DERMANYSSUS GALLINAE IN LAYING HENS FARMS IN ALGERIA: INFESTATION PREVALENCE AND MOLECULAR DETECTION OF *SALMONELLA*

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Abstract. *Dermanyssus gallinae* (Red mite) is the most important and common ectoparasite of laying hens and recognized as a vector of several pathogens. In order to estimate the infestation prevalence rate of Dermanyssus gallinae in layer housing and to evaluate its vectorial role in regard to *salmonella*. A study was carried out in 386 laying hen farms in four provinces located in north-eastern Algeria. A total of 32 pooled mite samples were examined for the presence of *Salmonella enterica*, *Salmonella typhimurium* and *Salmonella enteritidis* using PCR essay. Results showed that 14% of hen housing were infested by *D. gallinae*. *Salmonella enterica* DNA was detected in 8 samples (25%), including two *Salmonella enteritidis* (6.25%). These results indicate that *D. gallinae* can act as reservoir of *Salmonella*, allowing the propagation of this bacterium between successive bands and between different farms.

Keywords: *Dermanyssus Gallinae*, *Salmonella*, Layer Housing, Vector, Algeria.

INTRODUCTION

Dermanyssus gallinae (poultry red mite, PRM) is a common concern among poultry egg farmers in both developed and developing countries (Kim et al 2007) and remains an unresolved problem (Schulz et al 2014). The average prevalence in European countries was estimated at 80% (George et al 2015) with economic losses of approximately 130 million Euros annually (Van Emous et al 2006).

The poultry red mite is a haematophagous ectoparasite of poultry and wild birds (Sparagano et al 2009). It feeds on resting birds, mainly during the night. However, during the day, these mites hide in the crevices and cracks of walls and equipment. Under favourable conditions, the PRM life cycle is accomplished in a week, favouring the development of an intense short-term infestation, which may result in egg production dropping, mortality, reduced hen immunity and itching dermatitis in humans (Mul et al 2010). When the red mite was discovered, the problem was already very pronounced. Its ability to hide in crevices, which are difficult to reach by insecticide treatment, the prohibition of several acaricides use during the production cycle and the development of resistance to several insecticide products, makes eliminating this mite very difficult (Fischer et al 2014). Moreover, *D. gallinea* is a potential vector of several pathogens including Salmonella (Hamidi et al 2011) that cause the most frequent food-borne zoonoses. Poultry is the main source of infection for humans (Davies et al 2003).

Despite several studies in European and developing countries, describing different problems posed by this poultry pest (Sparagano et al 2014), there is no published work about red mite infestation in Algeria. The purposes of the present study were to evaluate the prevalence of mite infestation and subsequent economic losses the role of *D. gallinae* as a natural vector of *Salmonella*.

MATERIALS AND METHODS

Study area: The study was conducted during the period from September to December 2014 in four provinces (Bouira, Bordj Bouarreridj, Setif and Batna) in North-eastern Algeria. This region represents 70% of the global egg production in Algeria (Mezouane. 2010). The study region is characterized by dry and hot summers, with temperatures ranging from 30 to 40 °C, and by cold winters, with temperatures averaging from -2 to 8°C. Rainfall is moderate, varying between 400 and 600 mm per year.

Sampling: The parasites were collected directly from the cages and walls by a small spoon according to Cencek (2003) and placed in airtight plastic containers. The specimens were preserved in 70% ethanol solution.

Laboratory analysis: *D. gallinae* identification was made under an optical microscope at × 100 magnifications (Pritchard et al 2015) (Di palma et al 2012). In whole 32 Pooled samples of *Dermanyssus gallinae* of different age (contained each about 100 miles), each from one infested houses, were examined for the presence of *Salmonella enterica* (*S. enterica*) *Salmonella typhimurium* (*S. typhimurium*) and *Salmonella enteritidis* (*S. enteritidis*) using PCR essay. The mites taken from the 70 % ethanol were rinsed three times in 500 *µl* of sterile ultrapure water with vigorous shaking. Bacterial DNA was extracted using Dneasy Tissue Kit (QIAGEN) according to the manufacturer's instructions. Extracted DNA was stored at - 20°C.

PCRs were performed in a total volume of 30 *µl* containing 3.0 *µl* of buffer (10×, free Mg2þ), 2.0 *µl* of MgCl2 (25 mM), 1.0 *µl* of dNTP (2.5 mM), 1.0*µl* of forward and reverse primer mixture (stm-4495, sen-1392 and FS23) (5 mM), 1.0 *µl* of Taq DNA polymerase (1 U/mL), 3 *µl* of 5X Q solution, 3 *µl* of Coral Load and 2.5 *µl* of template DNA. PCR amplification was performed in thermal cycler with a cycling conditions consisting of a 5.0 min denaturation step of 94 ºC, followed by 35 cycles of denaturation (30 s, 94°C), annealing (30 s, 65°C) and extension (30 s, 72°C) and final extension step of 10 min at 72°C, followed by a final hold at 4 ºC. Amplification products were confirmed by electrophoresis using 2% agarose gels, stained with ethidium bromide, and then visualized under UV light (Liu et al. 2012). The specific primers used were presented in Table 1.

Table 1

The target genes and their sequencing primers (Liu et al 2012)

Target	Gene	Primer set name	Sequences (5'e3')	PCR product (bp)
S. typhimurium	STM4495	stm-4495	GGTGGCAAGGGAATGAA CGCAGCGTAAAGCAACT	915
S. enteritidis	SEN1392	sen-1392	GCCACTGTCTGATGCTCTTG GAAAGGCTCCGTGGTTAGT	656
S. enterica	srfC	FS23	GGCGTTAACCCACTCCAGTA TTACTGTGGGGAGAGCAACC	492

Statistical analysis: The statistical program used was R I 386 3.0.2 for Windows GUI front-end. ANOVA test was used to compare the prevalence of infestation between different regions. Chi-square test was used to study the influence of hens' age on prevalence of infestation by *D. gallinae*. Differences were considered as significant when P value was less than 0.05.

RESULTS

In total 54 out of 386 farms (14%) were infested by *D. gallinae*. Bouira region had a highest infestation rate (18.1%) in comparison with the three others regions (p< 0.001) (Table 2).

Table 2

D. gallinae infestation prevalence according to the different regions

Regions	Number of layer housing	Prevalence (%)	Probability
Bouira	88	18.1[a]	0.001
B.B. arreridj	102	11.7[b]	0.069
Setif	85	12.9[b]	
Batna	111	13.5[b]	
Total	386	14	

[ab] value marked with the same letter are not statically different

The age of the hens influences the prevalence of infestation by *D. gallinae*. The highest prevalence was reported in farms with older hens (>40 weeks), 25.9%, and lowest prevalence among farms with hens younger than 10 weeks (3.84%) (Table 3).

Table 3

Relation between age of hens and infestation by *D. gallinae*

flock age (Weeks)	Infestation prevalence	Probability
< 10	3.84%[a]	p< 0.001
10-20	13.8%[b]	p< 0.001
20-40	11.2%[c]	p< 0.001
>40	25.9%[d]	p< 0.001

[abcd] value not marked with the same letter are statically different.

In the infested housing the hen mortality rate and the drop in egg production were estimated at 10% and 2% respectively. PCR essay showed that 25% pooled mite samples (08/32) were positives for *Salmonella enterica* and 6.25% for *Salmonella enteritidis* (02/32). No positives case was recorded for *Salmonella typhimurium*.

DISCUSSION

Poultry farms infestation by *D. gallinae* in our study is less widespread in comparison with Tunisia and Morocco, which the prevalence of infested buildings was 38% (Gharbi et al 2013) and 55% (Sparagano et al 2009), respectively. In European countries the prevalence of infection exceeds 50%, it estimated at 50% in Kosovo (Hamidi et al 2011), 63.7% in Romania (Magdas et al 2006), 67% in France (Lubac et al 2003), 68% in Denmark (Sparagano et al 2009), 87% in the UK (Guy et al 2004) and 90% in Italy (Maragani et al 2012). In Asia, the prevalence is also high; it is 30.7% in Palestine Othman et al 2012, 39% in Iran (Yakhchali et al 2013) and 64.1% in China (Wang et al 2010).

European countries use an alternative system breeding that is more conducive to the development of this parasite than cage system. In 2014, over than 160 million laying hens were reared in non-cage systems (Chirico et al 2002) (Fiddes et al 2005) (Arkle et al 2006) .Whereas, in Algeria, the cage system has been employed in all farms, which give a lower prevalence revealed in this study. Also, the study was performed in semi-arid regions. According to Nordenfors et al (1999), *D. gallinae* thrives in an environment with high humidity (at least 70%), whereas it does poorly in arid conditions because it cannot fully retain moisture.

The higher infestation rate in the province of Bouira may be attributed to its climate more humid than the other regions. Sparagano et al. (2009) reported that smaller farms were most affected by *D. gallinae*, because they are associated with bad sanitation practices and hygiene management. Although, in our study, we didn't find a difference in hygiene regarding to housing size. In the present study, the older flocks are the most affected by the parasite. This finding is consistent with Mul et al. (2010) which concluded that non-infested flocks were significantly less older (45 weeks) than the infested ones (52 weeks), similar results are found by Gharbi et al. (2013).

The visited farms presented an acceptable hygienic measure that made the environment less favourable for red mite multiplication. (Othman et al. 2012) indicated that among the factors favouring a high prevalence in Palestine (38%) is the very low cleaning frequency, which is manually performed in all farms. In China, where the prevalence of infection is high (64%), 32% of farmers do not clean, 55% do not use water for cleaning and 20% do not use disinfectant between flocks (wang et al 2010). Similar habits were observed in the Netherlands (prevalence, 80%), where 43% of farmers do not use water to clean their buildings between flocks (Mul et al 2010). Red mite infection causes irritation and anaemia that manifest principally by decrease in egg production and death, in this study its can reach 10% and 2% respectively.

In a study realized in 06 battery cages showed a decline in egg production of 2-15%. While, Cosoroaba (2001) found a minor increase in mortality rate (0.08%) and a drop in laying of 20% in a 60-mile hens henhouse heavily infected. (Van Emous et al 2006) has reported that the death rate among the hens can rise from 1 to 4%, with a reduction in laying performance of up to 10%. The current study provides a molecular evidence for the involvement of wildlife red mite in the portage of *S. enterica* and *S. enteritidis* on laying hens farms.

The public health consequences and economic impact of *Salmonella* in the Algerian poultry breeding sector are unknown because there are no epidemiological surveillance systems or monitoring programs for *Salmonella* infections in this country (Bouzidi et al 2011). It has been shown that the mite can be infected through the blood meal or by cuticular contact (Valiente moro et al 2007) and because the red mite often hide under dry dropping which are frequently contaminated by *Salmonella* and feed several times in each life stage on hens which increase the risk to transmit *Salmonella* and other pathogen. Consequently, mites infected by *Salmonella* constitute a potential reservoir host of this bacterium, permitting it's persistent in the poultry house between flock cycles. Moreover, as *Salmonella* is a zoonotic agent, it remains to be clarified whether *D. gallinae* may act as a vector of *Salmonella* also to humans. The capacity of red mite to fasting for a long period time, more than six months (Hamidi et al 2011), involved an effective control of red mite before the introduction of new batches pullets.

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