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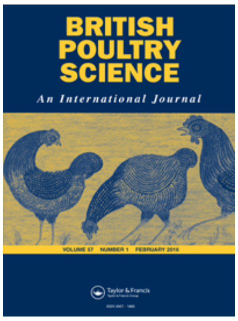
Article *in* British Poultry Science · December 2015

DOI: 10.1080/00071668.2015.1127893



British Poultry Science

ISSN: 0007-1668 (Print) 1466-1799 (Online) Journal homepage: http://www.tandfonline.com/loi/cbps20

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To cite this article: L. Do Carmo Rezende, N. R. Da Silva Martins, C. M. Teixeira, P. R. De Oliveira & L. M. Cunha (2016) Epidemiological aspects of lice (Menacanthus species) infections in laying hen flocks from the State of Minas Gerais, Brazil, British Poultry Science, 57:1, 44-50, DOI: 10.1080/00071668.2015.1127893

To link to this article: http://dx.doi.org/10.1080/00071668.2015.1127893

Accepted author version posted online: 08 Dec 2015.
Published online: 29 Feb 2016.

British Poultry Science, 2016
Vol. 57, No. 1, 44–50, http://dx.doi.org/10.1080/00071668.2015.1127893

Epidemiological aspects of lice (*Menacanthus* species) infections in laying hen flocks from the State of Minas Gerais, Brazil

L. DO CARMO REZENDE[1], N. R. DA SILVA MARTINS[1], C. M. TEIXEIRA[1], P. R. DE OLIVEIRA[1,*], AND L. M. CUNHA[2]

[1]*Departamento de MedicinaVeterináriaPreventiva, Escola de Veterinária da Universidade Federal de Minas Gerais, Campus da UFMG, Belo Horizonte, Minas Gerais, Brazil, and* [2]*Centro de Ciência Animal, Instituto de Ciências Exatas e Biológicas da Universidade Federal de Ouro Preto, UFOP, Campus Morro do Cruzeiro, Ouro Preto, Minas Gerais, Brazil*

Abstract 1. The epidemiology of chicken lice species such as *Menacanthus stramineus, M. cornutus* and *M. pallidulus* were studied during an observational, analytical and sectional survey, to determine predisposing factors for their occurrence in laying hen farms in the State of Minas Gerais, Brazil. A total of 431 houses on 43 farms were visited in 2012.

2. *M. cornutus, M. stramineus* and *M. pallidulus* occurred in 20.9%, 11.6% and 11.6% of farms, respectively. The frequencies of occurrence of *M. cornutus, M. stramineus* and *M.pallidulus* in poultry houses were 10.4%, 8.8% and 3.7%, respectively.

3. The epidemiological determinants for the occurrence of these species were investigated using Poisson or logistic regression models.

4. The region of the farm, the recent use of acaricides and the presence of birds, such as saffron finch (*Sicalis flaveola*), feral pigeon (*Columba livia*) and Guira cuckoo (*Guira guira*) around the farms were related to the epidemiology of *M. cornutus.*

5. Infestation by *M. stramineus* was associated with age of birds, number of birds per cage and the presence of Guira cuckoo and Chopi blackbird (*Gnorimopsar chopi*) near the poultry houses.

6. The occurrence of *M. pallidulus* was influenced by the type of facilities, presence of cattle egret (*Bubulcus ibis*) and free-range domestic hens around the farm.

7. The use of wire mesh nets in the houses and of forced moulting did not influence lice infestation.

INTRODUCTION

Lice are well adapted to live as permanent ectoparasites of birds and mammals. The order *Phthiraptera* (includes suborders *Amblycera* and *Ischnocera*) comprise species that feed on feathers and skin scurf (Guimarães et al., 2001). *Phthiraptera* of domestic chickens, also known as *Mallophaga* according to the old taxonomy, have been studied for their economic importance (Guerra *et al.*, 2008). Parasitised birds suffer skin irritation and inflammation, weight loss and a decrease in feed intake and egg production (De Vaney, 1976; Gless and Raun, 1959). Furthermore, these arthropods are a reservoir of *Pasteurella multocida* and may act in the transmission of pathogens (Derylo, 1970).

In Brazil, species of Amblycera, such as *Menacanthus stramineus* (Nitzsch, 1818), *M. cornutus* (Schommer, 1913) and *M. pallidulus* (Neumann, 1912) have been observed (Guimarães *et al.*, 2001). In a Brazilian survey, the association of the frequency of these ectoparasites and the type of facilities, host densities, cage system and practice of beak trimming were analysed and were related to a higher occurrence of infestations (Figueiredo *et al.*, 1993). Other studies demonstrated that infestation by *M. stramineus* was

Correspondence to: Lucas Maciel Cunha, Centro de Ciência Animal, Instituto de CiênciasExatas e Biológicas da Universidade Federal de Ouro Preto, UFOP, Campus Morro do Cruzeiro, CEP 35400-000, Ouro Preto, Minas Gerais, Brazil. E-mail: lucasmcunha82@gmail.com
Accepted for publication 22 October 2015.
*In memoriam.

higher in Leghorn hens kept in cages at higher densities and that beak trimming as a management practice may increase louse infestations (Mullens *et al.*, 2010). In a Pakistani study, older birds in small enclosures were more prone to *Mallophaga* infestation (Nadeem *et al.*, 2007).

There are few studies on epidemiological factors influencing prevalence or transmission of poultry lice. Our aim was to document occurrence of *Menacanthus* spp. in layer flocks in Minas Gerais, Brazil, and to examine associated flock and site characteristics.

MATERIAL AND METHODS

Survey characteristics

The study was conducted in layer farms in the State of Minas Gerais, Brazil, which has an area of 586 519 km^2 and a large range of climatic conditions. The climate in most regions surveyed in Minas Gerais is tropical, alternately wet and dry. To determine whether the climatic variations could affect the presence or absence of ectoparasites, the temperature (12–39°C) and relative humidity (≥30%) were monitored in cities near the farm. Farms where temperatures were below 5°C or above 40°C were not visited in order to avoid high variations in the severity of infestation related to the extreme climatic changes, which may influence the detection of ectoparasites.

The farms visited were generally similar, with the number of houses per farm ranging from 1 to 40 and an average number of 10 (SD = 5.49). Forty-one farms kept poultry in cage systems and produced breeding replacement growers and pullets.

Sampling and identification of arthropods

There is a national plan for poultry health in Brazil, which requires compulsory periodic inspection and georeferencing of commercial poultry flocks. (Properties producing poultry for home consumption on a small scale are characterised as subsistence producers and are not inspected.) The georeferencing data of the State Institute for Agricultural Sanitary Defence of Minas Gerais (*Instituto Mineiro de Agropecuária* – IMA) were used for determining the number of farms to be sampled in each region of the state.

The random selection of farms (conglomerates) and stratification of the samples were performed on the basis of the proportion of commercial laying farms in each of the 20 IMA administrative regions of the state. To calculate sample size, a hypothetical prevalence of 50%, a standard error of 5% and a finite number of farms were assumed. The farms were regarded as a conglomerate of active poultry houses (Medronho *et al.*, 2009) and from March to July 2012, 431 chicken houses on 43 farms were visited.

Inspections were performed in the poultry facilities and 20 chickens per house were individually examined – the visual examination was done by veterinarians trained for this purpose. Lice and feather samples were collected; in some cases, lice were collected using adhesive tape (Figueiredo *et al.*, 1993). Samples were packed in airtight plastic bags, taken to the laboratory, where they were stored at −20 ± 2°C until processing. In each house, only the presence or absence of the parasites was evaluated, and no quantification of infestations was made.

Lice and haematophagous mite specimens were prepared in lactophenol (Krantz, 1978) and mounted on slides with coverslips. Morphological characteristics were determined with an optical microscope (Olympus, Japan). The species of lice and mites were identified (Emerson, 1956; Guimarães *et al.*, 2001).

Questionnaire

To obtain information on predisposing factors for the occurrence of ectoparasite species, a questionnaire was completed during the visits (Cunha, 2013). It was prepared on the basis of a test–retest validation procedure, using kappa coefficient. The 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 or protective factors for the occurrence of infestations by *M.stramineus* was performed using logistic regression models, as demonstrated by Dohoo *et al.* (2003). Regarding *M. pallidulus* and *M. cornutus*, the evaluation of main epidemiologic factors was performed using Poisson regression models (Dohoo *et al.*, 2003). The choice of multivariate model for each species of parasite was performed on the basis of goodness of fit. These models were constructed using the software Stata 12.0 and considering the poultry houses as the statistical analysis unit. The preliminary selection of variables to be included in the model was performed using the Chi-square test, Fisher's exact test or univariate logistic regression, removing the variables with $P > 0.15$. Variables selected by univariate screening tests were used in multivariate logistic and Poisson regression models, except for those confounding or not significant ($P \leq 0.05$). The selection of the highest pseudo-R^2 values and the analysis of odds ratios (OR) and incidence rate ratios (IRR) confidence intervals

Table 1. *Variables selected by univariate statistical tests for their association with* Menacanthus *species in laying hen flocks in State of Minas Gerais, Brazil, 2012 (*P *< 0.15)*

Variable	*P*-value		
	M. cornutus	*M. stramineus*	*M. pallidulus*
Region	0.00*	0.000*	0.000*
Municipality	0.00*	____	0.000*
Farm	____	0.000*	____
Type of establishment (laying or replacement pullets and growers)	____	____	0.049*
Type of facilities (caged, or on the floor with or without bedding)	____	0.022*	0.149*
Primary genetic lineage in chicken house	____	0.000*	0.000*
Secondary genetic lineage in chicken house	0.000*	0.045*	____
Tertiary genetic lineage in chicken house	0.002*	0.016*	____
Age of chickens	0.138#	0.004#	0.056#
Number of chickens per farm	0.074#	0.103#	0.003#
Number of chickens per cage	____	0.000*	0.007*
Range of chickens per square metre	____	0.005*	____
Presence of smooth-billed ani (*Crotophaga ani*)	____	0.096*	0.003*
Presence of sparrows (*Passer domesticus)*	0.004*	____	____
Presence of feral pigeon (*Columba livia)*	0.000*	____	0.001*
Free range domestic fowl houses nearby	0.054*	____	0.000*
Presence of cattle egret (*Bubulcus ibis*)	0.020*	____	0.004*
Presence of Black Vulture (*Coragyps atratus*)	0.000*	____	0.105*
Presence of Guira cuckoo (*Guira guira*)	0.000*	0.000*	0.070*
Presence of saffron finch (*Sicalis flaveola*)	0.002*	____	____
Presence of Chopi blackbird (*Gnorimopsar chopi*)	0.027*	0.000*	0.123*
Presence of Ruddy ground dove (*Columbina talpacoti*)	____	____	0.043*
Other species of synanthropic birds	0.128*	____	____
Report of acaricides recently used	0.000*	____	____
Use of mineral oil	____	____	0.008*
Use of silica	0.000*	0.109*	____
Use of fire blowtorch	____	____	0.000*
Use of wire mesh nets	0.015*	____	____
Presence of *Lipeurus caponis*	____	0.004*	____
Presence of *Menacanthus pallidulus*	____	0.020*	____
Presence of *Menacanthus cornutus*	____	0.099*	____

*Pearson's chi-square test or Fisher's exact test.
#Univariate logistic regression.
____: $P > 0.15$

(95% significance – 95% CI) were also applied for variable selection and construction of the models. Variables with significant OR or IRR ($P \leq 0.05$) as well as variables, whose removal would disrupt the general model, were kept. The verification of logistic model adjustment was performed using Wald's test ($P \leq 0.05$) and Hosmer–Lemeshow's test (observational, analytical and sectional survey $P > 0.05$). The Pearson chi-square test for goodness of fit was used to verify the Poisson regression model's fitness. A check of the area under the receiver operating characteristic (ROC) curve was also considered for choosing the multivariate logistic regression model.

RESULTS

The frequencies of occurrence of *M. cornutus, M. stramineus* and *M. pallidulus* were 20.9%, 11.6% and 11.6% of the farms, respectively. For the poultry houses the frequencies of *M. cornutus, M. stramineus* and *M. pallidulus* were 10.4%, 8.8% and 3.7%, respectively. Characteristics selected in screening procedures regarding the three species of *Menacanthus* are shown in Table 1. Table 2

Table 2. *Variables associated with the occurrence of* Menacanthus cornutus *in commercial laying hen poultry flocks in the State of Minas Gerais, Brazil, 2012*

Variable	Incidence rate ratio	Confidence interval (95%)		P-value
Region	1.08	1.01	1.15	0.011
Presence of saffron finch (*Sicalis flaveola*)	2.25	1.13	4.45	0.020
Presence of feral pigeon (*Columba livia*)	3.36	1.05	10.71	0.040
Presence of Guira cuckoo (*Guira guira*)	18.18	6.31	52.34	0.000
Reports of acaricides recently used	0.73	0.60	0.89	0.002
Use of wire mesh nets around the poultry houses	0.21	0.025	1.74	0.149
Age of chickens	1.00	0.99	1.001	0.771

Number of valid observations: 423.
P-value of Pearson chi-square test for goodness of fit: 1.000.

presents the characteristics of risk for the occurrence of *M. cornutus* according to multivariate Poisson regression model.

Regarding the logistic models for *M. stramineus*, the presence of *M. cornutus* (95% CI = 0.00038–0.139, *P* = 0.001) was significant for some preliminary multivariate models. However, retaining this parasite in the models resulted in reduced sensitivity, leading to the exclusion of this variable. The presence of sparrows was also excluded from the general model due to the occurrence of perfect failure, as also observed with the variable range of chickens per square metre and use of silica. The characteristics included in the general model of verification of predisposing factors for the occurrence of *M. stramineus* are shown in Table 3.

In screening Poisson regression models for *M. pallidulus*, the use of mineral oil (IRR = 2.85, 1.03–7.85, *P* = 0.042), due to values of confidence intervals of the IRR, was considered to be a confounding variable and was excluded. The epidemiological aspects of *M. pallidulus* infestations are shown in Table 4. In the logistic and Poisson regression models, values of OR and IRR < 1 indicate that a variable is a preventative factor. Correspondingly, OR and IRR > 1 indicate that the variable is a predisposition factor for ectoparasite occurrence.

Table 3. *Variables associated with the occurrence of* Menacanthus stramineus *in commercial laying hen poultry flocks in State of Minas Gerais, Brazil, 2012*

Variable	Odds ratio	Confidence interval (95%)		P-value
Age of chickens	1.007	1.003	1.01	0.000
Number of chickens per cage	10.44	3.74	29.14	0.000
Presence of Guira cuckoo (*Guira guira*)	14.08	4.75	41.69	0.000
Presence of Chopi blackbird (*Gnorimopsar chopi*)	36.07	10.23	127.21	0.000

Number of valid observations: 365.
Region reference for the elaboration of odds ratio: Bambuí.
P-value for goodness of fit of the model in Wald's test: < 0.001.
P-value for goodness of fit of the model in Hosmer–Lemeshow's test: 0.2393.
Area under the ROC curve: 91.6%.

Table 4. *Variables associated with the occurrence of* Menacanthus pallidulus *in commercial laying poultry flocks in the State of Minas Gerais, Brazil, 2012*

Variable	Incidence rate ratio	Confidence interval (95%)		P-value
Type of facilities	7.58	1.71	33.49	0.007
Free-range domestic fowls nearby	11.58	3.71	36.10	0.000
Presence of cattle egret (*Bubulcus ibis*)	10.33	1.34	79.12	0.025

Number of valid observations: 431.
P-value of Pearson chi-square test for goodness of fit: 1.00.

DISCUSSION

The most frequent species per farm was *M. cornutus*, whereas per house, *M. cornutus* was the most frequent species of louse and the least diagnosed was *M. pallidulus*. A survey of lice in 44 laying hen establishments in the states of São Paulo, Pernambuco, Bahia, Alagoas and Paraná was performed, and *M. cornutus* was also the most frequent species there (Figueiredo *et al.*, 1993). A higher frequency of *M. cornutus* was also observed in the state of Rio Grande do Sul, a southern region of Brazil, as compared to other species of lice (Oliveira and Ribeiro, 1990). Guerra *et al.* (2008) reported the occurrence of *M. cornutus*, *M. stramineus* and *M. pallidulus* in chickens in the state of Maranhão, Northeastern Brazil. All these reports and results confirm the importance of lice for the Brazilian laying poultry industry.

In multivariate models for verifying epidemiological determinants for *M.stramineus* and *M. pallidulus*, variables related to the geographic distribution of the farms were not significant in Minas Gerais, suggesting that geographical location may not be important in the epidemiology of these species. However, in the case of *M. cornutus*, the variable "region" produced an IRR that classified it as a risk factor (IRR < 1) (Table 2). Although in this case it was possible to assess each region separately for the occurrence of *M. cornutus* due to the non-adjustment of Poisson models, one may infer that some regions probably do have a higher risk of infestation. The small number of poultry houses sampled within some regions did not contribute to the non-adjustment of the models. The variation of climatic conditions between States could explain the occurrence of *M. cornutus* and the values of the confidence interval of the variable "region" in the statistical model. Some authors have already reported the influence of temperature on the development of ectoparasites, which corroborates this hypothesis (Chen and Mullens, 2008; Halbritter and Mullens, 2011).

The number of chickens per cage was significant only as an *M. stramineus* predisposing factor (OR > 1) (Table 3), indicating that large number of chickens per cage increased the risk of occurrence of infestations. This is in agreement with other studies (Figueiredo *et al.*, 1993; Mullens *et al.*, 2010). A survey of 70 poultry farms in Pakistan also showed a higher occurrence of lice on chickens kept at high densities (Nadeem *et al.*, 2007). The relative densities of large numbers in small areas probably increase bird contact and thus facilitate dispersion of lice. Furthermore, overcrowded cages make it difficult for chickens to remove their ectoparasites (Figueiredo *et al.*, 1993).

The age of chickens was not a factor for the occurrence of *M. pallidulus* and *M. cornutus*, nevertheless, the age of chickens was retained to improve the fit of the model in terms of risk factors for the occurrence of *M. cornutus* (Table 2). However, in the case of *M. stramineus*, this variable was significant and retained in the logistic regression model as a risk factor (OR > 1) (Table 3). Other studies also confirm the influence of age on the occurrence of lice infestations (Nadeem *et al.*, 2007). Thus, older chickens have a greater probability of infestations.

Genetic lineage was not influential in the epidemiology of infestations by *Phthiraptera* in the *Menacanthus* genus, suggesting that other factors associated with the host are more important. However, a Brazilian study found that most hens parasitised by lice belonged to the strains Babcock and Isa Brown, though a nonstatistical technique was used to test this hypothesis (Figueiredo *et al.*, 1993).

The type of establishment (laying or production of replacement growers and pullets) was not significant in the epidemiological analyses, suggesting that the occurrence of lice is similar in laying hens and in pre-adult chickens. The type of facilities (with caged fowl or uncaged chickens on the ground with or without bedding) was a risk factor for the occurrence of *M. Pallidulus* (IRR > 1, Table 4). The occurrence of this parasite is higher in houses with birds on the floor than in houses with cages, probably due to the non-occurrence of this louse in farms with mechanical devices for removal of eggs and manure. These results are in agreement with another study (Figueiredo *et al.*, 1993).

The presence of nearby free-range birds was a predisposing factor for *M. pallidulus* (Table 4), probably because these fowls are often kept under a minimal sanitary control and could be a source of parasites to commercial laying hens. The presence of species of synanthropic birds such as saffron finch (*Sicalis flaveola*), Guira cuckoo, cattle egret (*Bubulcus ibis*), feral pigeon (*Columba livia*) and Chopi blackbird were also possible risk factors for the occurrence of *Menacanthus* lice, as shown in Tables 2–4. However, many studies report host specificity of these parasites for *Gallus g. domesticus* and some wild birds belonging to the species order *Galliformes* (Emerson, 1956; Guimarães *et al.*, 2001). Thus, we suggest that these birds are mechanical vectors or accidental hosts, responsible for the spread and dispersal of these parasites. In Brazil, the contact between synanthropic birds and laying hens is extremely close, which favours the spread of lice between houses. Axtell and Arends (1990) report the role of wild birds in lice dispersion, together with the activity and movement of people, infested equipment (mainly egg cartons) and rodents. The probability of synanthropic birds in spreading *Menacanthus* spp. is reinforced by occurrence of *M. stramineus* in *C. livia* in Bangladesh (Begum and Sehrin, 2011) and more studies of the role of these species are justified.

The use of wire mesh in chicken houses was not associated with the occurrence of *M. cornutus, M. stramineus* and *M. pallidulus* suggesting it did not prevent the entry of chewing lice associated with synanthropic birds. This is probably because wire mesh was not primarily to exclude synanthropic birds, but rather to contain growers and pullets in non-cage floor raised systems and to prevent the entry of other animals such as predators. Thus, many different sizes of mesh are used and some are not able to contain small species of synanthropic birds. The Brazilian National Plan for Poultry Health sets out a minimum mesh, but most of the farms did not conform to these requirements. Thus, in order to examine the influence of the use of wire mesh to prevent louse infestations, a suitable mesh size should be adopted.

The use of acaricides to combat haematophagous mites was evaluated and was a protective factor against infestation by *M. cornutus* (IRR < 1, Table 2), suggesting acaricides are also effective against *M. cornutus*, but not against other lice species. Mineral oil is used to combat haematophagous mites (Tucci and Guimarães, 1992) and should be a protective factor against lice infestation, but the use of this substance was a risk factor (IRR and OR > 1) for the occurrence of *M. pallidulus* (IRR = 2.85, 95% CI = 1.03–7.85, $P = 0.042$). It was a confounding variable, leading to its exclusion in the Poisson regression model. Probably acaricidic action of mineral oil is ineffective against *M. pallidulus* and other lice. The use of silica to combat ectoparasites was not significant, suggesting this measure may be more suitable for other avian ectoparasites, such as haematophagous mites.

The use of a blowtorch did not influence the occurrence of lice, probably due to the biological characteristics of these arthropods, since lice live and breed predominantly on the hosts and not on the house structures (Guimarães *et al.*, 2001). Furthermore, blowtorches are used at the end of the production cycle, which provides enough time for the recovery of lice populations.

Forced moulting is used to obtain a second production cycle in layers that would otherwise be discarded because of reduced productivity (Teixeira and Cardoso, 2011). It was not significant in univariate tests, so it was not used in the multivariate models. The moulting procedure may have lasted long enough to allow the population of lice to recover. However, in a Brazilian survey, the incidence of lice was higher in hens subjected to this procedure (Figueiredo *et al.*, 1993) possibly because the birds lost immunological resistance, making it more difficult to clear the ectoparasites. Moreover, the new feathers would provide better conditions for the development of immature lice stages.

We found no relationships between the presence of various species of lice, either with each other or with other ectoparasites, such as the mite *Ornithonyssus sylviarum*, in the houses, Thus, these species, though sharing the same ecological niche, do not seem to establish competitive relationships among themselves, even though another study observed hens to be parasitised by both mites and lice (Figueiredo *et al.*, 1993).

In conclusion, the incidence of *M. pallidulus* was higher in hens kept on the floor and in places where there were free-range chickens nearby. The presence of some species of synanthropic birds around the farms was a risk factor for the occurrence of *Menacanthus* spp., suggesting these birds behave as mechanical vectors of parasites or accidental hosts. More work is needed to verify the role of these birds on the transmission of lice in poultry farms. "Region" was a risk variable for *M. cornutus* infestation, suggesting that geographic localisation of farms influences infestation. The use of acaricides was a protective factor against *M. cornutus*, indicating that this practice could decrease arthropod populations. For *M. stramineus*, a large number of chickens per cage and an increase in bird age were risk factors. All this information is relevant for understanding the epidemiology of these species of ectoparasites in different poultry systems and should be helpful in the practical control of these ectoparasites.

ACKNOWLEDGEMENTS

The authors are grateful to *Instituto Mineiro de Agropecuária* (IMA) and farm owners for authorising poultry farm visits.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

FUNDING

We are indebted to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Comissão de Aperfeiçoamento de Pessoal (CAPES) and Pró-reitoria de Pesquisa da Universidade Federal de Minas Gerais (Prpq/UFMG) for financial support and fellowships.

ORCID

N. R. Da Silva Martins http://orcid.org/0000-0001-8925-2228

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