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Molecular identification by means of Seminested PCR of *Dermanyssus gallinae* circulating in Albania

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Abstract

In the present work a total of 5 sample sets of Poultry Red mites, collected in 5 different poultry (layers and broilers) farms of Albania, through the Cardboard method, were investigated. The mites previously macroscopically identified as *Dermanyssus gallinae* using morphological keys were investigated by molecular tools. These samples were analysed for the following identification of the *ITS* and *COI* genes by means of Seminested PCR. The SM PCR guaranties a major specificity and susceptibility. The phylogenetic identification of *Dermanyssus gallinae* was based on the detection of the ITS (internal transcribed spacers) gene and the specific genomic sequence COI (a portion of the cytochrome c oxidase gene of the mitochondrial DNA) gene. This study revealed no genetic diversities of *D. gallinae* distributed in Albania as all of the Sm PCRs assays resulted positive, where the 5 sample sets resulted positive (100%) either on the presence of *ITS* gene, so in the *COI* gene, which confirms them as *Dermanyssus gallinae* mites. This work represents a preliminary step for the implementation of larger epidemiologic investigation aimed to assess the extent of the circulation of *D. gallinae* in Albania, and to retrieve phylogenetic data about the mite populations.

Keywords: *Dermanyssus gallinae*, Albania, poultry farms, *ITS* gene, *COI* gene.

1. Introduction

Dermanyssus gallinae, order Mesostigmata, Acari, also known as the poultry red mite, is an obligatory blood-sucking parasite of both domestic and wild birds [5, 9]. In poultry farms, it causes irritation, loss of weight, reduction in egg production, anemia and death, leading to economic loss in the poultry industry [1]. It has been evidenced on more than 28 poultry species [7] and it is considered as a major concern in the poultry breeding, worldwide (1,5). The poultry red mite, *D. gallinae* has been involved in the transmission of many pathogenic agents, responsible for serious diseases both in animals and humans.

Nowadays, few effective methods are available to control the ectoparasite in poultry farms. Consequently, this is an emerging problem which must be taken into account to maintain good health in commercial egg production. [12]. In addition to causing 'direct' losses in poultry production systems, *Dermanyssus gallinae* has also been described as a potential vector of several bacteria and viruses of concern to poultry, among them several food borne pathogens as *Salmonella spp.* [6], *Erysipelothrix rhusiopathiae* (ER) [3] and the avian pox virus agent [2].

Dermanyssus gallinae is considered also as a biological vector of *S. Enteritidis* under experimental conditions and it may represent a suitable environment for the development of *Salmonella spp.* and could be an additional factor for the persistence of salmonellosis infection between successive flocks [11]. The poultry red mite, D. gallinae has been involved in the transmission of many pathogenic agents, responsible for serious diseases both in animals and humans. Nowadays, few effective methods are available to control the ectoparasite in poultry farms [12].

2. Material and Methods

Dermanyssus gallinae: A total of 5 sets mite samples, collected in Albania through the Cardboard method. Details about the samples are provided in table no. 1. Each sample was made of at least 100 mites, and they were collected from 5 different poultry farms in 4 regions of Albania, during april 2016. In all the farms, the chickens appeared healthy. Each sample was stored at room temperature until use.

Table 1. Details about the farms where mites were collected.

Number	DNA extraction Number in the Protocol Agenda	Poultry production type	Group age	Farm type	Region in Albania
1.	550	Layers	35 weeks	Intensive	Tirana
2.	551	Layers	20 weeks	Intensive	Durrës
3.	552	Broiler	4 weeks	Intensive	Korça
4.	553	Broiler	10- 12 weeks	Free Range	Krujë
5.	554	Broiler	8 weeks	Free Range	Tirana

DNA extraction: DNA was prepared from the set of each individual sample of mites obtained from each farm. The mite was homogenized with zirconia beads using TissueLyser II (Qiagen Inc., Chatsworth, CA, U.S.A.) in 20 *µl* of buffer 1 provided by Ten Minute DNA Release Kit-1 (Jacksun Easy Biotech Inc., Bronx, NY, U.S.A.), and DNA samples were prepared according to the manufacturer's instructions. The DNAs were stored at −30°C until use.

Seminested Polymerase chain reaction (PCR): Upon arrival, mites were crushed through mortar and pestle, and the homogenate was used fotDNA extraction by the mean of the Invitrogen Pure Link™ Genome DNA Mini Kit (Thermo-Fisher, Milan, Italy). The extracted DNA was used as a template for two seminedted PCRs targeting the *cytochrome oxidase subunit I* (COI) and *ribosomal internal transcribed spacer* (ITS), respectively. The PCRs were carried out by using primers previously designed in the host section, specific for *D. gallinae* (De Rojas et al. 2001). Specifically, the first step reactions were carried out in a mixture containing 1,5 U of Platinum Taq polymerase (Invitrogen, Milan, Italy), 1 x Buffer Maxima, 1.5 mM MgCl2, H_2O, 0.2 mM of each dNTP, and 25 pmol of each outer primer. After cycling, 1 µl of the mixture was used as a template for the second PCR step. It was carried out in a volume of 25µl, contained 1 U of Platinum Taq polymerase, 20 mMTris–HCl, 50 mMKCl, 1.5 mM MgCl2, 0.2 mM of each dNTP and12.5pmol of each primer. The thermal profile was 5 min at 95 °C followed by 30 s at 95 °C, 1 min at 60 °C, 2.5 min at 72 °C for 35 cycles, with a final extension of 10 min at 72 °C. For each assay, a negative control, with H_2O instead of DNA, was added. The PCR results were analyzed by electrophoresis at 7.5 V/cm for 60 min in 1.5% agarose gel followed by staining with ethidium bromide 0.5 µg/ml. Stained agarose gels were exposed to UV and image were digitalized by means of a GelDoc-It Imaging System (UVP, Upland, CA, US).

3. Results and Discussion

All PCRs resulted positive (Figure 1) either for the presence of *COI* gene, so for the *ITS* gene, on the genome of the tested sets of *Dermanyssus gallinae*. This preliminary study revealed no genetic diversities of *D. gallinae* distributed in Albania and the findings indicate that the mites are genetically related to the mites in European countries.

The number of samples are indicated on the top of the row. For each sample, the first step PCR has been loaded before the second step PCR, except for the 555 ITS. M: Marker; CN: Negative control. In addition, phylogenetic relationships among the mites in Albania and other countries indicate the possibility of interregional and oversea transmission of the mites. However, it should be noted that nucleotide sequences of *D. gallinae* have been determined for only a few areas despite their worldwide distribution. For a comprehensive understanding of intra- and international migrations of *D. gallinae*, further sequence analyses of the mites collected from all over the world are needed.

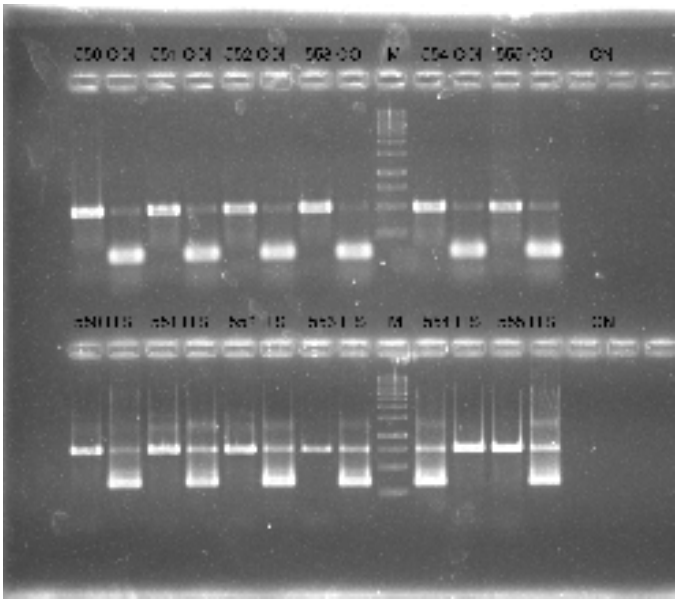

Figure 1. Results of the seminested PCRs.

Knowledge of the genetic make-up of mite populations within countries, together with comparative analyses of *D. gallinae* isolates from different countries, will provide better understanding of the population dynamics of *D. gallinae*. This will also allow the identification of genetic markers of emerging acaricide resistance and the development of alternative strategies for the prevention and treatment of infestations [8]. This work was supported by Cost ACTION FA1404.

5. References

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