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Teixeira, Cristina Mara; de Oliveira, Paulo Roberto
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Epidemiology of *Megninia* spp. in laying flocks from the State of Minas Gerais, Brazil

Epidemiologia de *Megninia* spp. em estabelecimentos de postura no Estado de Minas Gerais, Brasil

Leandro do Carmo Rezende¹; Lucas Maciel Cunha^{1*}; Nelson Rodrigo da Silva Martins¹;
Cristina Mara Teixeira¹; Paulo Roberto de Oliveira¹

¹Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais – UFMG, Belo Horizonte, MG, Brazil

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Abstract

Aspects related to the epidemiology of *Megninia ginglymura* were studied, in order to determine predisposing factors for the occurrence in layer chicken houses of commercial farms in the State of Minas Gerais, Brazil. An observational, cross-sectional, analytical survey was conducted for identifying major risks or protection factors for infestations. Four hundred thirty-one houses located in forty-three farms were visited in 2012. Regarding the farms, 17 (39.53%) had chickens infested by *M. ginglymura* and 1 (2.32%) presented *Megninia cubitalis*. The epidemiological aspects of the occurrence of *M. ginglymura* were investigated using multiple logistic regression models. The chicken house organization was an important protection factor involved in the occurrence of *M. ginglymura* (odds ratio [OR] = 0.85). The presence of subsistence breedings of poultry within the premises or at the proximity of a farm was characterized as a risk factor for the occurrence of infestations (OR = 3.92). The use of mineral oil (OR = 0.16) was considered protective against new infestations. Some regions of the state were characterized by a higher risk for mite infestation than others.

Keywords: Acari, Analgidae, feather mites, *Megninia ginglymura*, *Megninia cubitalis*.

Resumo

Aspectos relacionados à epidemiologia de infestações pelo ácaro *Megninia ginglymura* foram estudados com o objetivo de determinar os principais fatores para a ocorrência desse ectoparasito em galinhas poedeiras de granjas comerciais no Estado de Minas Gerais, Brasil. Um estudo observacional, analítico e seccional foi realizado para verificar os principais fatores de risco ou proteção para as infestações. Quatrocentos e trinta e um galpões distribuídos em quarenta e três granjas foram visitados, no ano de 2012. Em relação às granjas, 17 (39,53%) possuíam aves infestadas por *M. ginglymura* e uma (2,32%) apresentou galinhas infestadas por *Megninia cubitalis*. Os aspectos epidemiológicos para a ocorrência de *M. ginglymura* foram investigados, utilizando-se modelos de regressão logística múltipla. A organização dos galpões nas granjas foi verificada como um importante fator de proteção envolvido na epidemiologia dessa espécie (Razão de Chances=0,85). A presença de criações de galinhas de subsistência dentro das instalações das granjas ou nas proximidades (Razão de Chances =3,92) foi caracterizada como um fator de risco para a ocorrência de infestações. O uso de óleo mineral foi considerado um fator de proteção contra infestações por *M. ginglymura*. Algumas regiões do Estado de Minas Gerais foram caracterizadas como de maior risco do que outras para infestações por esse ácaro.

Palavras-chave: Acari, Analgidae, ácaros plumícolas, *Megninia ginglymura*, *Megninia cubitalis*.

Introduction

Some species of arthropods of the subclass Acari are important ectoparasites of birds (PROCTOR, 2003). The major feather mites of domestic chickens belong to the family Analgidae, especially to the genus *Megninia* (Acari: Analgidae). The main morphological

characteristic of this genus is the presence of a handle-shaped elevation on the proximal segment of legs (GAUD et al., 1985). *Megninia ginglymura* (Mégnin, 1877) and *Megninia cubitalis* (Mégnin, 1877) are the most common species of this genus described in chickens in Brazil. The morphological differences between these species are based on the observation of epimera and adanal setae. *M. cubitalis* has Y-shaped epimera, while *M. ginglymura* shows unfused epimera. The adanal setae of *M. cubitalis* are aculeiform,

*Corresponding author: Lucas Maciel Cunha. Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais – UFMG, Campus Pampulha, Av. Antônio Carlos, 6627, CP 567, CEP 31270-901, Belo Horizonte, MG, Brasil.
e-mail: lucasmcunha82@gmail.com

while in *M. ginglymura* they are inflated and relatively bigger (AMARAL et al., 1975; TUCCI et al., 2005).

Mites of the genus *Megninia* live on the host, laying their eggs on the feathers (HERNÁNDEZ et al., 2007). Adults and juveniles live and feed on the feathers. Mites are grouped into body parts such as head, chest, back and wings. Mite-infested feather barbules become rarefied and the follicles become swollen and erythematous (REIS, 1939).

In an outbreak in a laying poultry farm in the State of São Paulo a reduction in egg laying of around 20% and the appearance of itchy lesions caused by the mite saliva were observed. Furthermore, it was verified that the serous fluid released dries to form crusts, providing the opportunity to secondary bacterial infection, which may cause pyoderma. A fungal contamination of lesions may also occur (TUCCI et al., 2005).

Recently, the mite *Allopsoroptoides galli* (Psoroptoididae: Pandalurinae) was described in laying hens in Brazil (MIRONOV, 2013). The hens parasitized by *A. galli* show intense itching, desquamation and a marked drop in egg production (approximately 30%) (TUCCI et al., 2014). The blood profile is negatively affected, and biochemical changes are also observed in infected hens (BUIM et al., 2013). Although little studied, the occurrence of feather mite infestations in commercial laying hens is a subject of extreme health and economic relevance to veterinary medicine. Further research is necessary in order to develop better strategies for prevention and control of these ectoparasites.

Most reports of *M. ginglymura* in Brazil have described its occurrence in subsistence poultry, and little or no attention has been dedicated to commercial poultry flocks (TUCCI et al., 2005; REZENDE et al., 2013). Therefore, this study aimed at investigating the occurrence and main epidemiological aspects of infestations in chickens of commercial layer flocks in the State of Minas Gerais, Brazil. Each flock was analyzed for the presence or absence of *M. ginglymura* in poultry, but their quantification was outside the scope of this study. The epidemiological aspects of *M. cubitalis* were not analyzed due to the low frequency of occurrence.

Materials and Methods

Geographic area

The study was conducted in commercial laying chicken farms in the State of Minas Gerais, Brazil. This state, located in the southeastern region of Brazil, has an area of 586,522.122 km² and is characterized by large range of climatic conditions. Regions in the north of the state have higher temperatures than those located in the south. Most of the regions have a high-altitude tropical climate (GARCIA et al., 2007).

During the period of visits to the farms, the temperature (12.4 to 39.1 °C) and relative humidity ($\geq 30\%$) were monitored in cities where the farms were located. This monitoring was done in order to determine whether climatic variations could affect the presence or absence of ectoparasites in flocks. Farms located in cities with temperatures below 5 °C or above 40 °C were not visited.

Sampling

The implementation of a national plan for poultry health in Brazil has resulted in compulsory periodic inspection and geo-referencing of commercial poultry flocks. Small-scale poultry housing for family consumption only are referred to as subsistence breedings and are not covered by the national plan. The geo-referencing data of Agricultural Institute of Minas Gerais (IMA) were used for determining the number of farms to be sampled in each region of the State (Figure 1).

The random selection of farms and stratification of the samples was performed considering the proportion of industrial laying poultry farms in each of the 20 IMA administrative regions of the state. Thus, from March to July 2012, 431 chicken houses were visited in 43 farms. The number of houses per farm ranged from 1 to 40 and the average number was 10 (Standard Deviation= 5.49). Forty-one farms kept poultry in cage systems and 29 were intended to breeding replacement pullets.

The individual examination of 20 chickens per house and poultry facility inspection for ectoparasites and other arthropods were performed, with mite and feather samples collected. Samples were packed in airtight plastic bags, transported refrigerated to the laboratory and stored at -20 ± 2 °C until processing.

Mite specimens were prepared in Hoyer's medium (KRANTZ, 1978) and mounted between glass slides and coverslips. Morphological characteristics were determined using an optical microscope (Olympus, Japan). Species of genus *Megninia* were identified as described previously (GAUD et al., 1985; TUCCI et al., 2005). Differential diagnosis of the feather mite *Allopsoroptoides galli* (Acari: Psoroptoididae), which was recently described in chickens of commercial farms in Brazil (MIRONOV, 2013), was performed.

Questionnaire

During visits to the poultry establishments, an adapted questionnaire was applied in order to obtain information related to predisposing factors for the occurrence of ectoparasites (CUNHA, 2013). A preliminary version of the questionnaire, containing a total of 97 possible answers to objective and categorical questions, was prepared and submitted to a test-retest validation procedure, using kappa coefficient. 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 contained a total of 89 possible answers. This study was approved by the ethics committees of the Federal University of Minas Gerais - 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 *M. ginglymura* was performed using logistic regression models, as described by Dohoo et al. (2003). These models were constructed using software Stata® 12.0 and considered the chicken house as the unit of statistical analysis (HOSMER & LEMESHOW, 2000). The farms were used as conglomerate of active poultry houses for sample size calculation considering a hypothetical prevalence of 50%, a standard error of

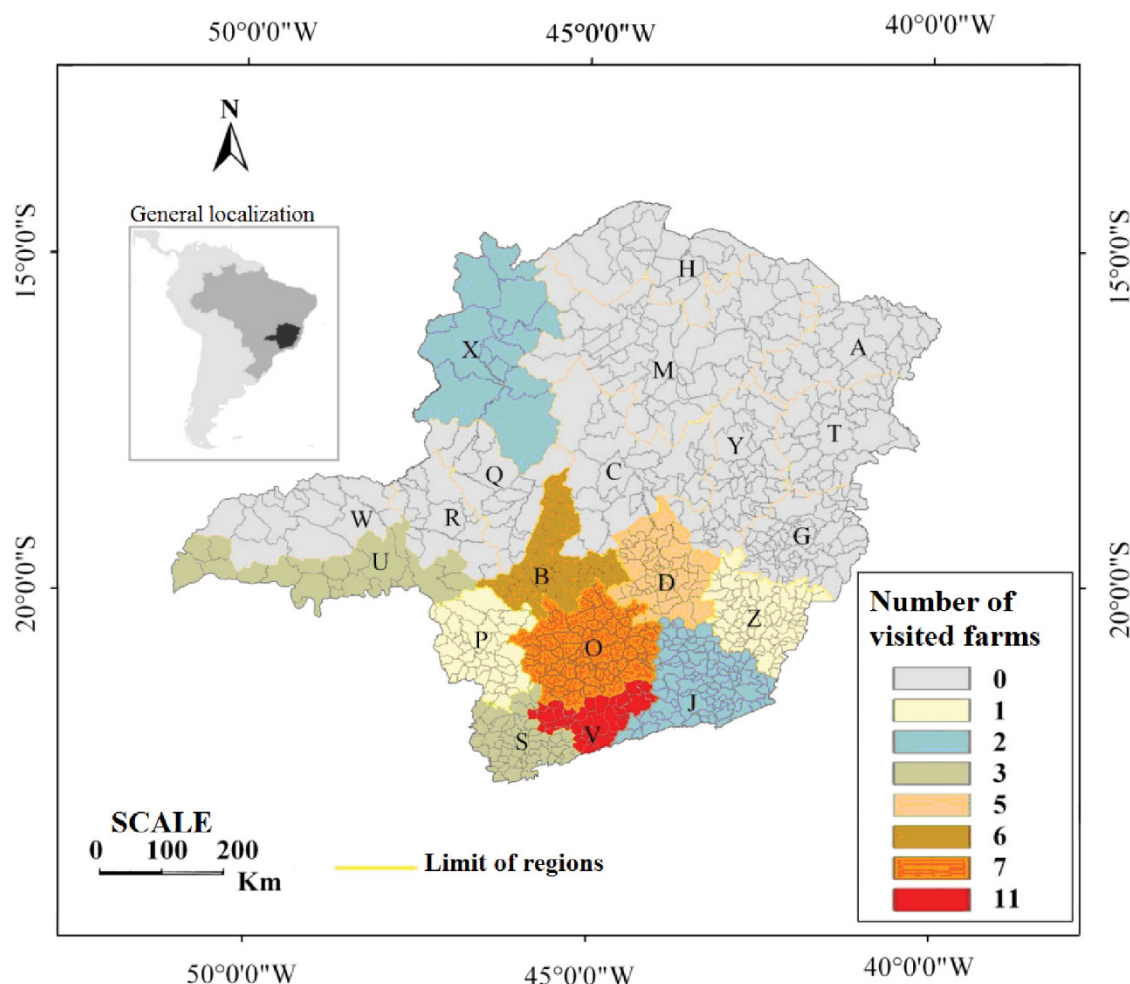


Figure 1. Map with the number of farms visited in regions according the Agricultural Institute of Minas Gerais (IMA), Minas Gerais, Brasil, 2012. A- Almenara; B- Bambuí; C- Curvelo; D- Belo Horizonte; G- Governador Valadares; H- Janaúba; J- Juiz de Fora; M- Montes Claros; O- Oliveira; P- Passos; Q- Patos de Minas; R- Patrocínio; S- Pouso Alegre; T- Teófilo Otoni; U- Uberaba; V- Varginha; W- Uberlândia; X- Unaí; Z- Viçosa.

5% and a finite number of farms. The quantification of the intensity of infestation in houses or on the hosts was not performed. Thus, only the verification of the presence or absence of *M. ginglymura* was performed in each house.

The preliminary selection of variables to be included in the logistic model was performed using the chi-square test, Fisher's exact test and univariate logistic regression, removing the variables with p-value less than 15% (DOHOO et al., 2003). Variables selected by univariate statistical tests were used in multivariate logistic regression models, except for confounding or not significant ($p \leq 0.05$). The characteristics with suspect hierarchical or clustering relation effect had the values of intra-class correlation (ICC) calculated. Variables with high values (≥ 0.40) were transformed in dummy variables. The analysis of odds ratios (OR) (95% significance) was also used for variable selection and construction of the models. Variables with significant OR ($p \leq 0.05$) or variables in which 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 test ($p > 0.05$). The area under the receiver operating characteristic (ROC) curve and the value of

the pseudo-coefficient of determination were also considered for choosing the logistic model (HOSMER & LEMESHOW, 2000).

Results

Eighteen (41.86%) farms and seventy-eight chicken houses (18.09%) showed infestations by mite of the genus *Megninia*. *M. ginglymura* was the most frequent species, being found in seventeen (39.53%) farms and in seventy-six houses (17.63%). *M. cubitalis* was found in one (2.32%) farm and in two houses (0.46%).

Table 1 presents the selected screening characteristics using the chi-square and Fisher's exact tests, and univariate logistic regression. The farm's geographic region, considered *a priori* a variable associated hierarchically with the occurrence of *M. ginglymura*, presented an ICC value of 60.56. Thus, this variable was decomposed (by creating dummy variables) to evaluate the regions separately. Chicken house organization within the farm was a significant factor in preliminary hierarchical logistic models ($p > 0.001$). Thus, this variable was allocated to a general logistic model. The

Table 1. Characteristics potentially associated with the occurrence of infestations by *Megninia ginglymura* selected by univariate statistical tests ($p < 0.15$).

Characteristic	p-value
Region*	0.000
Farm#	0.008
Chicken house organization*	0.024
Type of facilities*	0.055
Predominant chicken genetic lineage*	0.000
Number of chickens by cage*	0.095
Number of chickens by square meter*	0.095
Presence of rodents*	0.002
Presence of mice (<i>Mus musculus</i>)*	0.000
Presence of feral pigeons (<i>Columba livia</i>)*	0.000
Presence of subsistence chickens*	0.109
Presence of ruddy ground dove (<i>Columbina</i> sp.)*	0.000
Presence of cattle egret (<i>Bubulcus ibis</i>)*	0.102
Presence of chopi blackbirds (<i>Gnorimopsar chopi</i>)*	0.038
Presence of saffron finchs (<i>Sicalis flaveola</i>)*	0.016
Presence of other synanthropic birds*	0.000
Use of acaricides*	0.000
Biological control*	0.052
Use of mineral oil to control mites*	0.000
Use of silica to control mites*	0.000

* Pearson's Chi Square test/ Fisher's Exact Test. # Univariate Logistic regression.

predominant chicken genetic lineage ($p = 0.726$) and the differences among farms ($p = 0.944$) were not significant factors in preliminary models and were excluded from the general logistic model. The type of facilities ($p = 0.185$), number of chickens by cage ($p = 0.726$), number of chickens by square meter ($p = 0.590$), the presence of mice (*Mus musculus*) ($p = 0.696$) and other rodents ($p = 0.051$) were also excluded from the general logistic model for the same reason.

Regarding synanthropic birds, the only feature that remained in the general logistic model was the presence of subsistence domestic chickens ($p = 0.031$). The presence of saffron finch (*Sicalis flaveola*) ($p = 0.268$), cattle egret (*Bubulcus ibis*) ($p = 0.888$) and chopi blackbird (*Gnorimopsar chopi*) ($p = 0.992$) was excluded from the analysis due to lack of significance in preliminary models. The presence of ruddy ground dove (*Columbina* sp.), with OR equal to 0.14 (0.082 to 0.245, $p = 0.000$), and feral pigeons (*Columba livia*), with OR of 0.20 (0.11 to 0.36, $p = 0.043$), were not included in the general model due to $OR < 1$ ($OR = 0.131$). No association between the presence of wire mesh fencing for free-range birds and the occurrence of *M. ginglymura* was detected using univariate tests and multiple logistic regression models ($p = 0.770$).

Acaricide use did not show a significant value in logistic linear models ($p = 0.141$). Biological control ($p = 0.064$) or the use of silica ($p = 0.758$) also did not show significance for mite control in preliminary logistic models.

The characteristics associated with the occurrence of *M. ginglymura* in commercial laying poultry flocks in the State of Minas Gerais are presented in Table 2. The regions of Passos, Unai and Uberaba were excluded from the model. Logistic models were not used for analysis of *M. cubitalis* due to the low occurrence frequency.

Table 2. Characteristics associated with the occurrence of *Megninia ginglymura* in commercial laying poultry flocks in the State of Minas Gerais, Brazil (2012).

Characteristic	Odds ratio	Confidence interval (95%)		p-value
Chicken house organization	0.85	0.78	0.92	0.000
Presence of subsistence chickens	3.92	1.20	12.75	0.023
Use of mineral oil	0.16	0.036	0.71	0.016
Region of Belo Horizonte	40.19	4.29	376.28	0.001
Region of Juiz de Fora	10.55	0.49	223.09	0.130
Region of Oliveira	21.12	2.22	200.20	0.08
Region of Pouso Alegre	60.46	6.17	591.67	0.000
Region of Varginha	14.02	1.64	119.76	0.016
Region of Viçosa	1259.87	44.99	35280.43	0.000

Number of valid observations: 374; reference region for calculating odds ratios: Bambui; 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's test: 0.5129; model sensitivity (probability + infested): 27.63%; model specificity (probability - non-infested): 94.30%; area under the curve of the receiver operating characteristics (ROC): 83.94%.

Discussion

The vast majority of studies in Brazil characterize *Megninia* spp. as a parasite of little importance for the poultry industry due to low frequency of occurrence (REZENDE et al., 2013). However, the occurrence of such genus of mites, mainly *M. ginglymura*, was observed in 18.09% of commercial flocks studied, indicating relevant frequency. Mixed infestations of *M. ginglymura* and *M. cubitalis* were not observed in this study. These results are in disagreement to those of Tucci et al. (2005), who described mixed infestations by *M. cubitalis* and *M. ginglymura* in a farm in the State of São Paulo, Brazil. In other countries, the occurrence of these parasites in poultry varies, ranging from 2.6% in Israel (MUMCUOGLU & LUTSKY, 1990) to 89.6% in Cuba (HERNÁNDEZ et al., 2006).

The presence of synanthropic birds in nearby of houses was evaluated in order to assess the role as mechanical carriers of ectoparasites. Although the presence of avian species such as cattle egret and saffron finch did not appear as significant factors in preliminary models, the presences of ruddy ground dove, feral pigeons and other synanthropic birds were significant factors and showed OR lower than 1. The results suggest that there is a relationship between the presence of populations of *M. ginglymura* and the absence of birds in sheds. These results might result from the interference of confounding variables such as stricter biosecurity measures that were not approached in the study and that would reduce the presence of synanthropic birds in the farms.

The use of wire mesh fences to prevent the entry of birds in chicken houses was not associated with the absence of these mites in univariate statistical analysis. Wire mesh fencing was not

primarily added for contention of synanthropic birds, but was mainly used for containment of replacement pullets (pre-adult layers) in non-cage floor raised systems and for preventing the entry of other animals such as predators.

No association was observed between the use of acaricides and the occurrence of infestations by *M. ginglymura*. These results are possibly due to fact that these pesticides are used on the control of other species of mites. Thus, these products may have low efficacy to reduce infestations by *M. ginglymura* to undetectable levels.

In the logistic regression model for *M. ginglymura*, the spatial variable order of chicken house in farm was significant. The houses are usually numbered sequentially in increasing order in farms and those with lower numbers are located closer to the periphery or at the farm gatehouse. The results demonstrated that houses with higher order numbers have chances 1.17 times smaller to be infested by *M. ginglymura*. Probably, houses closest of the edges and entry of the farms, which have a lower order numbering, are more likely to be infested. The removed or centralized chicken houses, due the lower contact with the boundaries of farm, have less interaction with sources of contamination such as synanthropic hosts, avian with mechanic carrier function and fomites.

The presence of subsistence breedings of chickens at proximity increased the chances of infestations almost four times (Table 2). The proximity is determined when the subsistence chickens can keep contact with commercial poultry or when these breedings were kept inside or in neighborhood of commercial houses. These poultry are not of industrial lineages and are breeding for self-consumption at the farm and nearby. As generally known, the inexistence of health, preventive medicine and nutritional control in these chickens will favor the occurrence of parasitism and other diseases.

The use of mineral oil against ectoparasites was significant in logistic model and showed an OR lower than one. In sheds in which mineral oil was used, chances of infestations were approximately six times smaller. The use of mineral oil to combat insects, mites and other agricultural pests has been reported in the literature, including against hematophagous mites (GUIMARÃES & TUCCI, 1992). There is other reports of use of mineral oil against invertebrates such as *Dermanyssus gallinae* (Acari: Dermanyssidae) kept under laboratory conditions (MAURER et al., 2009). In the case of *D. gallinae*, there was a reduction of mite reproduction when mineral oil was used as control action (MAURER et al., 2009). Thus, hematophagous mite control would probably also reduce *M. ginglymura* population, due to inadequate reproduction conditions (GUIMARÃES & TUCCI, 1992).

The variable region had a high ICC value, which showed that there is a spatial distribution in the chances of occurrence of infestations. Thus, in order to evidenciate the individual influence of each region in the occurrence of these ectoparasites, this variable was decomposed in dummy variables. Thus, the logistic regression model analyzed each region individually avoiding biases that could occur when variable region is considered without discriminating each of the 20 regions of the State. The region of Bambuí was chosen as a reference for comparison with the other due to the fact that this is the region number one of the database and because it is the closest to the barycenter of the State of Minas Gerais. Also,

Bambuí has systems that have characteristics that make them adequately representative of the poultry industry in the state.

The regions of Belo Horizonte, Juiz de Fora, Viçosa, Pouso Alegre and Oliveira were considered as area of risk for the occurrence of mites due the $OR > 1$. Passos, Unaí and Uberaba regions had no infestation, leading to their exclusion from the model. Differences in the occurrence of feather mites among regions were also described by Jardim et al. (2012), in a study on the occurrence of quill mites on psittacines in conservation facilities in Brazil, observing a greater chance of occurrence in the State of Espírito Santo as compared to Minas Gerais. The occurrence of variations among regions could be attributed to climatic conditions, which would vary according to geographic location. In Figure 1, is presented a map of the State of Minas Gerais and its regions. The northern warmer and drier climate regions were removed from the logistic model due to absence of mite infestation. In contrast, the southern regions presented greater chances, in reason of a cooler and more humid climate. Hernández et al. (2007) also found influence of climatic conditions on the occurrence of mites, with an increase of the population in the rainy season. Other hypothesis to explain the increased risk of infestations in the south regions is the highest density of poultry farms in these areas, which could increase the chances of mite spreading. Regarding the value of the OR of area around Viçosa (Table 2), just one farm with diversified poultry houses profiles was visited (Figure 1). This farm had only one barn without infestations, which may have influenced partially in the OR value and in the cluster analysis after verification of intra-class correlation.

Conclusions

The high occurrence of mites of genus *Megninia* may be relevant for industrial chicken production in Minas Gerais, especially in laying hens, and suggests the need for periodic preventive monitoring. The evaluation of the economic impact of *Megninia* would be important. However, in order to adequately perform control, knowledge on biology and epidemiology is essential. This study verified that an important risk factor for the occurrence of infestations was the presence of subsistence breedings of chickens at the proximity of chicken houses. The results support the thesis that the role of synanthropic birds and the use of wire mesh fences had no significance on *Megninia* occurrence. Mineral oil also exerted a significant protective effect and must be further investigated.

Regarding the sample size and design of the study, more studies are needed mainly employing longitudinal designs at the same region. Prospective or retrospective studies may improve relevant information about intensity of infestation, seasonal dynamics and incidence of different species of the *Megninia* genus.

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