

EPIDEMIOLOGICAL DETERMINANTS OF *Chrysomya* (Diptera: Calliphoridae) INFESTATION IN LAYER FARMS OF MINAS GERAIS, BRAZIL

DETERMINANTES EPIDEMIOLÓGICOS DA INFESTAÇÃO POR *Chrysomya* (Diptera: Calliphoridae) EM GRANJAS AVÍCOLAS DE POSTURA DE MINAS GERAIS, BRASIL

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ABSTRACT: Aspects related to the epidemiology of Diptera belonging to genus *Chrysomya* were studied in order to determine predisposing factors for their occurrence in commercial laying hens farms from the state of Minas Gerais, Brazil. An observational, analytical and sectional survey was conducted to verify the main epidemiological determinants of infestations by *Chrysomya* spp. Forty-three farms were visited in 2012 and these 13 (30,23%) were infested by *Chrysomya putoria*, 4 (9,30%) by *Chrysomya megacephala* and 2 (4,65%) by *Chrysomya albiceps* and in 2 farms (4,65%) was not possible to identify the species of *Chrysomya*. The epidemiological determinants for the occurrence of infestations by *Chrysomya* spp. were investigated using multiple logistic regression models. Housing clustering type and its conditions were important factors involved in epidemiology of these species (OR=5.05, 95% CI = 2.66 to 9.58, p = 0.000). It was also observed that the increase of the humidity of manure leads to increase chances of infestations (OR = 1.81, 95% CI = 1.36 to 2.40, p = 0.000). The presence of the beetle “lesser mealworm” (*Alphitobius diaperinus*) (OR = 0.15, 95% CI = 0.067 to 0.36, p = 0.000) was characterized as a protective factor against infestations, probably due to predation behavior of the beetle. Some regions of the state were characterized as lower risk areas for *Chrysomya* spp. infestation as compared to other regions. Management measures, such as constant monitoring of water leaks on manure, adequate composting of dead poultry and periodic removal of manure could assist in decreasing the presence of *Chrysomya* spp. inside the laying houses.

KEYWORDS: *Chrysomya putoria*. *Chrysomya megacephala*. *Chrysomya albiceps*. Laying hen. Poultry manure management. Epidemiological determinants.

INTRODUCTION

The characteristics of systems of laying hens breeding in Brazilian poultry farms lead to accumulation of manure, which is an excellent substrate for the development of synanthropic flies (LOPES et al., 2008). Flies use the food debris, dead animals, broken eggs and accumulated manure to survive (NUORTEVA, 1963; PECK; ANDERSON, 1970; PRADO, 2003). Muscoid diptera are considered important vectors of pathogens for humans and other animals, due their eating, reproductive and synanthropic behavior. Moreover, those arthropods have ability to movement and fly long distances (BARREIRO et al., 2013; BLAAK et al., 2014).

Flies belonging to the genus *Chrysomya* (Diptera: Calliphoridae) (Robineau-Desvoidy, 1830) are important diptera for human and other animal health, since they have potential to carry several pathogens (YANG, et al. 2019). The species *Chrysomya megacephala* (Fabricius, 1794), known as ‘oriental latrine blowfly’ and *Chrysomya putoria* (Wiedemann, 1830), also known as ‘african latrine blowfly’, have been reported in environments associated with poultry production in Brazil (MONTEIRO; PRADO, 2000; LOPES et al., 2007).

There are few studies about the biology, ecology and predisposing factors of these arthropods in laying hens farms and of factors related to the epidemiology of infestations. Borges (2006) reported that the presence of synanthropic flies causes health problems for chickens and possible

economic damages to the poultry farms. Then, it is important to invest in epidemiological studies that can evaluate the predisposing factors for the presence of synanthropic flies in poultry systems. These studies may assist the implementation of measures to control more efficiently the infestations of flies in laying hens farms. Therefore, this study aimed to determine the factors involved in the epidemiology of infestations by flies of the genus *Chrysomya* in poultry farms of the state of Minas Gerais, Brazil.

MATERIAL AND METHODS

Geographic area

The study was conducted in commercial layer farms in the state of Minas Gerais, Brazil. From March to June 2012, 431 layer chicken houses were visited in 43 farms. The selection of farms and stratification of the samples were performed considering the proportion of commercial laying poultry farms in the 20 administrative regions of Instituto Mineiro de Agropecuária (IMA). A map with these regions was showed previously by Rezende et al. (2015) and Rezende et al. (2018). The state of Minas Gerais is located in the southeastern region of Brazil and has an area of 586.521,121 km², characterized by the large range of climatic conditions (IBGE, 2019).

Sampling

The inspection of poultry facilities was performed, and samples of manure, flies and other arthropods were collected. Samples were packed in airtight plastic bags, refrigerated and routed to the Ectoparasitosis Laboratory of Veterinary Medicine School of the Federal University of Minas Gerais (UFMG), where they were stored at -20 ± 2 °C until processing. Morphological characteristics were determined in a stereoscopic microscope (Olympus, Japan). Species of the genus *Chrysomya* were identified as indicated previously (McALPINE et al., 1987; CARVALHO; RIBEIRO, 2000; GUIMARÃES et al., 2001; AMAT et al., 2008; TRIPLEHORN et al., 2011; RAFAEL et al., 2012).

Questionnaire

During visits to poultry establishments, a questionnaire was applied in order to obtain information related to predisposing factors for the occurrence of flies. A preliminary version of the questionnaire, containing 97 alternatives of objective and categorical questions was prepared and submitted to a test-retest validation procedure, using kappa test. Questions with reliabilities ranging

from regular to excellent (kappa greater than 0.4, $p \leq 0.05$) or with unanimous answers were kept (LANDIS; KOCH, 1977). The final version of the questionnaire with 89 alternatives was applied and presented by Cunha (2013) and Rezende (2014). This study was approved by the ethics committees of the UFMG (COEP/UFMG Ethic Number 0238.0.203.000-11 and CETEA/UFMG 41/2011).

Statistical analysis

The simultaneous evaluation of different risk factors for the occurrence of infestations by *Chrysomya* spp. was performed using logistic regression models, as demonstrated by Dohoo et al. (2003). These models were constructed using the software Stata ® 12.0. The preliminary selection of variables was performed using the Chi-square test, Fisher's exact test and univariate logistic regression and the confounding variables and those with significance less than 15% ($p > 0.15$) were removed. Variables selected by univariate statistical tests were used in multivariate logistic regression models with significant ($p \leq 0.05$). The characteristics with suspect hierarchical or clustering relation effect had the values of intra-class correlation calculated. Variables with value equal or higher than 0,40 were then subdivided into dichotomous variables (dummy variables). The selection of the highest R² values determination coefficients and the analysis of the Odds Ratios confidence intervals (95% significance) were also used for variable selection and construction of the models. Variables with significant Odds Ratio ($p \leq 0.05$) or whose removal would disrupt the general model were kept. The verification of model fitness was performed using the Wald's test ($p \leq 0.05$) and the Hosmer-Lemeshow's Test ($p > 0.05$). The check of the area under curve - ROC (Receiver Operating Characteristic) and the value of the pseudo-coefficient of determination were also considered for choosing the logistic model.

RESULTS

The flies of the genus *Chrysomya* were the most frequent, and their presence was verified in 160 laying houses of 21 farms (48.83%). The most abundant specie was *C. putoria*, which was found in 30.23% of farms, followed by *C. megacephala*, which occurred in almost 10% of the farms. *Chrysomya albiceps* (Wiedemann, 1819) was the most uncommon specie of this genus, and its presence was verified only in two farms (4.65%).

Table 1 show selected characteristics in screening tests performed using the Chi-square,

Fisher's exact test and univariate logistic regression. The regions where are located each poultry farm, which was considered a priori as a variable hierarchically associated with the occurrence of *Chrysomya* spp., presented high values of intra-class correlation (73.76%). Thus, this variable was decomposed (creating dummy variables) and these areas were evaluated individually.

Preliminary logistic models were built for selection of variables. The use of mechanical

removal of manure ($p = 0.084$), the type of establishment ($p = 0.201$), the use of selective insecticides ($p = 0.267$), the existence of biological control ($p = 0.880$), the presence of beetles of the genus *Dichotomius* ($p = 0.380$), the occurrence of Uropodidae mites in manure of the layer chicken houses ($p = 0.399$) and the interval of manure removal ($p = 0.312$) lost significance and were removed from the general model.

Table 1. Characteristics potentially associated with the occurrence of infestations by *Chrysomya* spp. selected by univariate statistical tests ($p < 0.15$) in commercial laying poultry flocks of the state of Minas Gerais, Brazil, 2012.

Characteristic	p value
Region	0.000
Chicken house cluster	0.000
Type of establishment (Laying with mechanical removal or with manual removal, Replacement pullets or meat poultry)	0.000
Removal of manure	0.000
Humidity of manure	0.000
Manure removal interval	0.000
Number of chickens by cage (Density of hens)	0.000
Use of selective insecticides to flies combat	0.000
Mechanical removal of manure	0.000
Existence of biological control	0.000
Fumigation of the layer chicken houses with chemicals substances	0.065
Presence of <i>Alphitobius diaperinus</i>	0.097
Presence of <i>Dermestes ater</i>	0.000
Presence of <i>Hololepta</i> spp.	0.128
Presence of <i>Euspilotus</i> spp.	0.064
Presence of <i>Dichotomius</i> spp.	0.000
Presence of Uropodidae mites in manure	0.026

The use of fumigation of the layer chicken houses with chemicals substances was removed from the model due to occurrence of collinearity. The presence of beetles of the genus *Euspilotus* (Coleoptera: Histeridae) (Lewis 1907) (OR = 1.07×10^9 , 95% CI = 1.74×10^8 to 6.57×10^9 , $p = 0.000$) and *Hololepta* (Coleoptera: Histeridae) (Paykull, 1811) (OR = 3.31×10^7 , 95% CI = 6.10×10^5 to 1.85×10^9 , $p = 0.000$) and the number of poultry per cage (OR = 0.31, 95% CI = 0.12 to 0.77, $p = 0.012$) were considered confounders variables and were removed from the model. The presence of beetles

Dermestes ater (Coleoptera: Dermestidae) (De Geer, 1774) was removed due to non-occurrence of this species in layer chicken houses where was found *Chrysomya* spp.

Table 2 show the factors associated with the occurrence of *Chrysomya* spp. in the state of Minas Gerais. The regions of Juiz de Fora, Varginha and Viçosa were removed from the model due to non-verification of the presence of these flies in poultry farms of these regions. The regional of Unaí was removed due to occurrence of perfect success of the model, which indicates that in this region every

layer chicken houses and farms presented *Chrysomya* spp. infestations.

Table 2. Characteristics associated with the occurrence of *Chrysomya* spp. in commercial laying poultry flocks in state of Minas Gerais, Brazil, 2012.

Characteristic	Odds Ratio	Confidence Interval (95%)		p value
Chicken house cluster	5.05	2.66	9.58	0.000
Humidity of manure	1.81	1.36	2.40	0.000
Presence of <i>A. diaperinus</i>	0.15	0.067	0.36	0.000
Region of de Belo Horizonte	0.12	0.037	0.42	0.001
Region of Oliveira	0.06	0.020	0.17	0.000
Region of Passos	0.17	0.039	0.78	0.022
Region of Pouso Alegre	0.40	0.12	1.28	0.124
Region of Uberaba	0.07	0.022	0.26	0.000

Number of valid observations: 262; Region reference for the elaboration of Odds Ratio: Bambuí; P value for goodness of fit of the model in the Wald test: <0.001; P value for goodness of fit of the model in the Hosmer-Lemeshow test: 0.5758; Model sensitivity (Probability +I Infested): 78.67%; Model specificity (Probability -I Non-Infested): 84.82%; Area under the curve of the Receiver Operating Characteristics (ROC-Curve Receiver Operating Characteristic): 88.25%; Pseudo R² (pseudo coefficient of determination): 0.3732

DISCUSSION

The occurrence of *Chrysomya* spp. in laying hens farms in Minas Gerais is similar to other studies conducted in Brazil. LOPES et al. (2007) noted the presence of *C. megacephala* in a poultry farm located in the state of São Paulo. AVANCINI; SILVEIRA (2000) conducted a study about flies in poultry establishments in southeastern of Brazil and found *C. putoria* and *C. megacephala*. MONTEIRO; PRADO (2000), also conducted studies in laying farms in the state of São Paulo and verified the presence of *C. putoria*. BORGES (2006), in a study conducted in a poultry farm located in Minas Gerais, observed that *C. putoria* was the most common specie of synanthropic fly.

Some variables related to manure management lost significance and were removed of model, as the mechanical removal of manure ($p = 0.084$) and the interval of manure removal ($p = 0.312$). The periodic removal of manure also was excluded from the model due to occurrence of perfect failure. However, these characteristics should be reevaluated in further studies, since the removal of manure reduces the amount of substrate available for the development of flies. In addition, it is important to emphasize that there are two strands of manure management strategies in posture aviaries in Brazil. The first is to remove these wastes in a period lesser than three days, avoiding the changing of the larvae into pupae. The other is to remove after

a very long period, reducing the humidity of the manure and allowing the growth of predators, keeping the infestations in low levels. Both, if correctly used, give satisfactory results, but also may fail depending on other factors such as the presence of humidity or removal inefficiency.

The type of establishment (laying hens farm with manual removal, laying hens farm with mechanical removal and replacement chickens) also lost significance, highlighting the influence of non-automatic removal on the occurrence of *Chrysomya* spp. The use of selective insecticides, the fumigation of the facilities and the verification of biological control also not remained in the general model. These results suggest that other factors are more important for the occurrence of these flies. There is also the possibility of improper application of pesticides, what would lead to inadequate control of infestations.

The variables presence of beetles *Dichotomius* spp. (Coleoptera: Scarabaeidae) (Hope, 1838), *Dermestes ater*, *Euspilotus* spp. and *Hololepta* spp. not remained in the logistic model. Due to the values of the confidence intervals of their respective odds ratios, therefore the presence of beetles of the genus *Euspilotus* (OR= 1.07×10^9 ; 95% CI= 1.74×10^8 to 6.57×10^9 , $p = 0.000$) and of the genus *Hololepta* (OR= 3.31×10^7 ; 95% CI= 6.10×10^5 to 1.85×10^9 , $p = 0.000$) were classified wrongly as risk factors for the occurrence of *Chrysomya* spp. However, there are reports of the predation of eggs

of *C. putoria* by *E. modestus* (GIANIZELLA, 2000). Therefore, as predator, it is expected to behave as a protection factor against infestations and not as a risk factor. Then, the presence of beetles of these genus were considered confounders variables and were removed from the model. The presence of Uropodidae mites in manure ($p = 0.399$) lost significance. However, some authors report that these mites are predators of Diptera (AXTELL; ARENDS, 1990). The number of hens per cage (OR = 0.31, 95% CI = 0.12 to 0.77, $p = 0.012$) was characterized as a protective factor, what have not biological plausibility, leading to the exclusion of the logistic model.

Brazilian law defines as laying house a physics unit of poultry production composed of one or more sheds, which house a group of hens of the same species and age. The clusterings must have similar and appropriate management and should be isolated from other activities of poultry production through use of physical barriers (INSTRUÇÃO NORMATIVA n° 56 of 04 December, 2007). These variable (OR = 5.05, 95% CI = 2.66 to 9.58, $p = 0.000$) obtained an Odds Ratio with a confidence interval that indicates that in some clustering the chances of occurrence of infestations is almost 5 times higher than in other, even if it is located in the same farm. Thus, it is suggested that intrinsic characteristics of each chicken house clustering, which were not evaluated in this study, may influence the occurrence of infestations.

The humidity of poultry manure (OR = 1.81, 95% CI = 1.36 to 2.40, $p = 0.000$) was characterized as a predisposing factor for the occurrence of flies of the genus *Chrysomya*. Thus, the increase in moisture manure leads to increase in chances of infestations. The influence of humidity in populations of Diptera is also highlighted by Axtell; Arends (1990), who state that the parts of the laying houses where there are no water pipe leaks, the manure is drier and there are smaller populations of flies. Studies about other species of Diptera, such as *Musca domestica* (Diptera: Muscidae) (Linnaeus, 1758) have also shown that the aviaries with wetter manure are more appropriate to their development (BRUNO et al., 1993; STAFORD; BAY, 1987; LOPES et al., 2008). Oliveira et al. (2018) observed that the interval of removal of the waste, your humidity and the presence of other ectoparasites in shelters were associated with the high or moderate risk index for the presence of diptera.

The presence of the beetle *A. diaperinus* (Coleoptera: Tenebrionidae) (Panzer, 1797) (OR = 0.15, 95% CI = 0.067 to 0.36, $p = 0.000$), also known as 'Lesser Mealworm', was classified as a

protective factor against infestation. Thus, in the layer chicken houses where this beetle is found the chance of occurrence of *Chrysomya* spp. is 6.66 times lower. These results are probably due to the predator behavior of this beetle about Diptera's eggs and larvae. Santoro et al. (2010) suggest that *A. diaperinus* and *Carcinops troglodytes* (Coleoptera: Histeridae) (Paykull, 1811) are predators of Diptera such as *M. domestica* and *C. megacephala*. GIANIZELLA (2000) notes that adults of *Euspilotus modestus* (Coleoptera: Histeridae) (Erichson, 1834) consume, every day, approximately 26 eggs of *C. putoria*.

Some regions of the state of Minas Gerais were characterized as protective regions, which have less chance of to present infestations by *Chrysomya* spp. (Table 2). The regional of Unaí had perfect success of the model, which indicates that all laying houses of this region showed infestation by these flies, which led to exclusion of this regional of the model. These results strongly suggest that this region has a high risk of infestation. These differences among regions can be attributed, for example, to climate variations and differences among poultry establishments not evaluated in the study. As Pinto-Coelho (2000) stated, other factors may influence the occurrence of these arthropods, such as food availability, space, ecological relationships, genetic components, social interaction and dispersal of each species.

CONCLUSIONS

Flies of the genus *Chrysomya* are frequent in layer poultry farms in Minas Gerais, requiring specific sanitary management. Some differences between the laying houses and other variables that were not evaluated in this study, as relative humidity, temperature and precipitation, are important in the epidemiology of infestations by *Chrysomya* species and should be evaluated in future surveys. High levels of humidity of poultry manure increase the chances of infestations, and it is recommendable avoid excessive humidity of manure, checking leaks in water pipes and drinking fountains.

The presence of the beetle *A. diaperinus* is a protective factor against infestation by *Chrysomya* spp. Therefore, the application of pesticides in poultry farms should avoid the total elimination of populations of this beetle, once that the lesser mealworm is important in biological control of these flies.

Some regions of the state had lower chances of infestations by *Chrysomya* spp. when compared

to others regions. Some environmental, biological or management factors can evidence these results. Therefore, the study suggests the accomplishment of some management measures that could assist in decreasing the frequency of *Chrysomya* spp. in laying houses, such as constant monitoring of water leaks on manure, adequate composting of dead poultry and periodic removal of manure.

ACKNOWLEDGMENTS

The authors are grateful to Instituto Mineiro de Agropecuária (IMA) and farm owners for the visitation authorizations. We are indebted to CNPq, FAPEMIG and CAPES for financial support and scholarships.

RESUMO: Aspectos relacionados à epidemiologia das infestações por dípteros do gênero *Chrysomya* foram estudados com o objetivo de determinar os fatores predisponentes para sua ocorrência em granjas comerciais de postura do estado de Minas Gerais, Brasil. Foi realizado um estudo observacional, analítico e seccional para verificar os principais determinantes epidemiológicos das infestações por *Chrysomya* spp. Foram visitadas 43 granjas avícolas em 2012 e dessas 13 (30,23%) apresentaram infestação por *Chrysomya putoria*, 4 (9,30%) por *Chrysomya megacephala*, 2 (4,65%) por *Chrysomya albiceps* e em 2 granjas (4,65%) não foi possível identificar as espécies de *Chrysomya*. A verificação dos determinantes epidemiológicos foi realizada utilizando modelos de regressão logística múltipla. Diferenças entre os núcleos das granjas avícolas e que não foram avaliadas neste estudo, apresentaram-se importantes na epidemiologia destas moscas (OR=5.05 95% IC = 2.66 a 9.58, p = 0.000). Observou-se também que o aumento na umidade do esterco dos aviários aumenta as chances de ocorrência de infestações (OR = 1.81, 95% IC = 1.36 a 2.40, p = 0.000). A presença de coleópteros popularmente conhecidos como “Cascudinhos”, *Alphitobius diaperinus* (OR = 0.15, 95% IC = 0.067 a 0.36, p = 0.000), foi caracterizada como um fator protetor contra infestações, provavelmente devido ao comportamento predador deste coleóptero. Algumas regiões do estado apresentaram menores chances de ocorrência de infestações por *Chrysomya* spp. quando comparadas a outras regiões. Algumas medidas de manejo, como monitoramento constante de vazamentos de água no esterco, compostagem adequada de aves mortas e remoção periódica do esterco, poderiam auxiliar na diminuição da presença de *Chrysomya* spp. no interior dos galpões de postura.

PALAVRAS-CHAVE: *Chrysomya putoria*. *Chrysomya megacephala*. *Chrysomya albiceps*. Galinhas poedeiras. Manejo da cama de aviários. Determinantes epidemiológicos.

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