
DETERMINATION OF THE PRESENCE OF
***Ornithonyssus* spp. IN BREEDER HENS AND ROOSTERS**
IN THREE GENETIC LINES ON A FARM IN SOLANO
COUNTY, CALIFORNIA, USA

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ABSTRACT

Acarosis in birds may cause irritation, stress, reduction of food intake and, consequently, reduction in egg production and quality. In this sense, the *Ornithonyssus sylviarum* mite is considered one of the most aggressive mites to bird health due to its permanent behavior. The objective of this work is to determine the presence of *Ornithonyssus* spp. in three genetic lines of pedigree breeding chickens and roosters on a farm in the state of California, USA, and identify the species of *Ornithonyssus* spp. present in the three genetic lines studied. A sampling of 103 pedigree chickens and roosters was carried out to determine the presence of *Ornithonyssus* spp. The samples were collected from the feathers under the cloaca, preserved in 70% alcohol, then rinsed with 10% sodium hydroxide and evaluated, by direct observation through the microscope and stereoscope, in order to visualize and identify their morphological characteristics. The results show the presence of *Ornithonyssus* spp. in 99.0% (102/103) of the birds, identifying an average of 135, 121 and 30 mites per bird according to lines A, B and C, respectively. The species identified were *Ornithonyssus sylviarum*, *Ornithonyssus bursa* and *Ornithonyssus bacoti* in 89.8%, 1.4% and 0.8% respectively, with 8.1% *Ornithonyssus* spp. In conclusion, *Ornithonyssus* spp. was present in the pedigree breeding chickens and roosters in the three genetic lines. *Ornithonyssus sylviarum* was the predominant species.

KEY WORDS: Mites; laying hens; genetic lines; *Ornithonyssus sylviarum*.

INTRODUCTION

Mites in domestic poultry are gaining relevance due to recent research showing losses in productivity on poultry farms. In this respect, there are different species of mites that infest birds and that can accidentally also cause diseases in humans (Jofré et al, 2009).

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In the United States, *Ornithonyssus* spp. is considered the most harmful ectoparasite in the poultry industry (Axtell & Arends, 1990; Mullens et al., 2009), with *Ornithonyssus sylviarum* being a threat to egg production (Soares et al., 2008) and possibly one of the most important external parasites in commercial poultry farms (Lutsky & Ba-sela, 1982).

The transmission of *Ornithonyssus* spp. in birds can occur through contact with the facilities (Mullens et al., 2001; Mullens et al., 2009), as well as between birds or by the entry of wild birds or rodents (Mullens, 2009; Allymehr et al., 2012; Denmark & Cromroy, 2003). Once these mites enter the shed, they proliferate rapidly since they reproduce throughout the year and are able to form large populations in the hottest months (Barriga, 2002). Manifestation occurs causing irritation, weight loss, reduction in egg production and even death if the blood loss is considerable (Jordan, 1998).

On farms, the ectoparasites can go unnoticed and be underestimated, however they can cause significant damage to the health of birds. Therefore, the purpose of this study is to provide information on the detection and identification of *Ornithonyssus* spp. present in three genetic lines of pedigree breeding hens and roosters on a farm in the state of California, USA.

MATERIALS AND METHODS

The present investigation was carried out on a poultry farm in the county of Solano, Sacramento, in the state of California, United States during November 2016, when the average temperature was 13°C with an average relative humidity of 78%.

The studied population consisted of 124 45-week-old birds, 103 hens and 21 roosters. The genetic lines were: line A (silver gene), line B (meat) and line C (golden gene) containing 30, 27 and 46 birds respectively; these were housed in cages above floor level (2.7 ft = 82 cm), measuring approximately 1.5 m², with two nests for each family with carpeting to wear down claws (132 cm²). The birds received food and drinking water *ad libitum* automatically. All the birds underwent beak cutting (except for the roosters) by the cauterization method at approximately 10 days of age. In addition, it is noteworthy that the birds had not been dewormed.

A minimum sample size of 84 birds was calculated. However, 103 birds were sampled, where 17 roosters and 86 hens were selected randomly. The research variables were the presence of *Ornithonyssus* spp., and the genetic lines (A, B, and C), where the analysis sampling unit consisted of 8 to 10 feathers extracted once from the ventral part of the cloaca of the 103 birds. To obtain the samples, the Brazilian capture method was used, where each bird was captured individually, holding the wings vertically against the breast (López et al., 2015).

For the microscopic identification of the ectoparasites, samples were taken from the feathers located immediately above the cloaca (8 cm x 6 cm), stored in a container or Ziploc box containing 70% alcohol for the subsequent fixation and conservation (Owen et al., 2009; Vezzoli et al., 2016). The mites were sifted, extracted and placed in vials containing 70% alcohol for conservation, subsequent identification and assembly (Díaz & Menjivar, 2008). They were then transported to the Veterinary Parasitology Laboratory of the Scientific University of the South (Lima, Peru), where they were identified and rinsed for 5 minutes with slightly heated 10% sodium hydroxide (Villanueva et al., 2003).

For the microscopic identification of the mites, the taxonomic keys of Knee & Proctor (2006), Di Palma et al. (2012), Denmark & Cromvoy (2003) were used.

Statistical processing was performed using the Kolmogorov Smirnov test to identify if the average number of mites in the three genetic lines followed normal distribution and, upon confirmation, the variance (ANOVA) of the means of mites to determine differences. The differences ($P < 0.05$) between the three genetic lines were obtained with the Least Significant Difference (LSD) procedure. The frequencies were expressed in percentages.

RESULTS AND DISCUSSION

In the present study, a sample of 103 birds, both hens and roosters, 99% tested positive to *Ornithonyssus* spp. (Table 1). This may be due to several factors such as biosecurity, the type of production system, bird lineage, feeding, climate and bird immunity. This result is similar to that reached by Mullens et al. (2009), who noted a prevalence of 100% in the middle of January, and when the tests were completed, the prevalence remained high (>85%), besides, these birds had not received mite control treatment beforehand. In contrast, in China, a study carried out on poultry farms which had received treatment for mite control, presented a smaller amount (46.9%) of *O. sylviarum* in a sample of 236 birds (Wang et al., 2010).

Table 1. Presence of *Ornithonyssus* spp. on a poultry farm in California, USA

No. birds		
Sample	Positive	%
103	102	99

When evaluating the species of *Ornithonyssus* spp. in hens and roosters, *O. sylviarum*, *O. Bursa* and *O. bacoti* were identified in 89.8%, 1.4% and 0.8% respectively, with *Ornithonyssus* spp. in 8.1% classified with difficulty according to its species (Table 2). In short, *O. sylviarum* was expected to present a greater population due to its behavior characterized by permanence within the bird throughout its life cycle as compared to the species *O. bursa*, which lays its eggs in the nests and *O. bacoti* that is an ectoparasite characteristic of rodents and only accidentally present in birds and humans.

Table 2. Frequency of species of *Ornithonyssus* spp. on a poultry farm, California, USA, 2016

Species	Total (%)
<i>Ornithonyssus sylviarum</i>	89.8%
<i>Ornithonyssus bursa</i>	1.4%
<i>Ornithonyssus bacoti</i>	0.8%
<i>Ornithonyssus</i> spp.	8.1%

In sum, if wild bird entry in the sheds is not deterred, the hens and roosters are exposed, not only to mites, but also to other parasites and diseases. If there is no control of rodents, it is possible that *O. bacoti* may accidentally move to nests and birds. In addition, biosecurity must be carried out. In conclusion, regarding the presence of *Ornithonyssus* spp. in the three genetic lines studied on the poultry farm, *O. sylviarum* proved the most frequent, totaling 89.8%.

In relation to mite averages according to bird line, line A (Silver Gene) presented the highest mite average numbering 134.7, followed by line B (Meat) with 120.7, unlike line C (Golden Gene), presenting 29.6 mites / bird.

The Kolmogorov Smirnov test was subsequently applied to determine if the mite stocks of the genetic lines followed normal distribution. The means of the three genetic lines were of normal distribution. As of this result, the ANOVA test was utilized to determine if the means of the genetic lines were the same or different. Next, the total number of mites observed and the total number of birds was determined according to the genetic line (Table 3).

Table 4, shows the variance analysis of the three genetic lines, among the lines (intragroup) and within the lines (error), resulting in the ratio F. The calculated F value was greater than the F of the table, that is, the means of the genetic lines were not equal ($p < 0.05$). Therefore, Fisher's least significant difference test (LSD) was used to calculate if there were one or more unequal means in the genetic lines.

Table 3. Total number of mites in three genetic lines on a poultry farm, California, USA, 2016

No. mites	Line			Total
	A (n= 30)	B (n= 27)	C (n= 46)	
Total	4040	3258	1362	8660

Table 4. Variance analysis of three genetic lines on a poultry farm, California, USA, 2016

Variation source	G.L.	SMC	CM	F
Lines (A, B, C)	3-1=2	249,400	$\frac{249,400}{2} = 124,699.88$	$\frac{124,699.88}{7,073.62} = 17.629$
Error	103-3=100	707,361.62	$\frac{707,361.62}{100} = 7,073.62$	
Total	103-1=102	956,761.38		

G.L: degree of freedom; SMC: sum of average squares; CM: average squares; F: F reason.

In conclusion, there are statistical differences between lines A - C and B - C, but between lines A and B there is no difference since the calculated value is less than the critical value of the table (Table 5). That is, line C (Golden Gene) had a smaller amount of *Ornithonyssus* spp. As already mentioned, there are many factors that could determine the amount of *Ornithonyssus* spp. in birds, within them is the sex of the bird, genetic line and climatic factors, among others.

Table 5. Mite average differences in three genetic lines in a poultry farm, California, USA, 2016

Difference in average	Value	Critical value
$X_A - X_B$	134.7 - 120.7=14	44.3
$X_A - X_C$	134.7- 29.6=105.1	39.2
$X_B - X_C$	120.7-29.6=91.1	40.5

X_A , line A; X_B , line B; X_C , line C.

Investigations into why there are differences in population numbers of *O. sylviarum* in birds began in 1930 when Payne noted that some chickens in severely infested sheds remained free of mites. This observation was confirmed when Loomis et al., in 1970, saw the same behavior. Thereafter, certain hypotheses were presented; in 1974, Matthyse et al., explained that when there is local inflammation on the skin after the invasion and feeding of mites, it blocks access to blood, reducing the development, reproduction and survival of mites. In 2009, Owen et al., conducted an investigation on the inflammatory response in infested birds, where they evaluated the population of all stages and their changes against an infestation.

This study detected that satisfactory feeding of the nymph was not associated only with the size of the population. The ability to feed decreased with large populations in conjunction with extensive inflammation, i.e., “population x capillary distance” ‘ and this is related to birds that have the MHC B21 haplotype. In addition, it was noted that the negative impact of inflammation on the bird’s skin extends to affect the quality of mite progeny.

For decades it has been known that some animals are more resistant to infection than others. Pym (2008) mentions that, at present, this is not considered a priority with respect to other parameters of growth and production. However, it is a useful characteristic for selection programs since, by decreasing the number of mites, environmental pollution is reduced as well as the negative effects on production. In the 1980s, for example, De Vaney (1984) conducted research to show that genetic selection could be caused by genetic parasites. Therefore, he estimated a heritability for F1 of 0.18 at 2 weeks, 0.4 at 6 weeks, and 0.2 at 8 weeks post infestation. In his results he determines that genetic selection against *O. sylviarum* in a generation could not be effective.

In addition to the characteristics already mentioned, there are the morphological characteristics of the bird. In the management of laying hens, it is customary to schedule debeaking either on the first day or at 7 to 10 days of life to prevent behavior that may increase mortality by pecking, as well as food loss and selection. However, this practice prevents the birds from extracting mites to reduce body infestation. Mullens et al. (2010) evaluated whether the state of the beak determined the density of mites (*O. sylviarum*) in hens of the white Leghorn line. Their results showed that mite populations were 10 times lower in the hens that had intact beaks compared to hens with cut beaks. The chickens that had intact beaks, presented signs of skin irritation at the base of the cloaca which were not observed in the debeaked birds.

According to the results in this study, the hens of genetic line C presented the lowest amount of *Ornithonyssus* spp. on the farm and showed a slight bluntness in their beaks. It is probable that inadequate debeaking was performed allowing line C to diminish the quantity of *Ornithonyssus* spp. According to the literature, when there is no blight on birds, pecking increases in addition to other possible factors that interact generating cannibalism.

Yet line C showed no signs of cannibalism. Therefore, as proposed by Mullens et al. (2010) “the selection of more docile lines which can coexist without the need of debeaking” could be beneficial to farmers by reducing labor costs and treatment with acaricides. In conclusion, the presence of *Ornithonyssus* spp. was determined in the hens and roosters in the three genetic lines on the farm studied, where *O. sylviarum* was the most frequently found, with a percentage of 89.8%.

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