ANT22 - Aqueous Emulsion Control Method: Contribution to Rational Control of *Dermanyssus Gallinae* in Poultry

Aleksandar Pavlićević[1*], Radomir Ratajac[2], Tamaš Petrović[2], Aleksandra Tasić[3], Ivan Pavlović[3]

[1] *"AVES MIT" LLC, 24210 Subotica-Bajmok, Šandora Petefija 3, C Serbia*
[2] *Scientific Veterinary Institute „Novi Sad", 21000 Novi Sad, Rumenički put 20, Serbia*
[3] *Scientific Institute of Veterinary Medicine Veterinary Institute of Serbia, 11000 Belgrade, J.Janulisa 14, Serbia*

Abstract
Red poultry mite *Dermanyssus gallinae* (De Geer, 1778) is an ectoparasite that feeds on the blood of birds and mammals. Invasions of the ectoparasite *D. gallinae* in intensive poultry farming reach proportions unknown in the wild nature. They are classified as key health, economic, and ecological problems of the most intensive production of foodstuffs of animal origin. Annual losses worldwide are estimated at 3.92 billion USD and tend to grow further. This manifestation of the problem, to a large extent, results from inadequate control of *D. gallinae*. This study aims to optimize and rationalize the *D. gallinae* control by external application of inert oils. The ANT 22 method (working name) is based on "know-how" formulation and application technology. In this study, a manual application was carried out in the clinical conditions of 18 small industrial facilities (2,000 - 14,500 animals) of laying hens (cage system), with a total capacity of 151.300 laying hens, in a time frame of 25 months. The effects of *D. gallinae* control (suppression of infestation) in inhabited and empty non-conditional objects with one treatment lasted 4-10 months, and with two treatments lasted 8-10 months. The observed disadvantages of the method are common to inert oils, which can be minimized with care. The ANT 22 method is safe and does not develop resistance. It is a conditional method, which requires hygienic preparation and adequate application, and is optimal for empty buildings with rest. An important technical condition is the correctness of the applicator and a professionally performed application. Considering the control findings obtained so far, we believe that they justify testing the application of the ANT 22 method for control purposes in large industrial facilities, but also for the purpose of eradicating *D. gallinae.*

Keywords: ANT 22 method, *Dermanyssus gallinae* control, efficacy

1. Introduction

Red poultry mite *Dermanyssus gallinae* (De Geer, 1778) is an ectoparasite that feeds on the blood of birds and mammals. In terms of prevalence and impact, *D. gallinae* is classified as a key health, economic, and ecological problem of poultry farming [1,2]. Annual losses worldwide are estimated at 3.92 billion USD [3]. The trend of costs and prevalence of *D. gallinae* is unfavourable and has constant growth.

* Corresponding author: Aleksandar Pavlićević, +38163430864, zemljadrveca@gmail.com

The reasons for the extreme scale of infestation of *D. gallinae* in poultry farming (Figure 1) are 1 appropriate conditions for the parasite in poultry farming (such as a large number of hosts that cannot leave or defend themselves sufficiently, hidden places, technological connections that enable spread, temperature, and humidity); 2. exceptional biological properties of *D. gallinae* (especially reproductive power, resistance, adaptability, way of life (Figure 1); and 3. non appropriate approach to controlling *D. gallinae*. An inadequate approach for control of *D. gallinae* is the most important reason for developing this problem, and changing the approach is the basis of

its solution [4,5,6].
Control of *D. gallinae* is carried out by preparations with acaricidal effect, which can mainly be applied externally or by *per os* intake for the poultry. All types of applications have advantages and disadvantages and can complement each other. The aim of the research is to apply the ANT 22 (inert oils) method in the rational control of *D. gallinae* in small industrial facilities, by manual application, in application conditions when conditions for process optimization are lacking.
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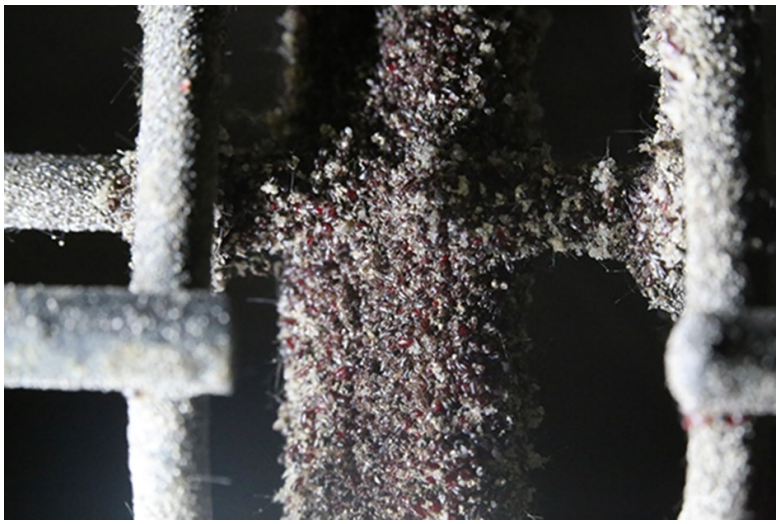

Figure 1. Aggregates of *D. gallinae* on cage (high intensity of infestation)
Aggregates are a way of life for *D. gallinae*, a place of reproduction and an important factor in the development of adaptations to the use of inert substances in control

Table 1 Categorization of the intensity of *D. gallinae* infestation

No	Intensity and extensity of infestation	Harmful impact on			Detection
		Health status of the flock	Production results	Staff	Visual review
1	-	No	No	No	They are not noticeable
2	+	No	No	No	Single or small number of individuals
3	++	No	No	No	Nail-sized lumps
4	+++	Yes, beginning	Yes, beginning	Yes	Larger clusters, up to the size of a palm
5	++++	Yes	Yes	Yes	Palm-sized or larger clumps, typical odour

2. Materials and methods

The ANT 22 method (working name) is based on "know-how" formulation and technology of application of inert oils [4].
The manual external application (with one nozzle), a spray method, was carried out in the study. The application was carried out with a hand-made applicator in the clinical conditions of 20 small industrial facilities (2,000 - 14,500 animals) of laying hens (conventional and enriched cage systems), with a total capacity of 169,800 laying hens in a time frame of 25 months (Table 1).
The intensity of the invasion was monitored by visual inspections of the poultry facilities and the health status of the flock, where the production results and the opinion of the farm staff were consulted.

3. Results and discussion

The results of the study are presented in Table 2.

Table 2. Overview of experimental facilities, performed ANT 22 treatments and control effects of *D. gallinae*

No	Capacity of facility*	Infestation intensity	Facility Full= 1; Empty= 0	Date of treatment	The effect suppression (months)	Comment
1	2,000	++	1	2022.02.16.	5	Hygiene not conditional
2	12,000	++ (+++)	1	2022.05.23. 2022.06.17.	8	-
3	12,000	+++ (++)	1	2022.05.30. 2022.06.03.	10	-
4	6,000	+++ (++)	1	2022.06.13. 2022.06.27.	10	-
5	6,500	+++ (++++)	1	2022.06.04. 2022.06.17.	8	-
6	7,700	++ (+++)	1	2022.06.06.	7	-
7	12,000	++ (+++)	1	2022.06.09. 2022.06.24.	8	-
8	10,000	+++ (++)	1	2022.06.20.	5	-
9	14,500	+++ (++)	1	2022.07.23.	6	-
10	7,700	++++	0	2022.07.22.	4	Hygiene not conditional
11	5,700	+++ (++++)	1	2022.11.28.	4	-
12	10,000	++ (+++)	1	2022.12.12.	5	-
13	7,700	++++	1	2022.12.27.	5	-
14	12,000	+++	0	2023.02.20.	10	-
15	14,500	+++ (++)	1	2023.04.18.	7	-
16	7,700	+++ (++)	1	2023.05.03. 2023.06.12.	10	-
17	10,000	+++ (++)	1/0	2023.05.30.	6	-
18	6,000	+++	0	2023.10.12.	(4) - in progress	Partially conditional

* Number of animals (poultry) in facility

The results of our research presented in Table 2 primarily include higher intensity invasions (+++, ++++) in the conditions of an inhabited facility. The ANT22 method, when applied with a single treatment, achieved predominantly weaker results in these situations. Therefore, the determination of the basic approach to the application of the ANT22 method is based on two treatments. The deviation to a larger number of treatments is a consequence of the failure of the applicator, i.e., inappropriate application of the method. The ANT22 method is designed so that when applied in empty conditional facilities with two treatments, it exhibits maximum effects. The results presented in Table 1 include the application of the method in empty facilities in only three cases, but not examples with two treatments, hygienically conditional facilities that will have a break in the application. In the described manner and under the stated conditions, we determined the existence of control (suppression) effects of *D. gallinae* in the range of 4-10 months. In terms of effectiveness, the ANT22 method can be classified as one of the most up-to-date products and a method of choice for controlling *D. gallinae.*

Control of D. gallinae by external application of products with acaricidal effects

External application (Figure 2) is the basic procedure for the application of agents with acaricidal action in the control of *D. gallinae* for the following reasons: 1. Safety, direct exposure of poultry to control measures is avoided; 2. Biosecurity, it prevents the introduction of parasites when buying used cages and equipment, and makes it impossible to transmit them through transport cages; 3. Prevention (acting in empty buildings can eliminate the invasion before the settlement of the new flock); 4. Greater exposure to parasites (highest hygiene requirements and

poultry do not cover surfaces); 5. Increased sensitivity to invasion (ceasing reproduction, passage of the most resistant egg and larval stages, hunger, behavioural changes). External application is a demanding procedure because it is necessary to ensure: 1. hygienic preparation of the area (regular technological procedure), 2. Professional application (applicator and trained executor), 3. Time, and 4. Application costs. Despite its demanding nature, properly implemented external applications many times more profitable through the effects of control. Due to the above, permanent improvement of external application creates a sound basis for better control of *D. gallinae.*

Selection and comparison of the most current products and methods for rational control of *D. gallinae*

Control of D. gallinae by acaricide Fluralaner

Since the beginning of the rapid development of intensive poultry farming (especially egg production), the control of *D. gallinae* has been predominantly based on synthetic neurotoxic chemical compounds - pesticides, specifically acaricides (insecticides in the broader sense). Toxicity is a key drawback of acaricides and consequent loss of efficacy through resistance [1,7,8]. The most current acaricide is fluralaner, the veterinary antiparasitic drug "Exzolt". It is used for the treatment of poultry, administered through drinking water, twice with an interval of 7 days. Fluralaner is highly effective 4 hours to 12 days after application and achieves total efficiency of 99.3-100% [9]. It has been estimated that this veterinary drug achieves more than 90% suppression and lasts up to 8 months (56 – 238 days) [10]. Studies of 3 fluralaner treatments 7 days apart have been conducted, and an efficiency of over 90% has been recorded [11].

Control of D. gallinae by SiO_2

The first exception to using acaricides in industrial poultry farming was made possible by SiO2 technology, which excluded the possibility of residues in eggs and meat. There is a wide range in the quality of SiO2-based preparations and their applications [12,13,14]. The best SiO2 products and technology were developed in Germany. Efficacy studies indicate up to 46 weeks of suppression of infestation [14]. Properties of SiO2 formulations are demanding application; low capacity of acaricidal effect for subsequently exposed; they act slowly, even mortally exposed individuals can lay fertile eggs; and their effect is affected by impurities and humidity [12,15,16]. SiO2 technologies may have a specific impact on exposed animals. In the Netherlands, they are banned due to the estimated harmfulness in the form of respiratory organ irritation [17]. Their application is optimized in program control by the combined application of powder and liquid form in an empty facility with a rest period of the facility when it can lead to the eradication of *D. gallinae* from production facilities [4].

Control of D. gallinae by Pulcap

Product *Pulcap* is the first dedicated product for the control of *D. gallinae* based on inert oils (P 547/17) [15,18]. Compared to the best SiO2 products, it has advantages in applicability, distribution, and penetration, as well as a faster effect on subsequently exposed individuals of *D. gallinae* and higher mortality per unit area. It has a long-lasting action on non-absorbent surfaces [12]. Differences in the effectiveness of SiO2 and product *Pulcap* are particularly pronounced in populated, highly infested facilities. So far, *Pulcap* has manifested the highest recorded *D. gallinae* control efficacy and confirmed eradication potential (eradication from production facilities). Eradication of *D. gallinae* by the product *Pulcap* is proven in facilities of industrial productions: breeding flocks, rearing and exploitation flocks [19]. The criteria for confirmation of eradication are that not a single individual of *D. gallinae* is found in inhabited buildings for a minimum of one year through detailed inspections (using a unique method of early detection with dust [20].

Control of D. gallinae by plant products

The external application of essential oils and other plant products to control *D. gallinae* is unjustified because they do not have an adequately prolonged acaricidal effect [13]. However, applying plant products through food and water is an interesting area because there are hints that it makes it possible to achieve not only a repellent effect but also a lethal effect on *D. gallinae*. Further research on this subject is needed.

Control of D. gallinae by ANT22 method

In addition to the outstanding properties and efficiency of the product *Pulcap*, the development

possibilities of inert oil formulations were observed with the aim of rationality and operability, as well as, eventually, usability and efficiency in field conditions. The main topic of the ANT22 method research is optimizing the application of inert oils to control *D. gallinae*. The ANT 22 method is recommended because of its safety, application possibilities, efficiency, economy, and durability.

The ANT 22 method is intended to apply two treatments in case of large invasions (+++, ++++) and one treatment to suppress more minor invasions (+, ++) of *D. gallinae*. The application may be different, depending on the economic situation (producer's decision), i.e. the current price of eggs. Optimizing the application is achieved with 2 related treatments in an empty building (cleaned, washed, and disinfected), after which it is important to rest the building. Research on the optimal application of the ANT 22 method, especially in order to confirm the possibility of eradicating *D. gallinae*, is not included in this study (they are ongoing) (Figure 2).

Figure 2. Experimental farms after ANT 22 treatment. A layer of water emulsion is observed on the cages and floor of the facility. The water emulsion is applied to important places in the life cycle of *D. gallinae*.

The tests of the ANT22 method carried out so far are primarily aimed at checking the concept itself and were carried out in a large part of the most challenging circumstances for the effect of these active substances (large invasions of *D. gallinae* in a populated building (Figure 3 and Figure 4). By comparison, such situations cannot even be rationally controlled by SiO_2 technology.

The results of the effectiveness of the ANT 22 method are not entirely comparable to those obtained with the *Pulcap*. The results of the product *Pulcap* were obtained in large industrial facilities by machine application (more demanding conditions), while the results of ANT 22 were obtained in small industrial facilities by manual application (where more detailed execution is possible). The mechanical application of ANT methods in intensive poultry farming requires technological improvements to existing application devices. Therefore, we must point out that *Pulcap* is today's most effective product in controlling *D. gallinae*. If similar or the same results are obtained in extensive industrial facilities, the ANT 22 method will have an advantage over *Pulcap* technology due to its economy and operability. Then it would be able to permanently, economically, and safely improve poultry farming in the field of *D. gallinae* control.

Figures 3 (left) and 4 (right) the effect of the ANT 22 method on the invasion of *D. gallinae*
On the feeder (Figure 3) and the supporting column of the cage (Figure 4), clusters of *D. gallinae* were directly exposed to the ANT 22 method
Dead bodies of *D. gallinae* remain at the exposure site for a long time.

The established effectiveness of one treatment of the ANT 22 method is 4 to 10 months, and two treatments ensure 8 to 10 months of *D. gallinae* infestation control. These results justify the application of the ANT 22 method in small-scale industrial poultry production facilities with cage systems. Concerning the properties shown so far, there is potential for using the ANT 22 method for *D. gallinae* control in large industrial facilities.
Disadvantages are common when using inert oils. A key disadvantage is the possible slipping of the egg belts. This problem is reduced by the correct selection and placement of nozzles and the work method. Immediately after the treatment, the floor is slippery, so care must be taken not to fall. It cannot be applied systematically in populated reproductive flocks, as the oils can block the breathing of hens' eggs. Adequate preparation of the empty facility for reproductive flocks will eliminate the need for treatment in exploitation. It does not affect eggs and larval stages of *D. gallinae* to a significant extent (common to all inert oils and many other products and methods). Therefore, for full effect, the ANT 22 technology is aimed at use in empty buildings. After the flock has moved out, *D. gallinae* lays eggs in an empty building for another ten days. The eggs hatch into larvae in 2-3 days, and after that, in 2-3 days, they move to the next stage that is sensitive to ANT 22 treatment. The problem of naturally resistant stages of *D. gallinae* is solved by appropriate cleaning, washing, and resting the object.
Rational control (pharmacotherapy) in the best possible way fits the pharmacological profile of products and methods with general requirements, specific situations and needs in the control of *D. gallinae*. The profile consists of important product characteristics and methods. We highlight safety, method and complexity of the application, hygienic conditions, efficiency, economy, resistance, and other disadvantages (Table 3).

4. Conclusions

The ANT 22 method is still under development; it is being developed to improve the formulation, application, and the applicator. Further development will ensure quality adaptation of the application in large industrial productions, a positive shift concerning the general shortcomings of the method (hygienic conditionality, the complexity of the application, etc.), and raise the overall quality of the external application.
Under the tested conditions, the results indicate that the ANT 22 method is competitive in itself and suitable as a basis for combination with other products and methods (program). The ANT 22 method is particularly suitable to prepare empty buildings, used cages and equipment, as well as transport cages (as a preventive measure). Based on the findings obtained so far, it is expected that in hygienically conditioned buildings with a rest period of the prepared buildings, with two treatments, the ANT 22 method will lead to the eradication of *D. gallinae* from production facilities.

Table 3. Comparison of selected active topical substances and methods

Active substance, product, method	Safety	Use	Complexity of application	Hygienic conditionality	Efficiency (months)	Economy	Resistance	Other short-comings
Fluralaner	Egg residues (safe but not desirable)	In a flock (inhabited building)	No	No	Control, not eradication	The highest price range	Yes (not permanently usable)	No
SiO2	No residue, possible impact on the exposed animals	Empty and inhabited building	Yes	Yes (highly demanding)	Control, possible eradication	Possibly high	No (possible behavioural adaptation)	Large invasions in a populated building
Pulcap	Safe	Empty and inhabited buildings, used cages and equipment, transport cages	Yes (lower)	Yes	Control, possible eradication	Possibly high	No (permanently usable)	Slippage of egg belts, slippery floor, cannot be applied systematically in reproductive flocks*
ANT 22	Safe	Empty and inhabited buildings, used cages and equipment, transport cages	Yes (lower)	Yes	One T: 4-7 months (10 in empty objects) Two T: 8-10 months The optimal application has not been tested. Control, eradication expected	Possible high, Potentially the most competitive	No (permanently usable)	Slippage of egg belts, slippery floor, cannot be applied systematically in reproductive flocks*
Phyto-products	Safe	In a flock (inhabited building)	No	No	Control, not eradication	Questionable	Probable	No

* The exploitation is in the text of the manuscript

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