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Short communication

Caseous lymphadenitis in sheep flocks of the state of Minas Gerais, Brazil: Prevalence and management surveys

A.S. Guimarães[a,d], N. Seyffert[b], B.L. Bastos[c], R.W.D. Portela[c], R. Meyer[c], F.B. Carmo[a,d], J.C.M. Cruz[a,d], J.A. McCulloch[b,e], A.P. Lage[a,d], M.B. Heinemann[a,d], A. Miyoshi[b], V. Azevedo[b,d,1], A.M.G. Gouveia[a,d,∗,1]

[a] *Laboratório de Bacteriologia Aplicada, Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais, CEP 31270-901, Belo Horizonte, MG, Brazil*
[b] *Laboratório de Genética Celular e Molecular, Departamento de Biologia Geral, Instituto de Ciências, Biológicas, Universidade Federal de Minas Gerais, CP 486, CEP 31270-901, Belo Horizonte, MG, Brazil*
[c] *Laboratório de Imunologia e Biologia Molecular, Departamento de Bio-Interação, Instituto de Ciências da Saúde, Universidade Federal da Bahia, CEP 40110-100, Salvador, Bahia, Brazil*
[d] *Grupo de Extensão da Pesquisa em Ovinos e Caprinos, Escola de Veterinária, Universidade Federal de Minas Gerais, CEP 31270-901, Belo Horizonte, MG, Brazil*
[e] *Laboratório de Polimorfismo de DNA, Instituto de Ciências Biológicas, Universidade Federal do Pará, CP 8607, CEP 66075-900 - Belém, Pará, Brazil*

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ABSTRACT

Corynebacterium pseudotuberculosis is the etiologic agent of caseous lymphadenitis, which is a serious, economically important problem for sheep production. We examined the sero-prevalence of infection by *C. pseudotuberculosis* and possible risk factors associated with caseous lymphadenitis in sheep herds of the state of Minas Gerais, Brazil. Samples were collected from 642 sheep from 97 farms. Sera of all of the sheep were tested with ELISA for antibodies against *C. pseudotuberculosis*. A questionnaire was applied to gather data on the farm, the sheep herd, the farmer, and individual animal data (breed, sex and age). This is the first sero-epidemiological survey for caseous lymphadenitis in sheep herds in Minas Gerais. We found a high real prevalence, much higher than that suggested from information obtained with the questionnaire, which points to the scarcity of vaccination against caseous lymphadenitis in the sample evaluated. Only a small proportion of the farmers declared that cases of this disease were present in their flocks. The frequency of seropositive sheep varied significantly with breed (χ^2 test, $P = 0.021$). Age group also significantly affected the percentage of seropositivity (χ^2 test, $P = 0.049$), the highest frequency being found in adult animals (more than 12 months old), when compared to the 5–12 months old group (χ^2 test, $P = 0.021$). The prevalence of infection with *C. pseudotuberculosis* in sheep in the state of Minas Gerais was estimated to be 70.9% (95% confidence interval (CI): 64.7–77.0%) and the prevalence of infected flocks being 95.9% (95% CI: 89.8–98.9%). We concluded that *C. pseudotuberculosis* infection is widely disseminated in sheep flocks in Minas Gerais and that caseous lymphadenitis control and eradication programs are necessary in this state.

∗ Corresponding author at: Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais - UFMG. Av. Antônio Carlos 6627 Caixa Postal 567, Campus da UFMG CEP 30123-970, Belo Horizonte, MG, Brazil. Tel.: +55 31 3221 6966.
E-mail address: aurora@vet.ufmg.br (A.M.G. Gouveia).

[1] These authors share responsibility as senior authors.

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1. Introduction

Caseous lymphadenitis is a chronic suppurative disease that mainly affects goats and sheep. Its etiological agent is *Corynebacterium pseudotuberculosis* and can cause debility in animals, presenting itself in cutaneous and visceral forms. The disease is distributed worldwide, with cases being reported in Europe, Australia, North and South America, Africa and the Middle East (Dorella et al., 2006).

Caseous lymphadenitis causes considerable economic losses, which range from condemnation of skins and carcasses, due to abscesses, to expressive losses in reproductive efficiency, as well as in wool, meat and milk production. Subclinical infections are also important, because they allow *C. pseudotuberculosis* to disseminate within and between herds (Paton et al., 1994). Also, caseous lymphadenitis can become a public health problem as it is a zoonosis (Peel et al., 1997; Join-Lambert et al., 2006). Resistance of *C. pseudotuberculosis* to antibiotics and its strenuous perseverance in the environment, associated with the difficulty in detecting infected animals, make caseous lymphadenitis hard to eradicate (Williamson, 2001).

The first case of caseous lymphadenitis in Brazil was reported in 1972 (Garcia et al., 1987) and even though this disease is found in Brazilian sheep herds, few epidemiological studies have been carried out in this country (Silva et al., 1982; Tinôco, 1983; Guimarães and Gouveia, 2006). There are no previous records of serological studies in herds from the state of Minas Gerais. The serological status of the herd is an indication of the presence of the infectious agent and can be used to orient control programs so that they are compatible with the actual infection rate.

Since 2000, commercial sheep husbandry has increased considerably in the state of Minas Gerais with the acquisition of animals from other regions of the country where caseous lymphadenitis is frequent, resulting in a considerable transit of animals into Minas Gerais (IBGE, 2009; ARCO, 2008). Also, the lack of species-specific sanitary legislation and the reduced availability of commercial immunogens in the Brazilian market limit the use of systematic vaccination against the etiological agent of caseous lymphadenitis (Guimarães and Gouveia, 2006).

The aim of this study was to assess the seroprevalence of infection with *C. pseudotuberculosis* in sheep in the state of Minas Gerais and looked for risk factors that could be associated with caseous lymphadenitis.

2. Materials and methods

2.1. Study area and sampling

The state of Minas Gerais is located in southeastern Brazil (Fig. 1). It has an area of 588,383 km^2, a mostly tropical climate, and a mean annual temperature of 21.2 °C. Annual rainfall varies from 1000 to 2000 mm, with well-defined dry and wet seasons. Sampling was organized at two levels: farms and animals. To calculate the number of herds that should be sampled, we used simple sampling with an estimated prevalence of 50%, a confidence interval of 95% and an error of 10% (Noordhuizen et al., 1997). Based on a combined list of sheep farms from the Association of Sheep and Goat Farmers of the State of Minas Gerais (Associação dos Criadores de Caprinos e Ovinos de Minas Gerais: Caprileite/ACCOMIG) and the state government agency for animal health (Instituto Mineiro de Agropecuária: IMA), 97 sheep farms were selected, representing 9 of the 12 mesoregions of Minas Gerais (Fig. 1). Animals were randomly selected and a fixed sampling of eight animals from each property was used (Bennett et al., 1991); for properties with less than eight sheep, all were sampled. The total animal sample was calculated as 776 sheep. Blood was collected by jugular vein puncture and the serum was separated and stored at −20 °C until used for analysis.

2.2. Data animals

A previously tested questionnaire (Pinheiro et al., 2000) was filled out for each herd, demanding data on the farm, the herd, the farmer, and individual animal characteristics (breed, sex and age). The questionnaires were completed and the sera collected in 2002 by IMA veterinarians.

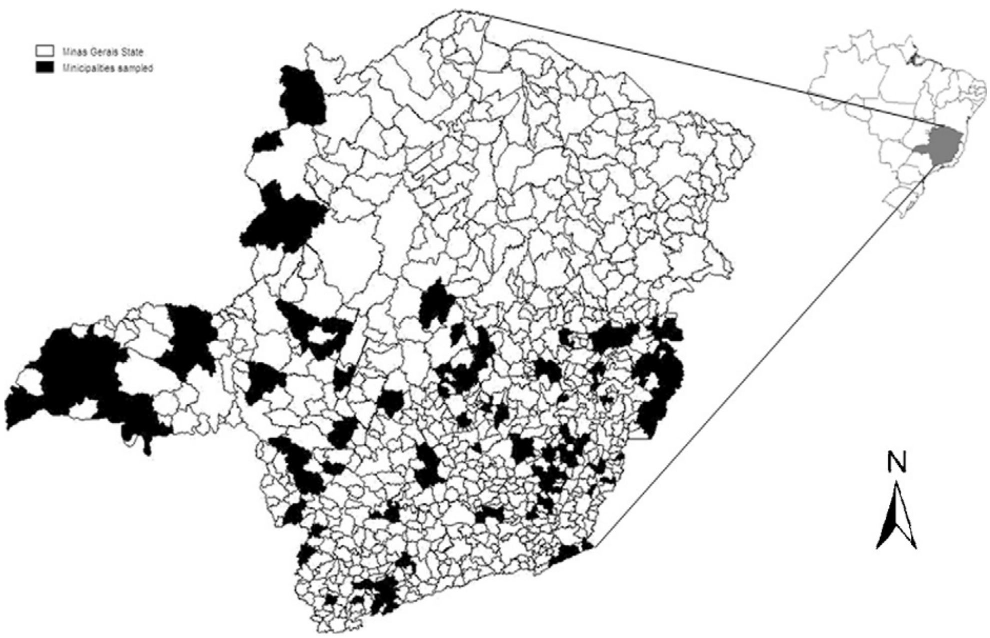

Fig. 1. Municipalities with sampled flocks in Minas Gerais State, Brazil.

Table 1
Frequency distribution of seropositive sheep based on ELISA for *Corynebacterium pseudotuberculosis* in Minas Gerais, Brazil, 2002.

Variable	Group	Positive (n)[a]	Positive (%)	Total tested (n)
Sex	Females	283 a	62.1	456
	Males	108 a	58.4	185
Racial group	PE[b]	22 ab	61.1	36
	PN[c]	212 a	66.7	318
	Mixed[d]	16 b	48.5	33
	Unknown[e]	128 b	55.2	232
Age (months)	≤4	11 ab	52.4	21
	5–12	90 b	53.9	167
	>12	291 a	64.1	454

[a] Different letters for the same variable indicate significant differences ($P<0.05$).
[b] PE: pure exotic, pure animals of foreign races.
[c] PN: pure national, pure animals of domestic races.
[d] Mixed: crosses of domestic and/or foreign races.
[e] Unknown: no race defined.

2.3. ELISA

An indirect ELISA for measuring seropositivity against *C. pseudotuberculosis* was carried out using microplates (Maxisorp, Nunc, USA) prepared with total secreted antigens of *C. pseudotuberculosis*, according to the procedures detailed by Seyffert et al. (2009). The sera were tested at a dilution of 1:100, in duplicate, with positive and negative controls included in all of the plates. The cut off point for the test was established based on the mean optical density reading (equal to OD_{450nm} 0.150) of sera from uninfected sheep from areas that are not endemic for caseous lymphadenitis, plus three times the standard deviation (Seyffert et al., 2009). All of the optical density values that differed more than 20% between duplicates were repeated for confirmation. Readings were made with a spectrophotometer (BIO-RAD, USA) at 450 nm.

2.4. Data analyses

A data bank was developed using software Epi-Info 6.04 (Dean et al., 1995). Serological data, prevalence calculations, confidence intervals, effects of delineation (D) and of the intra-group correlation coefficient (ρ) were calculated and analyzed according to Bennett et al. (1991). The results were weighted as a function of the size of the herd of origin (Bennett et al., 1991; Noordhuizen et al., 1997). The real prevalence of infected animals was calculated based on sensitivity and specificity values of 93.5 and 100%, respectively (Carminati et al., 2003). Aggregate sensitivity and specificity were calculated using the software Herdacc (Jordan, D.; Guelph, Ontario, Canada) (Martin et al., 1992; Jordan, 1996). Correlations were calculated between the frequency of seropositives and individual characteristics (sex, age and breed group) using the Chi-square test and an α error level of 0.05 (Epi-Info 6.04) (Dean et al., 1995; Sampaio, 2002). The following variables: individual identification of animals, technical assistance, participation in expositions, sanitizing of the navel, husbandry system, requisition of sanitary certification when purchasing animals, origin of the herd and reproduction management were tested to see how these affected the prevalence of lymphadenitis, using the software WinEpiscope® 2.0 (Noordhuizen et al., 1997; Thrusfield et al., 2001; Dohoo et al., 2003).

3. Results

Questionnaires were filled out and serum samples collected from 752 animals from 97 farms in 94 municipalities located in nine of the 12 mesoregions of the state of Minas Gerais (Fig. 1). Five of the farms had fewer than eight sheep. Serum samples from 110 animals were not analyzed because of operational problems (the tube broke, excessive hemolysis or insufficient serum volume). Thus, sera from 642 were available for analysis. Prevalence of infection with *C. pseudotuberculosis* among the 642 sheep with analyzable serum samples was estimated to be 70.9% (95% CI: 64.7–77.0%), the real prevalence was calculated as 75.8% and the prevalence of farms with infected animals was 95.9% (95% CI: 89.8–98.9%). The mean number of sheep analyzed per farm was 6.6, varying from 1 to 9. The observed effect of experimental design (D) was 3.4 and the intra-conglomerate correlation coefficient (ρ) was calculated to be 0.4. The frequency distribution of the sheep seropositive for caseous lymphadenitis, classified by sex, breed and age, is given in Table 1. Seropositivity did not significantly with gender ($P=0.386$). The animals were grouped as pure foreign (Texel, Suffolk and Merino), pure national (Morada Nova, Somális, and Santa Inês), mixed breed (crosses between national and/or foreign races) and undefined race, for the analyses. The frequency of seropositivity varied significantly among the four racial groups ($P=0.021$). Differences were also found between the pure national and pure foreign groups ($P=0.037$) and between pure national and undefined race ($P=0.006$). There was also a significant difference among age groups ($P=0.049$). The highest frequency was among adult animals (over 12 months), significantly higher than the 5–12 months old group ($P=0.021$). The criterion used to select variables for analysis of risk ratios was their potential influence on the epidemiology of caseous lymphadenitis. The main management practices identified and the risk analysis of variables that are predictors of risk for caseous lymphadenitis in the sheep herds are given in Tables 2 and 3, respectively.

4. Discussion

Our study was the first sero-epidemiological study of caseous lymphadenitis in sheep herds in the state of Minas Gerais. The prevalence we found was high, with a seropositivity of 75.8%. This contrasts with information supplied by farmers, as only a small number reported this disease in their herds and most did not vaccinate against caseous lymphadenitis (Table 2). Difficulty in detecting this disease, and management information tailored for and directed towards sheep farmers and the lack of a comprehensive control program for caseous lymphadenitis probably had an influence on the ignorance about this disease on the part of the sheep farmers.

Table 2
Principal management practices identified among the 97 sheep herds studied to determine caseous lymphadenitis incidence in the state of Minas Gerais, Brazil, 2002.

Variable	*n*	%
Reported vaccination against caseous lymphadenitis	0	0
Declared that they had caseous lymphadenitis in their herd	11	11.3
Slaughters animals at more than 12 months	23	23.7
Does not separate animals with clinical signs of caseous lymphadenitis	91	93.8
Does not separate animals by age group	89	91.7
Does not identify animals individually	75	77.3
No technical assistance	57	58.8
Participates in expositions	8	8.2
Asks for a sanitary certificate when purchasing sheep	18	18.6
Does not disinfect to navel of newborns	23	23.7
Extensive/semi-extensive rearing system	53	54.6

The high serological prevalence that we found, compared with the low rate of cases of caseous lymphadenitis reported by the farmers demonstrates that this disease is neglected in these farms or that the farmers are not prepared to handle it. Also, the long incubation period (up to 180 days) and the occurrence of the visceral form of this disease (Williamson, 2001; Valli and Gentry, 2007; Dorella et al., 2006), which is detected only by serological tests during *post-mortem* exams or at slaughter, contribute to the fact that farmers have little concern for this disease, increasing the risk of dissemination of the infectious agent.

Few epidemiological studies have been made on caseous lymphadenitis in sheep in Brazil. We found only two such studies, one in the southern region of the country and the other in the Northeast. Silva et al. (1982) examined 9410 sheep from eight municipalities in the state of Rio Grande do Sul; these had been butchered with federal inspection. They found a frequency of positivity of 1.5% among castrated males and 8.1% in ewes. Tinôco (1983) reported a frequency of 36.5% of sheep farms that reported infection with caseous lymphadenitis, based on questionnaires answered by sheep farmers. We found a much higher prevalence among sheep herds and individual sheep in Minas Gerais, though the results are difficult to compare due to differences in methodologies, region and time of year.

The lack of serological studies to determine the prevalence of caseous lymphadenitis in other Brazilian states and the lack of data on the damage caused by this pathogen to farms and slaughterhouses, reinforces the notion that the actual economic importance of this disease for ovine breeding in Brazil is underestimated. In Australia, Paton et al. (1994) estimated losses of 4.1–6.6% in wool production, with annual losses of 17 million Australian dollars, without including subclinical losses, which are important for farmers, because they negatively impact on production, though the extent of such damage is unknown (Paton et al., 2003).

Table 3
Risk analysis of variables that are predictors of risk for caseous lymphadenitis in sheep farms in Minas Gerais.

Sheep farm characteristic	Prevalence ratio	Confidence interval
Animals not individually identified	1.060	1.001–1.122
No technical assistance	1.064	0.992–1.141
Participate in expositions	1.067	1.008–1.129
Extensive/semi-extensive management system	1.082	1.002–1.168

Among the farms that were sampled, 95.9% (93/97) had at least one positive sheep. Nearly all of the sampled farms had animals infected by *C. pseudotuberculosis*; among the 94 municipalities, 90 (95.7%) had at least one positively-testing animal. Diagnosis of herds based on finding a single animal to be infected was possible due to its high sensitivity and specificity, calculated as a function of the characteristics of the diagnostic procedure and the observed and expected levels of prevalence (Martin et al., 1992; Jordan, 1996).

The estimate of the prevalence of animals infected by *C. pseudotuberculosis* in Minas Gerais was also acceptable, as the actual prevalence calculated from the ELISA data, 75.8%, was within the estimated confidence interval for prevalence. The intracluster correlation coefficient (ρ) value found in this study was within the range observed by McDermott and Schukken (1994), which varied from 0.0017 to 0.46, but was larger than that estimated by Otte and Gumm (1997), (0).20, and than that observed by many other authors (McDermott and Schukken, 1994), between 0.01 and 0.15, for most infectious diseases. This reflects the lack of homogeneity in the distribution of the disease in each flock, which had a high variation from herd to herd, from 12.5 to 100% in infected flocks (data not shown), although the great majority of them were infected.

Design effect is calculated based on sampling error, which is higher in cluster sampling (Bennett et al., 1991). In our study, it was mainly influenced by the irregular size of the samples in each herd. This resulted from differences in herd size, which varied from 2 to 1843 sheep (mean 80.7 sheep, data not shown), and material lost between collection and laboratory analysis. Although sampling and blood collection were well conducted, some problems with the transportation of sera resulted in the loss of many serum samples, which influenced the design effect.

Only four of the flocks were not found to bear animals presenting infection. Samples obtained from those herds may not have been large enough to detect the disease. The high frequency of herds with infected sheep again shows that caseous lymphadenitis is widespread in the state of Minas Gerais, which is the state with the second largest sheep population in the southeastern region of Brazil (www.ibge.gov.br).

The prevalence of this disease in adult sheep in Australia was over 61% before they began vaccinating for caseous lymphadenitis (Middleton et al., 1991). It then fell to 20–29% (Paton et al., 2003). Currently, three commercial immunogens are available in Brazil; however, few farmers systematically vaccinate their sheep for caseous lymphadenitis or have no information on the health situation of their herds, especially regarding the serological status for the disease, which is fundamental for developing control strategies (Dorella et al., 2009). It is apparent that a control program that includes vaccination would be quite useful for reducing infection with *C. pseudotuberculosis* in this state.

Seroreactivity did not differ significantly between males and females ($P > 0.05$, Table 1), as was also found by Silva et al. (1982). Seropositivity was more frequent in the pure local breeds. There were significant differences in the comparison between pure local and no defined race, and also between pure local and mixed breed (Table 1). The race Santa Inês and the group "no defined race" are the main contributors to the racial mix of the Minas Gerais sheep population, which was mainly founded by animals from the northeastern region of Brazil (ARCO, 2008), where the incidence of caseous lymphadenitis is high and sheep and goats are often kept together (Tinôco, 1983; Pinheiro et al., 2000). Pure-bred animals are generally reared in an intensive system and many such sheep are sold in auctions and expositions, favoring the dissemination of the infectious agent when these animals are used to form or genetically improve herds.

When buying sheep, only 11.7% of the farmers asked for health certification, and most were not aware of the importance of this measure to maintain the health of their herds. Among producers who asked for health certificates, they were concerned with brucellosis, tuberculosis, rabies and anthrax, which are normally a concern for transit of bovines. None of the sheep farmers indicated that they required that the animals they purchased to be free of caseous lymphadenitis.

The high frequency of serologically positive animals in the upto 4-month-old category and the lack of a significant difference in this rate when compared to adult animals (Table 1) may be due to acquisition of infection early in the life of the animal, as hinted by the high frequency of infection in young animals (Table 1). This suggests that control efforts, to reduce or eliminate transmission of *C. pseudotuberculosis*, should be directed towards this age group.

Seropositive frequency was highest among adult animals (Table 1). O'Reilly et al. (2008) demonstrated that besides contact with the purulent material from abscesses, and environmental contamination, respiratory transmission (aerosols) can influence the maintenance of the infectious agent in the herd. Among the 97 farms that were sampled, 23 (23.7%) slaughtered the sheep at a later age, at 12 months or more; also, 93.8% (91/97) did not adopt control measures, such as segregation of clinically affected animals, and they did not separate the animals by age group (Table 2), which would explain why caseous lymphadenitis is common in sheep over 1-year-old. These conditions are favorable for the dissemination of the infectious agent, because along with high risk of contact with infected purulent material, the probability of pulmonary lesions is higher in older animals (O'Reilly et al., 2008).

Determining risk factors associated with prevalence helps with the planning of control programs, in order to attend market requirements for more and improved quality sheep meat and reduce losses for farmers. We found prevalence ratios of above one for the variables: lack of individual animal identifications, lack of technical assistance, participation in expositions and extensive/semi-extensive rearing system (Table 3). Nevertheless, these variables should not be considered key risk factors, because their indication of seropositivity prevalence was only marginally above 1; also, for one of them, lack of technical assistance, the confidence interval ratio included unity (Noordhuizen et al., 1997; Dohoo et al., 2003; Medronho et al., 2006).

These results could be a consequence of the type of study, transversal, which is not the best for identifying risk factors, and the high frequency of farms with seropositive animals, as only four herds were not seropositive (Dohoo et al., 2003; Medronho et al., 2006).

Even though they were not identified as significant risk factors for caseous lymphadenitis in sheep herds in the state of Minas Gerais, changes in the management schemes for the variables with ratios above 1 (Table 3) could help control the disease. The correct identification of animals results in improved control of the herd, because it allows separation of animals found to be infected, with introduction of specific management measures for controlling caseous lymphadenitis. This requires involvement of the farmer and monitoring of the control program for each farm, as well as training and education for farm workers. Participation in expositions and auctions is always a risk factor for the introduction of caseous lymphadenitis. These events are important for the commercialization of breeding stock and ewes.

Nevertheless, due to a lack of a routine laboratory diagnostic procedure that would attend to sanitary legislation, diagnosis is based on clinical exams alone. The sensitivity of this procedure is low, which allows animals without clinical signs characteristic of caseous lymphadenitis, both visceral and subclinical, to participate in events, disseminating *C. pseudotuberculosis*. Therefore, participation in events should be restricted to only those farms that use techniques that are sensitive enough for the diagnosis of caseous lymphadenitis, such as ELISA, for selecting animals. Increased observation of animals, associated with individual identification, even in extensive rearing operations, can help avoid factors that predispose towards this disease, such as exposition to mechanical agents that cause skin lesions, including barbed wire, narrow gates and passageways, cutting edges of feeding stations, and spiny vegetation, all of which are found in rural properties in the state of Minas Gerais.

Our findings support the notion that control of caseous lymphadenitis is done without effective diagnosis that would be needed for segregation and discard of seropositive animals. The lack of these measures and of vaccination, which we found in our study, allows for the dissemination of this infectious agent throughout the production network. Consequently, we conclude that caseous lymphadenitis is amply disseminated in the sheep population of the state of Minas Gerais and is overlooked by most farmers, favoring the endemicity of this disease.

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