monitoring of the *D.gallinae* population at least once a month with ten AVIVET traps per poultry house. The use of ten traps is based on the research of Nordenfors and Chirico (Nordenfors and Chirico, 2001).

Monitoring should be performed at a minimum in the periods with the highest risk for *D.gallinae* for fast development, i.e., when temperatures start to increase or remain high (roughly March to April till October for Northern Europe). Moreover, as already mentioned it is of crucial importance for the control of *D.gallinae* to observe (very) low numbers. Therefore monitoring should be performed from the beginning till the end of a production round.

The first time, when AVIVET traps are hung in a poultry house, it is important to determine the sites for hanging the traps. These sites must be well distributed across the whole poultry house, easy to reach for both the farmer and the veterinarian and not easy to reach for the hens, but they must also be in the shadow and near shelters or "highways" (busiest "roads" to the hens) of *D.gallinae.*

To compare results within a poultry house, all traps must be oriented in the same way i.e., horizontally or vertically. Horizontal orientation is preferred, because fewer mites will get lost when the traps are removed.

Each new monitoring point in time in the same poultry house must be carried out using the same locations. To achieve this, it is convenient to make a quick map, leave the cable ties of the preceding monitoring in place, and/or to make an easily seen mark there.

AVIVET traps should be placed in the poultry house for 48 hours, just as validated in this study. Moreover, the trap is very useful for research purposes to compare control methods.

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