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*in memoriam

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Occurrence and Epidemiology of *Fannia* Spp. (Diptera: Fanniidae) in Laying Poultry Farms in State of Minas Gerais, Brazil

ABSTRACT

Aspects related to epidemiology of flies belonging to *Fannia* genus were studied in order to determine predisposing factors for its occurrence in layer hens houses of commercial farms in the state of Minas Gerais, Brazil. An observational, analytical and sectional survey was conducted to verify major epidemiological aspects associated to infestations. This research was accomplished starting from an adapted secondary database of and structured with information on the presence and absence of ectoparasites and arthropods, characteristics of chicken houses, the poultry, type of facilities, geographical location and the management. The study was conducted in four hundred and thirty one houses located in forty three farms visited in 2012 year in the state of Minas Gerais. Ten farms (23.25%) and sixty eight houses (15,77%) were observed the presence diptera belonging to *Fannia* genus. The specie *Fannia admirabilis* was found in four farms (9,30%), *Fannia albitarsis* in two farms (4,65%) and only in one farm (2.32%) was observed the presence of *Fannia canicularis*. For three farms 6,98% was no possible to identify the species only *Fannia* genus. The epidemiological aspects for the occurrence of these flies were investigated using Poisson regression models. Characteristics such as farm, city of farm, presence of Histeridae beetles, type of facility (cage, on the floor with or without bedding) and the existence of mechanical removal of manure were associated with the occurrence of *Fannia* spp.

INTRODUCTION

In traditional egg production systems, laying hens are housed in high densities, leading to accumulation of manure under the cages, making it an excellent substrate for the development of synanthropic flies (Lopes *et al.*, 2008). Environments modified by humans are associated with the presence synanthropic diptera. Flies utilize feed residues, dead birds, broken eggs, and accumulated excreta for growth and survival (Nuorteva, 1963; Peck & Anderson, 1970; Prado, 2003). Muscoid diptera are considered important vectors of human and animal pathogens due their eating and reproductive habits and synanthropic behavior. Moreover, those arthropods are capable of moving and flying for long distances (Barreiro *et al.*, 2013; Blaak *et al.*, 2014).

Axtell (1999) describes that, among the flies of the *Fannia* genus, the most widespread species is *Fannia canicularis*, which may be a major pest in some regions. The biological cycle from egg to the adult stage of this fly requires 15-30 days, which is longer than that of the *Musca domestica*. *Fannia* genus larvae are frequently found in the manure together with other fly larvae. This species often flies slowly in circles inside poultry houses, are able to disperse to neighboring farms, and may harbor human and avian disease microorganisms. A few other species of *Fannia* with very similar appearance and behavior



as *F. canicularis* are found in chicken houses in some regions and are most readily differentiated by details of larval spine arrangement and type.

Muscoid flies, especially those belonging to the Muscidae, Fanniidae and Anthomyiidae families, are among the insects most commonly associated with human and animal production environments (Carvalho *et al.*, 2002). Many fly species associated with manure in egg-laying farms have been previously described (Axtell & Arends, 1990), because the accumulation of waste under the laying cages provides a favorable environment for the development of diptera of the *Fannia* genus. In Brazil, *F. canicularis* was detected in poultry farms in the municipality of Pelotas, state of Rio Grande do Sul (Bicho *et al.*, 2004) and the species *F. trimaculata* and *F. pusio* were described in municipalities of the state of São Paulo (Bruno *et al.*, 1993). In the state of Minas Gerais, Lomônaco & Prado (1994) captured some diptera species, including *F. trimaculata* and *F. pusio*, in a layer farm of the municipality of Uberlândia. There are few studies on the biology, ecology, and predisposing factors for the presence of these arthropods in layer farms, as well as on the factors related to their infestation epidemiology. Most studies on flies in Brazilian poultry farms evaluate species such as *M. domestica* and *Chrysomya* spp. (Lopes *et al.*, 2007). Lopes *et al.* (2008) verified the presence of other fly species in a layer farm in the state of São Paulo, including *Drosophila repleta*, *M. domestica*, *Ophyra* spp., *Hermetria illucens*, *F. canicularis*, *Chrysomya megacephala*, as well as Sepsidae species.

Considering the few studies on the occurrence of *Fannia* spp. in layer farms and the economic importance egg production in Brazil, this study aimed at investigating the occurrence of *Fannia* spp. in layer farms in the state of Minas Gerais and analyzing the epidemiological aspects and factors that may favor the presence of these flies.

MATERIAL AND METHODS

This survey was performed using the secondary databases of Cunha (2013) and Rezende (2014), which were adapted to include information on ectoparasites and arthropods, layer house characteristics, chicken, the type of facilities, geographic location, and management practices. The study was conducted in commercial laying chicken farms in the state of Minas Gerais, which is located in the southeastern region of Brazil and has an area of 586,519.727 km², characterized by the large range of climatic conditions.

A number of 431 poultry houses belonging to 43 layer farms were visited between March to June 2012. House relative humidity and temperature were measured during the visits. Considering that climatic variations may influence the occurrence of ectoparasites, farms located in areas which environmental temperatures may be below 5°C or above 40°C were not visited.

The number of layer farms in each region of the state was determined using the georeferencing data of the Agricultural Institute of Minas Gerais (IMA) (Figure 1). The ratio between the numbers of layer farms registered in each IMA administrative region and the total number of commercial layer farms in the state of Minas Gerais were used to stratify the sample. The map showing the evaluated regions was previously published by Cunha (2013). The number of layer houses per farm ranged from 1 to 40, with an average of 10 (standard deviation = 5.49). In 41 farms, layers were housed in cage systems. Out of the 431 houses surveyed, 29 were used for rearing replacement pullets. Sample size was calculated as a conglomerate (*i.e.* type of cluster) of active poultry houses, considering 50% hypothetical prevalence of arthropod pests or ectoparasites, 5% standard error, and a finite number of farms.

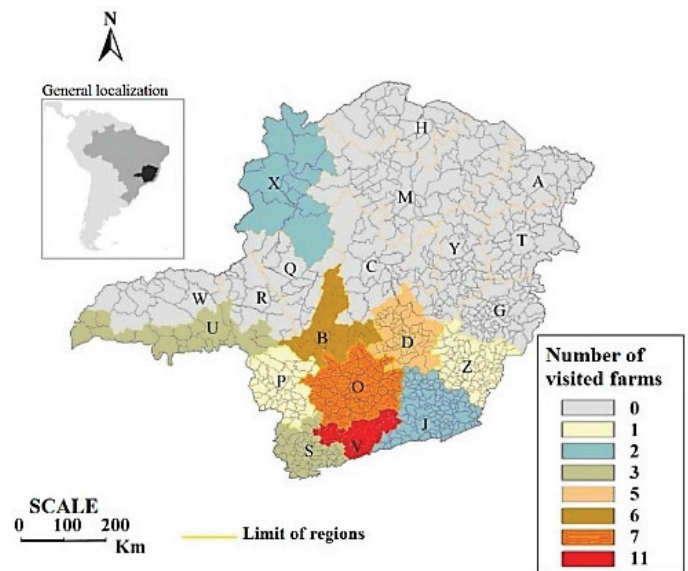


Figure 1 – Map with the number of farms visited in regions according the Agricultural Institute of Minas Gerais (IMA), Minas Gerais, Brasil. A- Almenara; B- Bambuí; C- Curvelo; D- Belo Horizonte; G- Governador Valadares; H- Janaúba; J- Juiz de Fora; M- Montes Claros; O- Oliveira; P- Passos; Q- Patos de Minas; R- Patrocínio; S- Pouso Alegre; T- Teófilo Otoni; U- Uberaba; V- Varginha; W- Uberlândia; X- Unai; Z- Viçosa.

Simultaneously, different risk and protective factors for the occurrence (presence or absence) of *Fannia* spp. infestation were evaluated using Poisson regression models and considering layer houses as the sampling unit (Dohoo *et al.*, 2003). The intensity



of *Fannia* spp. infestation in the layer house or in the poultry was not measured. The following variables were evaluated: geographic location (region, municipality), farm type (egg production, replacement pullet rearing, broiler production), production system (cages, on the floor with or without litter), number of birds per house (n), housing density (birds/m²), biological parasite control (absence or presence), manure removal (presence or absence), and the presence Diptera predatory arthropods (Coleoptera as *Hololepta* spp., *Euspilotos* spp, *Carcinops* spp., *Dermestes ater* and Histeridae beetles). The Poisson regression models were built using the software Stata® 12.0 (Dohoo *et al.*, 2003).

Fly samples were collected during the inspection of the poultry house with the aid of open plastic bags containing an attractive insecticide. Bags were sealed and submitted to the Ectoparasitosis Laboratory of the School of Veterinary Medicine of the Federal University Minas Gerais, where they were stored at -20 ± 2 °C until processing.

The adult specimens of *Fannia* spp. and of other arthropods were examined under a stereoscopic microscope (Olympus, Japan) and identified according to their morphological characteristics utilizing identification keys (Mcalpine *et al.*, 1987; Carvalho, *et al.*, 2002; Bouchard, *et al.*, 2005; Wendt & Carvalho, 2009; Bouchard *et al.*, 2011; Triplehorn & Norman, 2011).

The variables included in Poisson regression models were first selected using the Chi-Square test, Fisher's Exact test or Univariate Logistic Regression for removal of confounding variables and those with significance higher than 15% ($p > 0.15$). The highest coefficients of determination (R²) and the analysis of the Incidence Rate Ratios (IRR) confidence intervals (95% significance - 95% CI) were used to select the variables to be included in the models. Variables with IRR values lower than 1.0 are considered protection factors and those with values higher than 1.0 are risk factors. Variables with significant IRR ($p \leq 0.005$) or which removal would disrupt the general model were included. The goodness of fit of the Poisson regression model was verified by the Pearson's Chi-Square test.

RESULTS AND DISCUSSION

The presence of flies of the *Fannia* genus in 10 (23.25%) poultry farms, and the species *F. admirabilis*, *F. albitarsis*, and *F. canicularis* were identified (Table 1). However, *Fannia* species were not detected in three

farms (6.98%). These results indicate different *Fannia* species were present in laying poultry farms and are relevant when considering the health aspects of egg production. Other studies have also reported the presence of *Fannia* spp. in poultry facilities (Povolny, 1971; Lopes *et al.*, 2007). Lomônaco & Prado (1994), in a survey conducted in poultry farms in Uberlândia, Minas Gerais, verified the presence of *F. pusio* and *F. trimaculata*. In addition to these species, *F. canicularis* also was found in poultry farms in the state of São Paulo (Bruno *et al.*, 1993). Monteiro & Prado (2000) also reported the presence of *F. pusio* in farms located in São Paulo. In the United States, *F. canicularis* and *F. femoralis* were associated with poultry environments (Anderson & Poorbaugh, 1964; Axtell & Arends, 1990) and in Argentina, *F. albitarsis* was associated with poultry production environments (Perotti 1998).

Table 1 – Frequency of observations of *Fannia* spp. in commercial layer flocks in state of Minas Gerais, Brazil, 2012.

Flies	Number of positive houses	Frequency
<i>Fannia</i> spp.	68	15.77%
<i>Fannia admirabilis</i>	40	9.30%
<i>Fannia albitarsis</i>	20	4.65%
<i>Fannia canicularis</i>	10	2.32%

Table 2 shows the variables selected during screening of epidemiological determinants of the occurrence of *Fannia* spp. flies in poultry farms of Minas Gerais. The region variable was removed from the final model because the preliminary multivariate statistical

Table 2 – Variables potentially associated with the occurrence by *Fannia* spp. selected by univariate statistical tests ($p < 0.15$).

Characteristic	p value
Region	0.000*
Municipality	0.000#
Farm	0.000#
Production system (Cage, on the floor with or without bedding)	0.042*
Number of birds per house	0.027#
Housing density (number of chickens per cage)	0.002*
House type (laying hens, replacement pullets or broiler)	0.180*
Use of selective insecticides for fly control	0.000*
Presence of biological control	0.000*
Use of mechanical removal of manure	0.008*
Presence of <i>Hololepta</i> spp.	0.013*
Presence of <i>Euspilotos</i> spp.	0.113*
Presence of <i>Carcinops</i> spp.	0.008*
Presence of <i>Dermestes ater</i>	0.111*
Presence of Histeridae beetles	0.000*
Presence of <i>Chrysomya putoria</i>	0.002*
Presence of Uropodidae mites in manure	0.000*

*Chi-square test/ Fisher's Exact test.

#Univariate logistic regression.



models showed it was not significant ($p=0.377$), which suggests that the distribution of poultry farms in different regions of the state of Minas Gerais is not a determining factor for the occurrence of infestations of these flies. On the other hand, the municipality where the farms are located ($p=0.000$) significantly influenced the occurrence of *Fannia* spp., and was included in the final model, indicating higher risk of infestation in some municipalities than in others (Table 3). The preliminary analysis showed the variable poultry farm significantly influenced *Fannia* spp. occurrence and was maintained in the final model (Table 3). Poultry farm was considered as a protective factor, with an IRR of 0.86, indicating that some farms presented infestation risk probably due poultry farm specificities, such as management practices, manure removal, geographic location and application of ectoparasite control. Poisson regression models were built considering the clustering relationship between region, farm, and house, but they did not show appropriate fit. Therefore, it was not possible to evaluate with more details the influence of geographical distribution on the epidemiology of flies of the *Fannia* genus.

Table 3 – Variables associated with the occurrence of *Fannia* spp. in commercial layer flocks in state of Minas Gerais, Brazil, 2012.

Characteristic	Incidence Rate Ratio	Confidence Interval (95%)		p Value
City	1.38	1.26	1.51	0.000
Farm	0.86	0.80	0.91	0.000
Presence of Histeridae beetles	0.016	0.0024	0.107	0.000
Type of establishment	0.43	0.22	0.83	0.012
Use of mechanical removal of manure	0.26	0.11	0.62	0.002

Number of valid observations: 431.

p-value of Pearson Qui-Square test for goodness of fit: 1.000.

Pseudo R² (pseudo coefficient of determination): 0.2989.

Farm type ($p=0.992$) and housing density ($p=0.652$) were not significant in the preliminary analysis and were removed from the final model. The confidence interval of the Incidence Rate Ratio obtained for the total number of birds per house (IRR = 0.99; 95% CI = 0.99992 to 0.99993; $p=0.012$) allowed its classification as a protective factor. However, this result is not biologically plausible, as a higher number of hens increases the amount of manure produced, which consequently increases possibility of attraction and development of flies. As the number of hens per farm was expected to be a risk and not a protective factor, it was considered as confounding variable and was excluded from the statistical models.

The use of selective insecticides was removed from the final model due to the lack of significance ($p=0.994$) in the preliminary analysis. The application of biological control ($p=0.993$) and the presence of arthropods, such as *Hololepta* spp. ($p=1.000$), *Euspilotus* spp. ($p=0.999$) and *Carcinops* spp. ($p=0.998$), that could be involved in this process were not significant in the preliminary regression models and were removed from the final model. However, when the presence of these beetles was analyzed as a group (Histeridae family), it showed to be a significant ($p=0.000$) determinant for the occurrence of *Fannia* spp. Therefore, the presence of Histeridae beetles is a protective factor against the infestation by these flies (Table 3), that is, in houses where the presence of these beetles was detected, *Fannia* spp. occurrence was about of 62.5 times smaller. This may be explained by the possible predation of *Fannia* spp. eggs and larvae by Histeridae beetles in the manure, as reported for other fly species, such as *M. domestica* and *Chrysomya* spp. (Hulley & Pfeleiderer, 1988; Lopes *et al.*, 2007; Pinto & Ribeiro, 2011).

The presence of Uropodidae mites (IRR = 2.65; 95% CI = 1.57 to 4.45; $p=0.000$) was wrongly characterized as a risk factor for the occurrence of *Fannia* spp. due to values of the confidence interval presented. However, an IRR with a confidence interval lower than one (protection factor) was expected as species of this family of mites are often reported as predators of dipteran eggs and larvae (Axtell & Arends, 1990; Al-Dulaimi, 2002; Rodrigueiro & Prado, 2004). Therefore, the presence of Uropodidae mites was considered as a confounding variable, which resulted in its exclusion of the final regression model. The variables presence of the beetle *Dermestes ater* ($p=0.798$) and of the diptera *C. putoria* ($p=0.284$) were not significant in the preliminary Poisson regression analysis and were removed from the general model.

Manure removal was considered as a protective factor against infestations by *Fannia* genus (Table 3), indicating the risk of occurrence of *Fannia* spp. was 3,8 time smaller when this practice was applied. Manure removal dramatically and immediately reduces the volume of substrate for fly eggs, larvae, and adult development, disrupting fly biological cycle. Peck & Anderson (1970) also reported that manure availability is an important factor for the occurrence of flies in poultry establishments.

Preliminary analysis showed significant influence of house type (laying hens, replacement pullets; $p=0.180$) was and remained in the final regression model (Table 3), which indicated that replacement pullet houses had about 2.3 times lower risk of being infested with *Fannia* spp. than layer houses. Differences among facilities (farms with manual egg collection, farms with



automated egg collection, replacement pullets) and management practices may account for these results.

CONCLUSIONS

The results show that flies of the genus *Fannia* spp. frequently infest layer farms in the state of Minas Gerais. House type, municipality where farms are located, and poultry farm significantly influence the epidemiology of infestations. The presence of Histeridae beetles in the manure and manure removal are protective factors against the occurrence of *Fannia* species. Therefore, adequate monitoring and manure management, as well as the possible predation by Histeridae beetles may aid the control of *Fannia* spp. infestations in poultry farms.

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